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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF BILASTINE AND MONTELUKAST IN TABLET DOSAGE FORM

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Abstract:

A simple and selective LC method is described for the determination of Bilastine and Montelukast in tablet dosage forms. Chromatographic separation was achieved on a Waters AcquityC18(50mm x2.1 mm ID) 1.8µm using mobile phase consisting of a mixture of 55 volumes of mixed Phosphate Buffer pH 3.5: Acetonitrile (75:25) %v/v with detection of 265nm. Linearity was observed in the range 20-60 µg/ml for Bilastine (r^2 =0.9995) and 10-30 µg/ml for Montelukast (r^2 =0.9997) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. From the above experimental results and parameters, it was concluded that, this newly developed method for the simultaneous estimation Bilastine and montelukast was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

Keywords: *Bilastine, Montelukast, RP-HPLC, Simultaneous estimation.*

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INTRODUCTION:

Bilastine is a novel new-generation antihistamine that is highly selective for the H1 histamine receptor, has a rapid onset and prolonged duration of action. Bilastine is a selective histamine H1 receptor antagonist (Ki = 64Nm).[1] During allergic response mast cells undergo degranulation which releases histamine and other subastances. By binding to and preventing activation of the H1 receptor, bilastine reduces the development of allergic symptoms due to the release of histamine from mast cells.[2] IUPAC Name of Bilastine is 2-[4-(2-{4-[1-(2-ethoxyethyl)-1H-1,3-benzodiazol-2-yl] piperidin-1-yl} ethyl) phenyl]-2-methylpropanoic acid. Chemical Formula of Bilastine is $C_{28}H_{37}N_3O_3$. Molecular Weight of Bilastine is 463.622 $g{\cdot}mol^{-1}$. Bilastine is soluble in the organic solvent chloroform at a concentration of approximately 30 mg/ml.

Montelukast is a leukotriene receptor antagonist used as part of an asthma therapy regimen, to prevent exercise induced bronchoconstriction, and to treat seasonal allergic rhinitis.[3] Cysteinyl leukotrienes (CysLT) like LTC4, LTD4, and LTE4, among others, are eicosanoids released by a variety of cells like mast cells and eosinophils. When such CysLT bind to corresponding CysLT receptors like CysLT type-1 receptors located on respiratory airway smooth muscle cells, airway macrophages, and on various pro-inflammatory cells like eosinophils and some specific myeloid stem cells activities that facilitate the pathophysiology of asthma and allergic rhinitis are stimulated.[4] IUPAC Name is 2-[1-({[(1R)-1-{3-[(E)-2-(7-chloroquinolin-2-yl) ethenyl] phenyl}-3-[2-(2-hydroxypropan-2-yl) phenyl] propyl] sulfanyl} methyl) cyclopropyl] acetic acid. Chemical Formula is $C_{35}H_{36}ClNO_3S$. Molecular Weight is 586.183 $g{\cdot}mol^{-1}$. Montelukast sodium is a hygroscopic, optically active, white to off-white powder. Montelukast sodium is freely soluble in ethanol, methanol, and water and practically insoluble in acetonitrile.

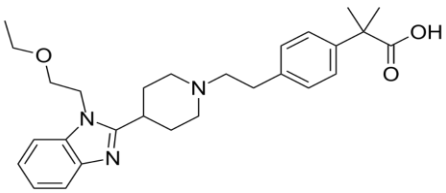

Figure 1: Structure of Bilastine

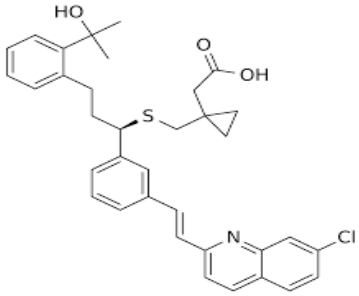

Figure 2: Structure of Montelukast

The literature survey revealed that There are very few methods reported in the literature for analysis of Bilastine and Montelukast alone or in combination with other drugs in the pure form and pharmaceuticals formulations by RP-HPLC [6-10], RP-UPLC [11], UV [12]. In view of the need for a suitable, cost-effective RP-HPLC method for routine analysis of Simultaneous estimation of Bilastine and Montelukast in Tablet dosage form, attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Bilastine and Montelukast. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the Simultaneous estimation of Bilastine and Montelukast in Tablet dosage form by using RP-HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form. To apply the developed method for the simultaneous estimation of Bilastine and Montelukast in Tablet dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: Bilastine and Montelukast were Gift samples obtained from Madras pharmaceuticals, Chennai. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 265 nm with column Waters AcquityC18(50mm x2.1 mm ID) 1.8µm, dimensions at 35^0C temperature. The optimized mobile phase consists of Phosphate Buffer pH 3.5: Acetonitrile

(75:25) %v/v Flow rate was maintained at 0.5 ml/min and run time for 5 min.

Preparation of solutions:

Diluant Preparation:
Mobile phase is used as Diluant.

Preparation of Standard solution:
About 125 mg of BILASTINE and 100mg of Montelukast were weighed into a 100 mL volumetric flask, to this 70mL of mobile phase was added, sonicated and the volume was made up with the mobile phase. Pipetted 5 mL of the clear solution in to 50 mL volumetric flask and make up volume with mobile phase.

Preparation of Sample solution:
Crush more than 20tablets then weigh a quantity of powder equivalent to 125mg of BILASTINE and 100mg of Montelukast in 100 mL volumetric flask and add70mL of mobile phase then sonicated it for 30min intermittent shacking after 30min make up volume with mobile phase. Pipetted 5 mL of the clear solution in to 50 mL volumetric flask and make up volume with mobile phase. Filter the solution through 0.45µm filter paper. The resulting solution is used to record the chromatogram

Procedure:
20µL of the standard, sample are injected into the chromatographic system and the areas for Bilastine and Montelukast peaks are measured and the %Assay are calculated by using the formulae.

METHOD:
The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 0.5 ml/min for 5 minutes to equilibrate the column at 35ºC temperature. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into Waters AcquityC18(50mm x2.1 mm ID) 1.8µm, the mobile phase of composition Phosphate Buffer pH 3.5: Acetonitrile (75:25) %v/v was allowed to flow through the column at a flow rate of 0.5 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1,2.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Bilastine and Montelukast in their tablet dosage form. The result obtained for Bilastine and Montelukast was comparable with the corresponding labeled amounts and they were shown in Table-3.

Validation of Analytical method:
Linearity and Range: The linearity study was performed for the concentration of 62.5 to 188.5 µg/mL and 100 to 300 µg/ml. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 4,5.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Bilastine and Montelukast and calculate the individual recovery and mean recovery values. The results are shown in table 6,7.

Precision Studies: precision was caliculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The resulte are shown in table 8.
Ruggedness: To evaluate the intermediate precision of the method, Precision was performed by different analyst. The standard solution was injected for five times and measured the percentage assay in HPLC. The %RSD for the area of five replicate injections was found. The resulte are shown in table 9.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.4 ml/min to 0.6 ml/min. The results are shown in table 10.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The resulte are shown in table 11.
LOD = 3.3σ/S and

LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

RESULTS AND DISCUSSION:

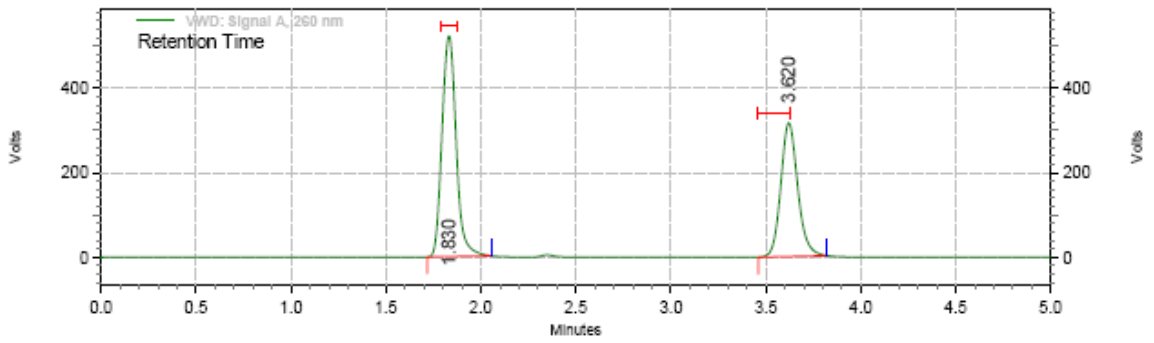

Figure 3: Standard chromatogram

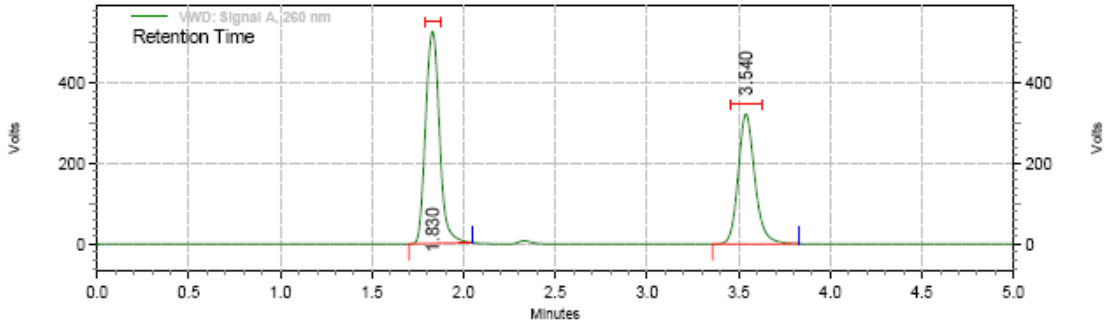

Figure 4: Sample chromatogram

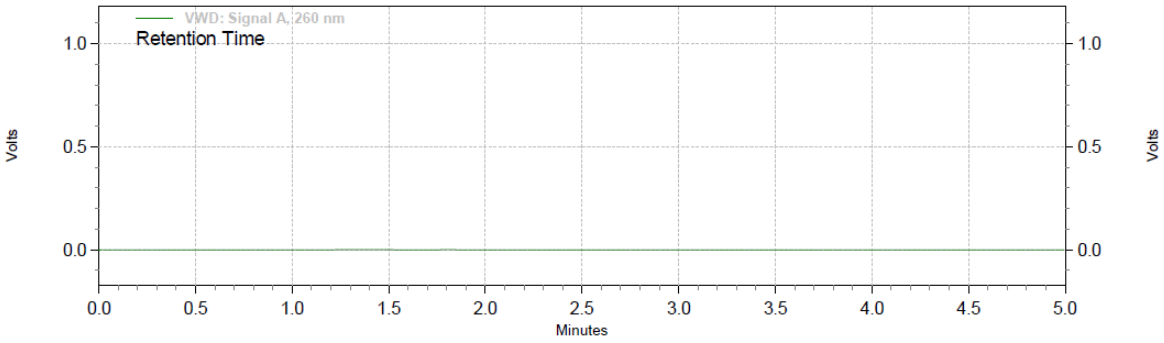

Figure 5: Blank chromatogram

Table 1: System suitability parameters Bilastine

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	1.830	46340258	2945	1.21
2	1.827	46001582	2942	1.17
3	1.823	46033474	2949	1.18
4	1.830	46101716	2999	1.14
5	1.833	45923870	3041	1.15
6	1.827	45780149	3032	1.14
Mean	1.828	46030175	-	-
SD	0.003	187570	-	-
%RSD	0.2	0.4	- -	-

Table 2: System suitability parameters Montelukast

Injection	Retention time	Peak area	Theoretical plates	Tailing factor	Resolution
1	3.540	34134250	7587	1.14	13.25
2	3.580	33405725	7902	1.16	11.85
3	3.567	33639716	7664	1.18	11.72
4	3.580	33494709	7828	1.19	11.84
5	3.597	33681271	7886	1.19	11.95
6	3.573	33505619	7912	1.17	11.90
Mean	3.573	33643548	-	-	-
SD	0.019	260754	-	-	-
%RSD	3.540	34134250	-	-	-

Table 3: Assay results for Bilastine and Montelukast

Drug	Label claim(mg)	Amount found(mg)	% Assay
BILASTINE	125	124.1	98.8
MONTELIKAST	100	98.6	98.6

Table 4: Linearity results for Bilastine

S.No	Concentration (µg/mL)	Area
1	62.5	25055621
2	100	36806942
3	125	45311483
4	150	52619670
5	188.5	63933476

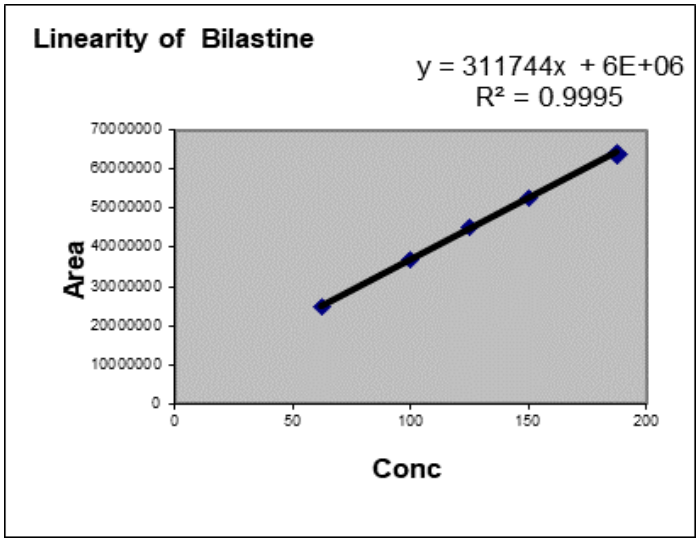

Figure 6: Linearity graph for Bilastine

Table 5: Linearity results for Montelukast

S.No	Concentration (µg/mL)	Area
1	100	16194209
2	160	26535716
3	200	33633340
4	240	39910697
5	300	49953204

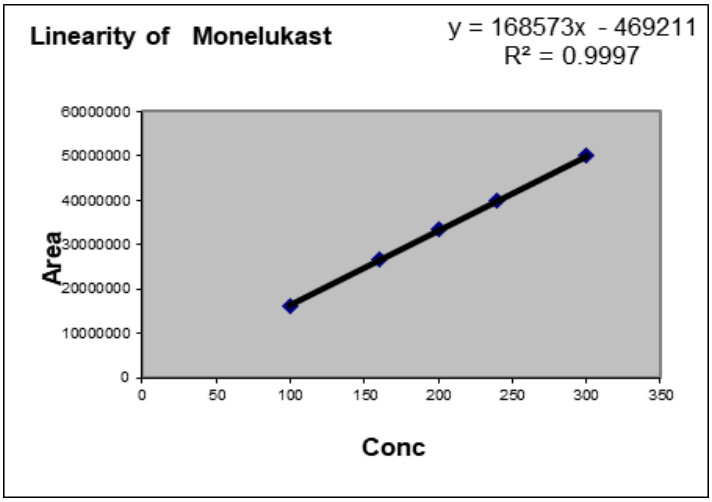

Figure 7: Linearity graph for Montelukast

Table 6: Showing accuracy results for Bilastine

%Recovery	Amount present (µg/mL)	Amount found (µg/mL)*	Percent Recovery *	% Mean Recovery
50%	62.50	62.66	100.3	99.9
100%	125.0	123.75	99.4	
150%	188.5	188.17	100.4	

Table 7: Showing accuracy results for Montelukast

%Recovery	Amount present (µg/mL)	Amount found (µg/mL)*	Percent Recovery *	% Mean Recovery
50%	100	100.94	99.1	99.8
100%	200	198.58	100.7	
150%	300	300.95	99.7	

Table 8: Precision results for Bilastine

Injection	BILASTINE		MONTELUKAST	
	Area	%Assay	Area	%Assay
1	45741313	98.8	33365477	98.9
2	45787695	98.9	33411360	99.1
3	46080749	99.6	33638194	99.7
4	45802928	99.0	33304993	98.7
5	45286836	98.8	32828676	98.3
6	45786681	98.9	33039426	98.0
Average	-	98.8	-	98.6
SD	-	0.6	-	0.9
%RSD	-	0.6	-	0.9

Table 9: Precision results for Montelukast

S.NO	Name	RT	Area	Height
1	Montelukast	4.302	1401475	100274
2	Montelukast	4.305	1401345	100078
3	Montelukast	4.325	1402415	98425
4	Montelukast	4.315	1404775	98165
5	Montelukast	4.312	1408614	98154
Mean			1491354	
Std.dev			5882.5	
%RSD			0.38	

Table 10: Ruggedness results of Bilastine and Montelukast

BILASTINE	%Assay	MONTELUKAST	%Assay
Analyst 01	99.36	Analyst 01	100.15
Analyst 02	99.27	Analyst 02	100.38
% RSD	0.27	% RSD	0.57

Robustness results

Table 11: Results for Robustness of BILASTINE and MONTELUKAST

Chromatographic changes		Theoretical Plates		Tailing factor		Resolution
		BILASTINE	MONTELUKAST	BILASTINE	MONTELUKAST	Between BILASTINE & MONTELUKAST
Flow rate (mL/min)	0.4	3588	9450	1.1	1.2	4.5
	0.6	2455	7299	1.0	1.1	4.6
Temperature(°C)	25	3541	9524	1.1	1.2	4.4
	35	3652	9536	1.0	1.1	4.5

Table 12: LOD, LOQ of Bilastine and Montelukast

Drug	LOD	LOQ
Bilastine	0.16	0.995
Montelukast	0.55	1.635

CONCLUSION:

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Bilastine and Montelukast in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of in pure and its pharmaceutical dosage forms.

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