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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF BILASTINE AND MONTELUKAST IN BULK DRUG AND ITS PHARMACEUTICAL DOSAGE FORM

BHANDARE AN[1*] AND NANNOR KM[2]

1: PG Scholar, M. Pharmacy (Pharmaceutical Quality Assurance), PRES's College of Pharmacy (For Women), Chincholi, Nashik

2: Assistant Professor, PRES's College of Pharmacy (For Women), Chincholi, Nashik

*Corresponding Author: Ms. Arati N. Bhandare: E Mail: bhandarearati@gmail.com

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ABSTRACT

Several spectrophotometric and HPLC methods have been reported for determination of Bilastine and Montelukast in drugs and in pharmaceutical dosage forms. Hence, in the present study, a new, sensitive, suitable and robust reversed-phase high performance liquid chromatography method was developed and validated for the determination of in Bilastine and Montelukast bulk drug and in tablet formulation. In RP-HPLC method, Methanol and Water (60:40 % v/v) was used as mobile phase, at a flow rate of 1.0 ml/min, on HPLC system containing UV- detector with Openlab EZchrome software and Agilent, poroshell C18, 150 mm X 4.6 mm ID, 5 µm. The detection was carried out at 225 nm. The method gave suitable retention time i.e. 2.89 min for Bilastine and 6.07 min for Montelukast. The results of analysis in the method were validated in terms of Filter study, Solution stability, specificity, Linearity, accuracy, precision (Repeatability and intermediate precision), limit of detection, limit of quantification and robustness. A simple and precise method was developed for the assay of Bilastine and Montelukast in bulkdrug and in tablet formulation.The method need regular reagents for doing analysis and also less time consuming, it can be performed routinely in industry for routine analysis of bulk and marketed product of Bilastine and Montelukast.

Keywords: RP-HPLC, Bilastine, Montelukast, Validation, Analytical Method

INTRODUCTION

A current new antihistamine called Bilastine discerns highly for H1 histamine receptor with rapid onset but long-term action period, which comes under the selective Histamine of H1 receptor Antagonist (Ki = 64Nm) **[1]**. At the time of sensitive feedback pole, cells release histamine and other substances by undergoing degranulation. Bilastine, on adhering to and protecting against the H1 receptoractivation, can slow the progression of acute symptoms by releasing histamine from mast cells **[2]**. The IUPAC name for the Bilastine was "2- [4-(2- 4- [1-(2-ethoxyethyl)-1 H-1,3-benzodiazol-2-yl] piperidin-1-yl ethyl) phenyl] -2-methylpropanoic acid", the Chemical Solution for the Bilastine drug was $C_{28}H_{37}N_3O_3$. Bilastine has a Molecular Weight of 463.622 $g.mol^{-1}$. Bilastine is soluble in the natural solvent chloroform at a concentration of about 30 mg/ml. Montelukast is a leukotriene receptor villain that partly is used in daily asthma therapy, to prevent the workout caused by bronchoconstriction, as well as in hay fever treatment **[3]**. Cells such as pole cells, eosinophils release CysLT (Cysteinyl Leukotrienes) especially LTD4, LTC4, LTE4, and even Eicosanoids. Once these CysLT binds accordingly with CysLT receptors such as the type-I CysLT receptors present over the smooth muscular tissue cells of breathing air passage, Macrophages' air passage & even pro-inflammatory cells such as eosinophils, few myeloid stem cells can aid in asthma pathophysiology and boosting of allergic rhinitis **[4]**. IUPAC name is 2- [1-(methyl) cyclopropyl] acetic acid. Chemical Formula is C35H36ClNO3S. Molecular Weight is 586.183 g.mol−1. The physical properties of Montelukast salt are its hygroscopic nature, optical activity, white to beige colored powder. Montelukast sodium is highly soluble in ethanol and methanol, but is slightly soluble in water and acetonitrile **[5]**. In the estimation of the Bilastine and Montelukast drug, both individual and other drug combination forms there are many works of literature works with different techniques have been reported, such as RP-HPLC **[6-10]**, RP-UPLC **[11]**, UV **[12]**. Because of the requirement for an appropriate and economical RP-HPLC method for daily analysis of both Bilastine and Montelukast as tablet dosage form, as being accessible, specific, accurate, and cost-efficient analytical technique, this has been considered for the current study, Comparative to literature survey the developed method runtime is less and Retention times for both the drugs were very less when compare to reported works. Further, the validation of the suggested above method was performed by keeping the ICH standards.

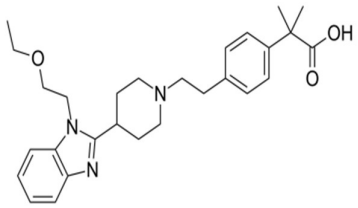

Figure 1: Chemical structure of Bilastine

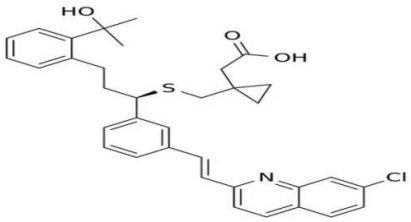

Figure 2: Chemical structure of Montelukast

MATERIALS AND METHODS

Chemicals and Reagents

Bilastine and Montelukast samples were gifted from the yarrow pharmaceuticals Mumbai.water, Methanol required for HPLC analysis were bought from 'Merck' Mumbai.

Conditions for Chromatographic Analysis and Equipment

A 1260 Infinity II HPLC system was used to perform chromatography that contains an auto- sampler, a UV detector, and a software application named Openlab EZChrome. At 225 nm, the analysis was performed with the column Agilent poroshell C18 150 mm length x 4.6 Internal Diameter x 5 µm particle size, dimensions at 400C temperature level. The isocratic mobile phase contains Methanol and also Water (60:40%v/v). The flow rate at 1ml/min was conditioned at a run time of about 10 min.

Preparation of the Individual Bilastine Standard (Stock Solution)

In order to prepare stock solution, weighed accurately 10 mg Bilastine and transferred into 20 ml volumetric flask, added 15 ml of methanol and sonicated to dissolve the standard completely and diluted up to the mark with methanol (500 PPM). Further diluted 0.8 mL to 20 mL with methanol. (20 PPM)

Preparation of the Individual Montelukast Standard (Stock Solution)

In order to prepare stock solution, weighed accurately 10.37 mg Montelukast Sodium (Equivalent to 10 mg of Montelukast) and transferred into 20 ml volumetric flask added 15 ml of methanol and sonicated to dissolve the standard completely and diluted up to the mark with methanol (500 PPM). Further diluted 0.8 mL to 20 mL with methanol. (20 PPM)

Preparation of Sample Solution: (Tablet)

Weighed 20 tablets transferred in mortar pestle and crushed to fine powder. Mixed the contents with butter paper uniformly. Weighed the powder material equivalent to 10 mg of Montelukast and 20 mg of Bilastine (262.4 mg of powder material). Transfer it in a clean and dried 50 mL of volumetric flask, added 35 ml of methanol sonicated it for 15 minutes with intermittent shaking. Made the volume up to the mark with methanol. Filter the solution through

suitable 0.45µ syring filter discarding 3-5 mL of filtrate. Further diluted 2 ml of filtrate to 20 ml with mobile phase. (20 PPM of Montelukast and 40 PPM of Bilastine)

Procedure

20 µl of the sample was infused into the HPLC instrument, and the peak areas for Bilastine and Montelukast were measured. The formulae are then used to calculate the percent Assay.

RESULTS AND DISCUSSION

The proposed work involves the development and validation of the selected Bilastine and montelukast drug determination concurrently with the aid of RP-HPLC method that is in both bulk and combined formulations according to ICH guidelines Q2 (R1).

Analytical Method Validation

The method was developed and validated by parameters such as precision, accuracy, LOD, LOQ, Robustness, system suitability as per the ICH standards.

System Suitability Parameters

The system suitability parameters such as retention time, USP theoretical plate count, USP Tailing were assessed. Mobile phase has been sent via column at flow rate at 1ml / min to equilibrate the column at the desired temperature level. The solution of about 20 µl has been injected to the column by using Agilent Poroshell C18 column with the given dimensions of 150 mm length × 4.6 mm internal diameter × 5 µm particle size. Methanol and Water at 60:40 ratio was taken as mobile phase in order to reach chromatographic separation between two drugs. The system suitability parameters have been mentioned in the **Table 1**.

Pharmaceutical Formulation Assay

The development of the technique was used to determine the estimation of Bilastine and Montelukast tablet dosage form. The resulting data of the drugs were compared with that of the label claim of the drugs and was represented in the **Table 2**.

Linearity and Range

The present work includes the concentration levels from 100 ppm-500 ppm and 1 ppm-5ppm. The concentration the drug is directly proportional to the response of the analyte and at each concentration level, the area of the analyte has been taken to calculate the co-relation co-efficient. The chromatographic system was injected with the drug concentrations mentioned above. The peak areas were calculated for all the concentration levels. The plotting of graph against the concentration vs peak area. The calculated co-relation co-efficient values has been mentioned in **Tables 3, 4** and graphs in **Figure 6 and 7**.

Accuracy Study

With the aid of recovery study, the accuracy has to be determined. The recovery study was performed at 3 concentration levels as 50%, 100%, and 150%. The standard solution is further injected into

chromatographic instrument. The recovery of Bilastine and Montelukast has been calculated from the known concentrations of spiked amounts. The values are mentioned in **Tables 5 & 6**.

Precision Study

Precision was determined with co-efficient variance of six replicate injection based on requirement. The standard solution has been injected five times and gauged in HPLC. From this %RSD of the area for 6 times has been observed. The outcome has been represented in **Tables 7 and 8**.

Robustness study

The robustness has been calculated by modifying the flow rate, composition of the mobile phase, according to the optimized conditions. Flow rate has been lowered to 1.1 ml/ min and elevated to 0.9 ml/ min. Mobile phase has been reduced to 10% and increased to 10%. Results were mentioned in **Tables 9, 10, 11, 12**.

LOD and LOQ Results

The analyte's lowest concentration of analyte has been injected for calculating the detection level and quantification level. The LOD and LOQ levels were calculated from the calibration curves by using the below mentioned formulas based on ICH standards. The results are shown in the **Table 13**.

LOD = 3.3 x SD/slope,

LOQ = 10 x SD/slope

Table 1: Parameters of System Suitability

Parameters	Bilastine	Montelukast
Retention time	2.89	6.07
USP Plate count	5342	4628
USP Tailing	1.11	1.10

Table 2: Results of Bilastine and Montelukast assay

Label Claim (mg)		% Assay
Bilastine	20	98.97
Montelukast	10	99.71

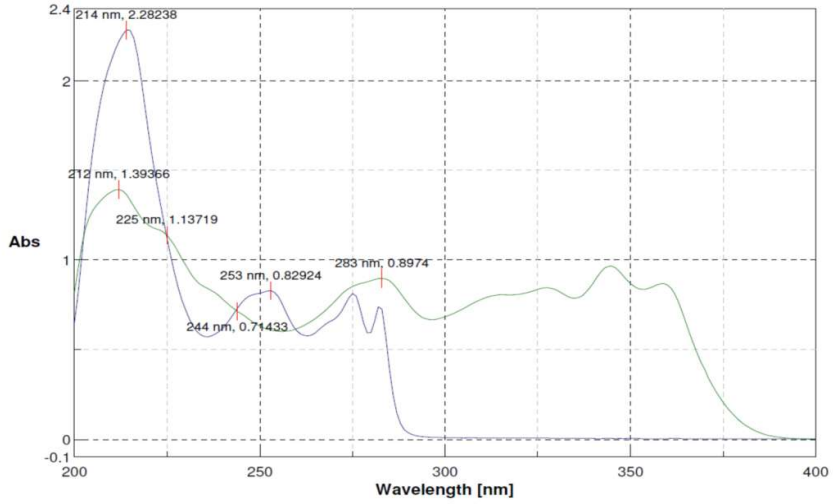

Figure 3: Overlay UV spectrum of Bilastine & Montelukast

Table 3: Bilastine linearity results

S. No	Sample Name	RT	Area	Height
1	Injected Concentration-10%	2.88	532563	5119
2	Injected Concentration-50%	2.88	2648560	5170
3	Injected Concentration-100%	2.91	5364774	5296
4	Injected Concentration-125%	2.89	6689046	5217
5	Injected Concentration-150%	2.91	7994823	5158

Y = 133538.878 x + -5341.172,(R^2)=0.99997

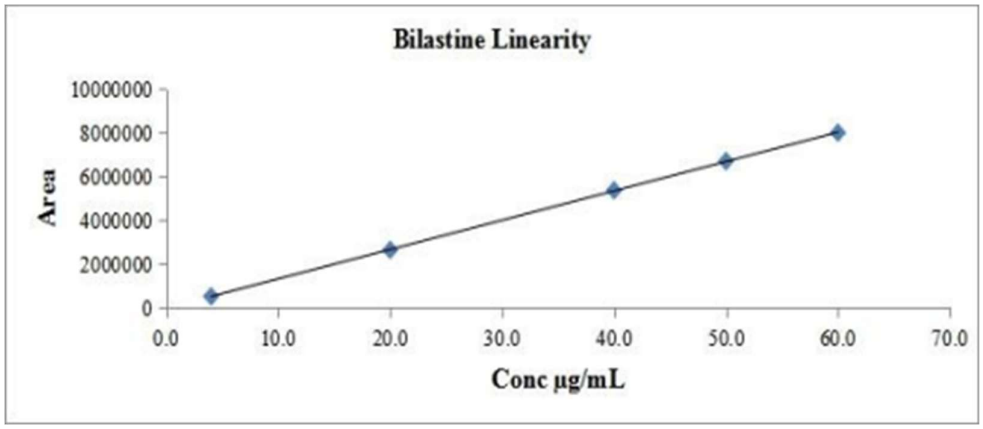

Figure 4: Linearity graph for Bilastine

Y = 311782.762 x + 12701.246, (R^2)=0.99998

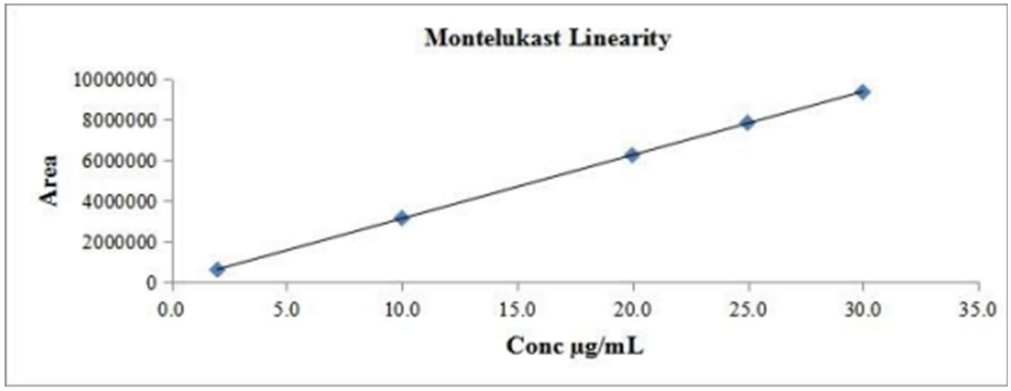

Figure 5: Linearity graph for Montelukast

Table 4: Montelukast linearity results

S. No	Sample Name	RT	Area	Height
1	Injected Concentration-10%	6.88	619248	4573
2	Injected Concentration-50%	6.04	3149617	4436
3	InjectedConcentration-100%	6.07	6240432	4603
4	InjectedConcentration-125%	6.06	7826147	4813
5	InjectedConcentration-150%	6.08	9348007	4787

Table 5: Accuracy results for Bilastine

%Concentration at specification level	Bilastine Area	Amount added in mg	Amount retrieved in mg	% Recovery	Mean Recovery
50%	2670251	20	20.11	99.06%	
100%	5386014	40	39.63	99.83%	99.40%
150%	8020452	60	59.66	99.32%	

Table 6: Accuracy results for Montelukast

50%	3210362	10	10.06	99.21%	
100%	6286048	20	19.82	99.44%	99.48%
150%	9360253	30	29.90	99.78%	

Table 7: Bilastine precision results

S. No	Sample Name	Test Sample (mg)	Area	%Assay
1	Bilastine	262.8	5302562	99.14
2	Bilastine	262.1	5196304	97.41
3	Bilastine	262.3	5315820	99.57
4	Bilastine	262.4	5249604	98.30
5	Bilastine	261.9	5328140	99.96
6	Bilastine	262.6	5389256	100.83
Mean				99.20
Std. dev.				1.2174
% RSD				1.227

Table 8: Montelukast Precision results

S. No	Sample Name	Test sample(mg)	Area	%Assay
1	Montelukast	262.8	6157921	98.34
2	Montelukast	262.1	6283205	100.61
3	Montelukast	262.3	6093252	97.49
4	Montelukast	262.4	6223581	99.54
5	Montelukast	261.9	6109475	97.90
6	Montelukast	262.6	6213047	99.30
Mean				98.86
Std. dev.				1.163508
% RSD				1.177

Table 9: System Suitability Results of Bilastine (Flow Rate)

S. No	The Flow Rate in ml/min	Result of system suitability	
		USP theoretical plate count	USP Tailing Factor
1.	1.1	4884	1.08

Table 10: System Suitability Results of Montelukast (Flow Rate)

S. No.	The Flow Rate in ml/min	Result of system suitability	
		USP theoretical Plate count	USP TailingFactor
1.	1.1	4275	1.09

Table 11: System Suitability Results for Bilastine (Mobile phase)

S. No.	Organic composition changes in Mobile Phase	Result of system suitability	
		USP theoretical Plate count	USP Tailing Factor
1.	10% Less	5354	1.11
2.	Actual	5329	1.12
3.	10% More	5346	1.11

Table 12: System Suitability Results for Montelukast (Mobile phase)

S. No.	Organic Composition changes in Mobile Phase	Result of system suitability	
		USP theoretical Plate count	USP Tailing Factor
1.	10% less	4624	1.10
2.	Actual	4632	1.10
3.	10% More	4626	1.09

Table 13: LOD, LOQ Results of Bilastine and Montelukast

Drug	LOD	LOQ
Bilastine	0.534	1.618
Montelukast	0.233	0.707

CONCLUSION

As per the ICH standards the RP-HPLC method has been developed and validated and the method has been rapid, sensitive, simple, specific, accurate, economic, ethical, cost effective, precise, robust for

concurrent estimation and quantification of Bilastine and Montelukast in marketed dosage form. By using this method analysis can be carried out for the regular batch analysis of pure and pharmaceutical dosage forms and quality monitoring. The final results of the recovery studies were found to be accurate indicating no interferences from the excipients.

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