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Original Article

Analytical Method Development and Validation for Simultaneous Estimation of Bilastine and Montelukast Sodium in their Combined Dosage form by Derivative UV-Spectroscopy and RP-HPLC Method

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ABSTRACT:

Objective: To develop and validate simple, accurate and Derivative UV-Spectroscopy and Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for the simultaneous determination of Bilastine and Montelukast Sodium (MNT) in bulk and pharmaceutical formulations. **Experimental approach**: In the first derivative UV method methanol was used as a diluent and quantification was achieved at 257 nm and 365 nm over the concentration range of 4-20 µg/mL and 2-10 µg/mL for Bilastine and Montelukast sodium respectively. In RP-HPLC method mixture of Acetonitrile and 20mM Potassium Dihydrogen Orthophosphate buffer (pH 3.5) in the ratio of 80:20 v/v was used as mobile phase, at a flow rate of 1.2 ml/min**. Findings and Discussion:** The linearity range for Bilastine and Montelukast sodium was found to be 10-50 µg/ml and 5- 25µg/ml respectively. The correlation coefficient value for both the methods were greater than 0.996. Accuracy of the methods was determined by recovery studies and it was found to be in the range of 98- 101%. The % RSD values for all the validation parameters were less than 2 % for both the methods. **Conclusion:** Both the developed methods are equally significant and can be used for simultaneous estimation of Bilastine and Montelukast sodium in their combined dosage form.

Keywords: Bilastine, Montelukast Sodium, First Derivative UV-Spectroscopy, RP-HPLC, Validation.

1. INTRODUCTION

Bilastine is a new, well-tolerated, nonsedating H1 receptor antihistamine. Clinical studies have shown that Bilastine is as efficacious as other nonsedating antihistamines in allergic rhinoconjunctivitis and chronic urticaria in individuals from 12 and 18 years of age, respectively [1]. Chemically it is, 2-[4-[2-[4-[1-(2-ethoxyethyl)benzimidazol-2-yl]piperidin-1-yl]ethyl]phenyl]-2-methylpropanoic acid (Figure 1).

Montelukast is a specific cysteinyl leukotriene receptor antagonist belonging to a styryl quinolines series with the chemical name 2-[1-[1(R)-[3-[2(E)-(7-chloroquinolin-2-yl) vinyl] phenyl]-3[2-(1-hydroxy-1-methylethyl) phenyl] propylsulfanylmethyl] cyclo-propyl] acetic acid sodium salt (Figure 2). It is mainly used to control and prevent symptoms caused by asthma (such as wheezing and shortness of breath) and in allergic rhinitis [2].

Drug Combination Bilastine and Montelukast Sodium were approved by CDSCO on 11th of March, 2020. Drug Combination Bilastine and Montelukast Sodium used for the treatment of allergic rhinitis and mild to moderate asthma [3].

Literature review revealed that several methods for analysis of Bilastine and Montelukast Sodium either alone or with other drugs by RP-HPLC [4-10], UPLC [11-13] and HPTLC [14-15] have been reported. Only one Method for simultaneous estimation of this combination has been reported by UV Spectroscopic method [16]. But there was no method for simultaneous estimation of this combination by RP-HPLC reported till now.

So, the aim of the present work was to develop the simple, economical, accurate, reliable RP- HPLC and First Derivative UV-Spectroscopic method for the estimation of Bilastine and Montelukast sodium in bulk and combined dosage form. This method was validated as per the ICH guidelines.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Bilastine and Montelukast Sodium were obtained from SYNOKEM PHARMACEUTICALS Ltd and AMBIX HEALTHCARE LLP (Ahmedabad) respectively as a gift samples. All Tablet BILUKAST-M containing 20 mg Bilastine and 10 mg Montelukast was purchased from local pharmacy. Analytical grade and HPLC grade solvents were used in UV-Spectroscopic method and RP-HPLC method respectively.

2.2 Instrumentation

Shimadzu UV-1700 double beam spectrophotometer connected to a computer with Shimadzu UV-Probe 2.10 software installed was used for all the spectrophotometric measurements. Shimadzu HPLC System equipped with UV detector was used for chromatographic separation of drugs. The samples were weighed on an electronic balance (A×120) by Shimadzu.

2.3 Spectrophotometric conditions for first derivative UV-Spectroscopy

2.3.1 Preparation of standard stock solution

Accurately weighed 10 mg of Bilastine (BIL) and Montelukast Sodium (MNT) was transferred into 10 ml volumetric flask separately and volume was made up to the mark using methanol to get concentration of 1000 µg/ml for both the drugs.

2.3.2 Preparation of working standard solution

From the standard stock solution of Bilastine and Montelukast Sodium 1 ml were taken out separately into 10 ml volumetric flask and volume was made up to the mark using methanol to get concentration of 100µg/ml for both the drugs.

2.3.4 Selection of analytical wavelength

A zero order UV spectrum (D^0) of MTK and BIL was obtained by scanning 10 µg/ml of both the drugs at 200-400 nm on a double beam UV/Visible spectrophotometer. These zero order spectra were converted into their respective first derivative (D^1) spectra using UV Probe software itself. The transformation of zero order spectra to first derivative spectra was done using a $\Delta\lambda = 5$nm and scaling factor of 1.Thus, it was observed that estimation of BIL was possible at 257 nm which was zero crossing point of MNT and estimation of MNT was possible at 365 nm which was zero crossing point of BIL.

2.3.5 Preparation of series for calibration curve for 1st derivative method

To prepare the linearity range of Montelukast sodium 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1ml were withdrawn from the working standard solution of Montelukast sodium and taken into 10ml volumetric flasks and the volume was made up with methanol to prepare a series of solutions having concentration in the range of 2-10 µg/ml. Similarly to prepare the linearity range of Bilastine 0.4ml, 0.8ml, 1.2ml, 1.6ml and 2ml were withdrawn from the working standard solution of Bilastine and taken into 10ml volumetric flasks and the volume was made up with methanol to prepare a series of solutions having concentration in the range of 4- 20 µg/ml.

2.3.6 Analysis of marketed formulation

20 tablets of formulation (BILUKAST-M) containing 20mg of Bilastine and 10mg of Montelukast Sodium were weighed accurately. The average weight of tablets was found and tablets were powdered. The tablet powder equivalents to 10mg of Montelukast Sodium was weighed and transferred into 100ml volumetric flask and volume was made up to the mark using methanol to get 100 µg/ml solution. The content was filtered through the whatman filter paper to get clear solution.

From the clear sample stock solution, 1 ml was withdrawn and taken into 10 ml volumetric flask and volume was made up to the mark using methanol to obtain 10µg/ml of Montelukast Sodium and 20 µg/ml of Bilastine. The resulting solutions were analyzed for drug content by D^1 spectrophotometric method at 365 nm and 257 nm for MTK and BIL, respectively. Assay was repeated 6 times and standard Deviation was calculated.

2.4 Chromatographic conditions

2.4.1 Selection of analytical wavelength

The Standard solutions of Bilastine and Montelukast Sodium were scanned using the UV-Spectrophotometer in the range of 200nm to 400nm.

From overlain spectra, both the drugs showed good sensitivity at 283nm. Overlay spectra are shown in Figure 3.

ANALYTICAL – C18 Hyper Chrome ODS-BP Column was used having dimensions of 250mm x 6mm, and an internal diameter of 5µm. The optimized mobile phase used was Acetonitrile : Potassium Dihydrogen Orthophosphate Buffer (80:20) at Ph 3.5adjusted with Orthophosphoric acid (OPA). A flow rate of 1.2 ml/min was set and the wavelength of detection was set to 285nm. A manual Rheodyne 7725 Injector valve with a fixed injection volume of 20µL was used and the run-time was set to 12 minutes at ambient temperature. Mobile phase was sonicated for 10 mins to degas the solution.

2.4.2 Construction of calibration curve

From the working standard solution of Bilastine and Montelukast Sodium (100 µg/ml), accurate volume was taken out into 10 ml volumetric flask separately to get 10- 50 µg/ml of Bilastine and 5- 15 µg/ml of Montelukast Sodium. Volume was made up to the mark using mobile phase and solution was sonicated for 10 mins.

2.4.3 Analysis of marketed formulation

Twenty tablets were accurately weighed and finely powdered. Tablets powder weight equivalent to 20 mg of Bilastine and 10 mg of Montelukast sodium accurately was weighed and transferred to a into 100 ml volumetric flask and volume is made up to the mark using methanol. The mixture was sonicated for 10 min and diluted up to the mark with methanol. The content was filtered through the whatman filter paper to get clear solution. From the clear sample stock solution, 1 ml was withdrawn and taken into 10 ml volumetric flask and volume was made up to the mark using mobile phase to obtain 10 µg/ml of Montelukast Sodium and 20 µg/ml of Bilastine. Solutions were sonicated for 10 mins.

2.5 Method Validation

2.5.1 Linearity and calibration curve

Linearity was checked by diluting standard stock solution at six different concentrations. MNT (2-10 µg/ml) and BIL (4-20 µg/ml) concentration ranges were taken for linearity study and for RP-HPLC method MNT (5-25 µg/ml) and BIL (10-50 µg/ml) were taken. The linear regression analysis obtained by plotting the D^1 absorbance (for UV) and peak area (for HPLC) of analyte vs. concentration. Each dilution was prepared sextaplicate, and scanned in the range of 200-400 nm against the methanol as a blank, and average of six first derivative (D^1) absorbance value was taken for calibration curve and linearity study in case of UV method. In RP-HPLC all the solutions were injected 6 times and average of six peak area was taken for calibration curve.

2.5.2 Accuracy

Recovery studies for the UV method were conducted using the Standard Addition Method by taking a nominal concentration of 6µg/ml for Montelukast Sodium and 12µg/ml for Bilastine from the formulation (test sample) and then spiking this solution by 50%, 100% and 150% of standard drug (API). For RP-HPLC accuracy were performed by taking 15 µg/ml for Montelukast Sodium and 30 µg/ml for Bilastine from the formulation (test sample) and then spiking this solution by 80%, 100% and 120% of standard drug (API). Each concentration was analyzed six times, and average recoveries of added standard drug were measured.

2.5.3 Precision

For performing intraday precision (repeatability), and inter-day precision (intermediate precision) three concentrations including lower, middle and upper limits 2, 6, and 10 µg/ml for MNT and 4, 12 and 20 µg/ml for BIL were taken in UV method and in RP-HPLC 5, 15 and 25 µg/ml for MNT and 10, 30 and 50 µg/ml for BIL were taken and analyzed three times on the same day for intraday precision, and on 3 different days (first, second and third) at the same concentration levels.

2.5.4 Limit of Detection and Limit of Quantification

The value of LOD and LOQ were calculated according to 3.3σ/S and 10σ/S respectively, where σ is the standard deviation of the y-intercepts of the regression lines and S is the slope of the calibration curve.

2.5.5 Specificity

The specificity of the method was determined by comparing the spectra (for UV) and chromatogram (for RP-HPLC) of the standard and sample solutions of Bilastine and Montelukast sodium.

2.5.6 System Suitability parameters (for RP-HPLC)

The system suitability tests which were applied for the RP-HPLC Method include the following parameters

- Retention Time
- Theoretical Plates
- Asymmetry Factor
- Resolution between the peaks

Retention times of both BIL and MNT as well as Resolution between the peaks were having a %RSD value less than 2 which depict that the method is acceptable.

2.5.7 Assay of Marketed formulation

The validated UV spectrophotometric and RP-HPLC methods were used in the analysis of the marketed formulation BILUKAST-M with a label claim of 20 mg for BIL and 10 mg for MNT per tablet. The results for the assay show good agreement with the label claims.

2.5 8 Comparison of methods

Developed UV-Spectroscopy method and RP-HPLC methods were compared by applying paired two tail t-test to the assay results. By applying t-test to both the methods it was found that t_{stat} values were less than the $t_{critical}$ values and P values were greater than applied alpha value ($P>0.05$). It denotes that there are no significant difference between the means of the methods. So, both the developed method are equally significant and can be applied for simultaneous estimation of these drugs in their combined dosage form.

3. RESULTS AND DISCUSSION

Calibration curve was found to be linear at the concentration range of 4-20 µg/ml (BIL) and 2-10 µg/ml (MNT) for UV method and 10-50 µg/ml (BIL) and 5-25 µg/ml (MNT) for RP-HPLC. Correlation coefficients, slope and intercept of the regression equation are presented in Table 1. Calibration graph of RP-HPLC method and UV method for BIL and MNT are shown in figure 4 and figure 5. Overlay chromatogram of linearity in RP-HPLC is shown in Figure 6. Overlay spectra of First Derivative UV method is shown in Figure 7. Accuracy results of Bilastine and Montelukast sodium for UV Method and RP-HPLC are shown in Table 2 and Table 3 respectively. All the methods showed good recoveries of drugs. %RSD were also found to be less than 2. Precesion results of Bilastine and Montelukast are shown in Table 4 and Table 5. Results of Limit of Detection and Limit of Quantification of Bilastine and Montelukast sodium are presented in Table 6. Specificity of the RP-HPLC method was checked by the comparing blank, API and sample chromatogram. Blank chromatogram and API chromatogram are shown in Figure 8 and figure 9

respectively. Chromatogram of sample is shown in figure 10. Retention times of both BIL and MNT as well as Resolution between the peaks were having a %RSD value less than 2 which depict that the method is acceptable. System suitability parameters are presented in Table 7. Assay was performed by both the methods and results are presented in Table 8. The comparison of methods denotes that there is no significant difference between the means of the methods. So, both the developed method is equally significant and can be applied for simultaneous estimation of these drugs in their combined dosage form Table 9.

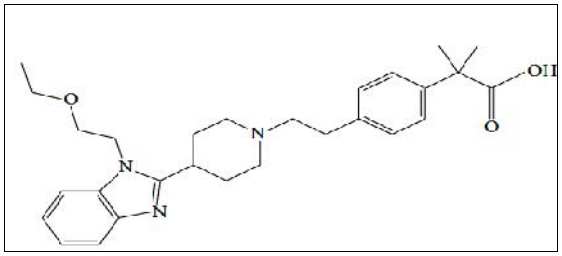

Fig 1: structure of Bilastine

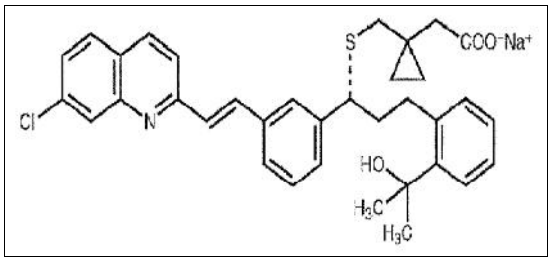

Fig 2: **Structure of Montelukast Sodium**

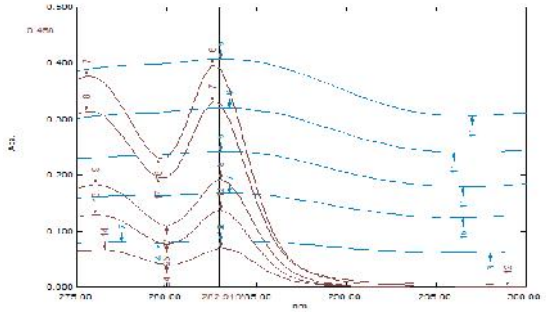

Fig 3: Selection of wavelength for RP-HPLC

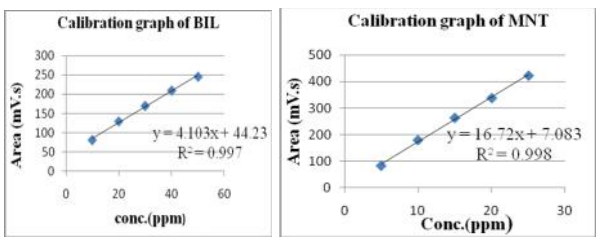

Fig 4: **Calibration graph of RP-HPLC (a) BIL. (b) MNT**

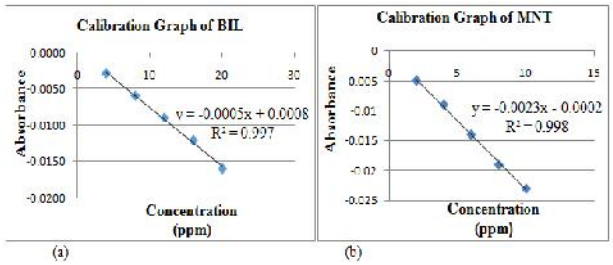

Fig 5: Calibration graph for UV Method (a) BIL, (b) MNT

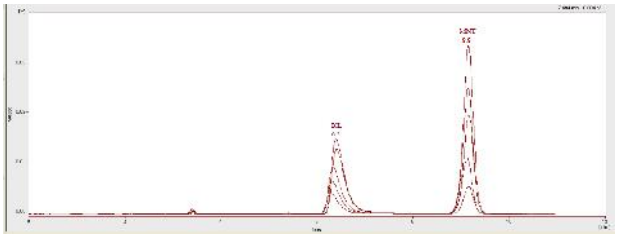

Fig 6: Overlay chromatogram of Linearity in RP-HPLC

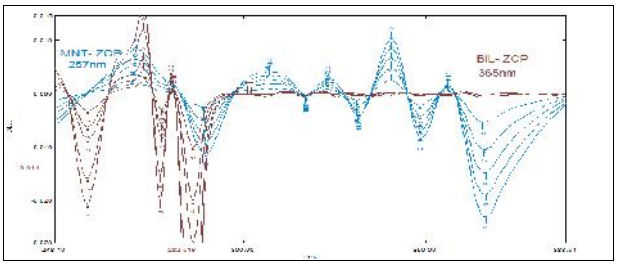

Fig 7: Overlay first derivative spectra of UV method

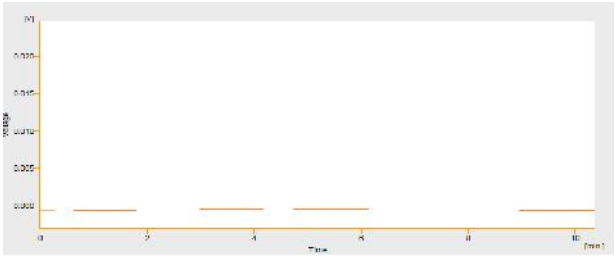

Figure 8 Blank chromatogram

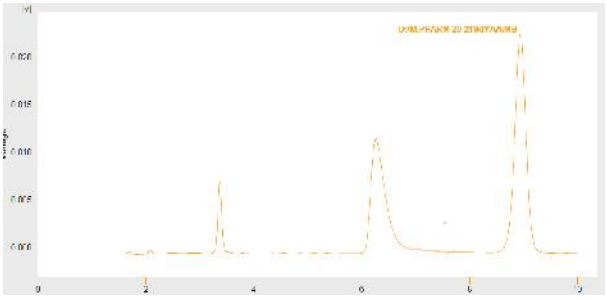

Figure 9 Chromatogram of API

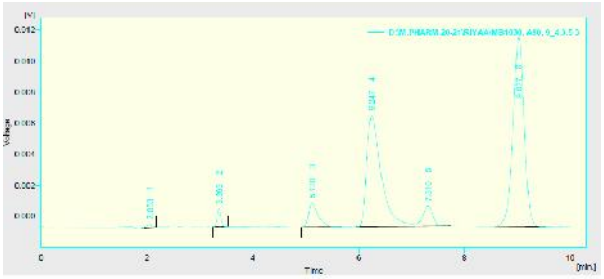

Fig 10: Chromatogram of sample

Table 1: Linear regression data of calibration curve

Parameters	UV method		RP-HPLC Method	
	BIL	MNT	BIL	MNT
Concentration range	4-20 µg/ml	2-10 µg/ml	10-50 µg/ml	5-25 µg/ml
Slope	-0.0005	-0.0023	4.103	16.72
Intercept	0.0008	-0.0002	44.23	7.083
Correlation co-efficient (r^2)	0.997	0.998	0.997	0.998

Table 2: Accuracy results of BILASTINE for UV and RP-HPLC method

Parameters	BILASTINE					
	UV Method			RP-HPLC method		
% spiked	50	100	150	80	100	120
Concentration of test	12	12	12	30	30	30
Concentration of std added	6	12	18	24	30	36
Concentration recovered	18.18	23.87	29.87	54.31	59.53	66.01
% Recovery ± SD	101.02 ± 0.092	99.45 ± 0.214	99.56 ± 0.449	100.57 ± 0.964	99.22 ± 0.6	100.02 ± 0.029

Table 3: Accuracy results of Montelukast Sodium for UV and RP-HPLC

Parameters	MONTELUKAST SODIUM					
	UV Method			RP-HPLC Method		
% spiked	50	100	150	80	100	120
Concentration of test	6	6	6	15	15	15
Concentration of std added	3	6	9	12	15	18
Concentration recovered	9.06	12.1	14.86	26.98	29.97	32.95
% Recovery ± SD	100.66 ± 0.023	100.83 ± 0.051	99.06 ± 0.150	100.07 ± 0.069	99.00 ± 0.237	99.09 ± 0.289

Table 4: Precesion results for Bilastine

UV Method	INTRADAY			INTERDAY	
	Conc.(µg/ml)	SD(n=3)	%RSD	SD(n=3)	%RSD
	4	5.8E-05	1.97	0.00001	0.36
	12	5.8E-05	0.64	0.0001	1.12
	16	0.00012	0.71	0.00015	0.94
RP-HPLC method	10	1.09	1.33	1.07	1.29
	30	1.49	0.87	1.35	0.78
	50	1.69	0.67	2.21	0.9

Table 5: Precesion results for Montelukast Sodium

UV Method	INTRADAY			INTERDAY	
	Conc.(µg/ml)	SD(n=3)	%RSD	SD(n=3)	%RSD
	2	0.00006	1.16	0.00006	1.19
	6	0.0001	0.7	0.00015	1.08
	10	0.00012	0.53	0.00015	0.67
RP-HPLC method	5	0.43	0.5	1.34	1.54
	15	1.76	0.66	2.24	0.84
	25	1.28	0.3	1.83	0.43

Table 6: LOD and LOQ results for UV and RP-HPLC

UV- Method	Drug	LOD (µg/ml)	LOQ (µg/ml)
	Bilastine	0.369	1.118
	Montelukast Sodium	0.128	0.389
RP-HPLC Method	Bilastine	0.858	2.6
	Montelukast Sodium	0.06	0.193

Table 7: system suitability parameters

Parameters	BILASTINE	MONTELUKAST SODIUM
Retention Time	6.202	8.87
Asymmetry Factor	1.72	0.932
Resolution	9.082	4.76
Theoritical plates	2734	8868

Table 8: Assay results

UV-Method	Formulation BILUKAST-M	Amount Lebeled (mg)	Amount Estimated (mg)	%Recovery ± SD (n=6)	%RSD
	Bilastine	20	19.64	98.2 ± 0.122	0.62
	Montelukast Sodium	10	9.88	98.93 ± 0.041	0.521
RP-HPLC Method	Bilastine	20	19.89	99.45 ± 0.321	1.63
	Montelukast Sodium	10	10.13	101.3 ± 0.131	1.31

Table 9: Applied t-test results

Parameters	Bilastine		Montelukast sodium	
	RP-HPLC	UV Method	RP-HPLC	UV Method
Mean	99.45	99.21	99.875	100.31
Variance	0.00141	0.33	0.0033	0.777
Observations	6	6	6	6
Hypothesized mean difference	0		0	
D_f	5		5	
t_{stat}	1.026		-1.207	
P(T<=t) two tail	0.351		0.281	
$t_{critical}$ two tail	2.57		2.57	

4. CONCLUSION

A convenient and rapid method for simultaneous estimation of Bilastine and Montelukast Sodium in combined dosage form has been developed and validated. Out of the results it can be concluded that the proposed analytical methods are

equally significant and can be used for the estimation of Bilastine and Montelukast sodium in their combined dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day and interday % RSD coupled with excellent recoveries. Hence, these methods can be conveniently adopted for routine analysis of Bilastine and Montelukast sodium in pure form and its dosage forms.

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