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Analytical Method Development and Validation for the Estimation of Montelukast and Bilastine in Bulk and Pharmaceutical Tablet Dosage Form by RP-HPLC

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ABSTRACT

A novel, specific, accurate, rugged, precise reversed-phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative determination of Montelukast in active pharmaceutical ingredients and in its Pharmaceutical dosage form by using Phenomenex LunaC18 (4.6mm x 150mm, 3µm)column with a mobile phase containing a mixture of Acetonitrile and Potassium dihydrogen phosphate buffer adjusted to pH-2.8 with Ortho phosphoric acid in the ratio of 25:75%v/v. The flow rate was 0.9 ml/min and effluent were monitored at 220 nm and a peak eluted at 3.174 min and column oven temperature was maintained ambient. Calibration curve was plotted with a range from 10- 30 µg/ml. The LOD and LOQ values of Montelukast were found to be 1.3µg/ml and 3.9µg/ml respectively. The percentage recovery of the Montelukast and bilastine was found to be within the limits. The developed RP-HPLC method was validated according to the current International Conference on Harmonization (ICH) guidelines for specificity, LOD, LOQ, linearity, accuracy, precision, intermediate precision and robustness. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Montelukast and Bilastine in bulk drug and in its pharmaceutical dosage form. The proposed method were applied for the analysis of tablet formulations, to improve QC and assure therapeutic efficacy.

Keywords: Montelukast and Bilastine, RP-HPLC, Accuracy, Validation, ICH Guidelines.

INTRODUCTION

Coronavirus disease (COVID-19): CO' stands for corona, 'VI' for virus, and 'D' for disease. Formerly, this disease was referred to as '2019 novel coronavirus or '2019-nCoV.' The COVID-19 virus is a new virus linked to the same family of viruses as Severe Acute Respiratory Syndrome (SARS) and some types of the common cold.A highly contagious respiratory disease caused by the SARS-CoV-2 virus. SARS-CoV-2 is thought to spread from person to person through droplets released when an infected person coughs, sneezes, or talks. It may also be spread by touching a surface with the virus on it and then touching one's mouth, nose, or eyes, but this is less common. The most common signs and symptoms of COVID-19 are fever, cough, and trouble breathing. Fatigue, muscle pain, chills, headache, sore throat, runny nose, nausea or vomiting, diarrhea, and a loss of taste or smell may also occur. The signs and symptoms may be mild or severe and usually appear 2 to 14 days after exposure to the SARS-CoV-2 virus. Some people may not have any symptoms but are still able to spread the virus. Most people with COVID-19 recover without needing special treatment. But other people are at higher risk of serious illness. Those at higher risk include older adults and people with serious medical problems, such as heart, lung, or kidney disease, diabetes, cancer, or a weak immune system. Serious illnesses may include life-threatening pneumonia and organ failure. Research is being done to treat COVID-19.

Montelukast inhibits viral reproduction by promoting widespread mutations in the replication of viral RNA by RNA-directed RNA polymerase. It is metabolized into a ribonucleoside analog that resembles cytidine, β-D-N4-Hydroxycytidine 5′-triphosphate (also called EIDD-1931 5′-triphosphate or NHC-TP).During replication, the virus's enzyme incorporates NHC-TP into newly made RNA instead of using real cytidine. Oral montelukast and bilastine was found to be effective for the treatment of Covid-19, without evident safety concerns, when initiated within 5 days after the onset of signs or symptoms in this population of no hospitalized, unvaccinated adults who were at risk for progression to severe disease. Montelukast and bilastine is indicated for the treatment of mild-to-moderate coronavirus disease (COVID-19) in adults with positive results of direct SARS-CoV-2 viral testing, and who are at high risk for progression to severe COVID-19.

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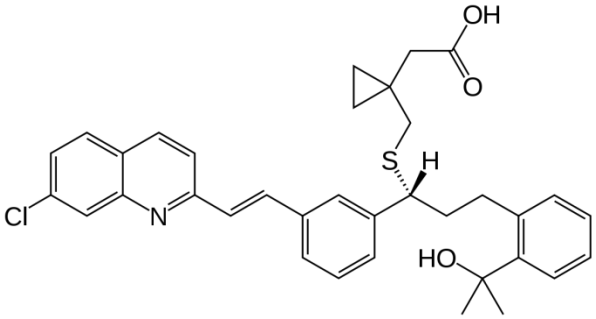
1.MONTELUKAST
Molecular structure



Molecular Formula: $C_{35}H_{36}ClNO_3S$

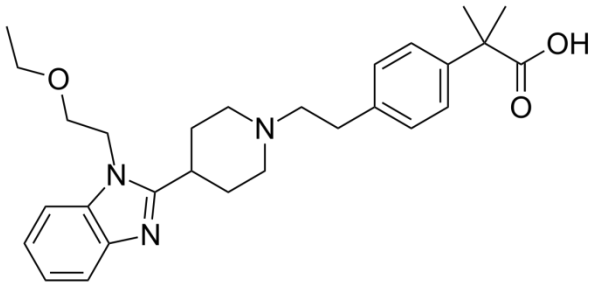
Molecular weight: 586.183

Solubility: Soluble in water, alcohol, and in methanol.

pKA : 4.4

2. BILASTINE

Molecular structure



IUPAC Name: 2-[4-(2-{4-[1-(2-ethoxyethyl)-1H-1,3-benzodiazol-2-yl]piperidin-1-yl}ethyl)phenyl]-2-methylpropanoic acid.

Mol Formula : $C_{28}H_{37}N_3O_3$

Molecular Weight : 463.622

Category : 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.

Solubility : Soluble in water

Melting Point : > 190 °C

pKa : 4.06

Table No. 1: Marketed Drug Formulation

S.NO.	Drug Name	Brand Name	Label Claim	Company Name
1	Motelukast and bilastinie	Lupin	200mg	jaiwik

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MATERIALS AND METHODS

Instruments used:

The liquid chromatographic system used was WATERS, software: Empower Alliance 2695 separation module 996 PDA detectors, Phenomenex LunaC18 (4.6mm x 150 mm, 5 µ) column, P^H meter Lab India, Ultrasonicator-Denver.

Chemicals used:

A gift sample of montelukast was procured from Sure Labs, Hyderabad, HPLC grade water, methanol, and Potassium dihydrogen phosphate were purchased from MERCK laboratories, Mumbai.

Method Development

Preparation of Standard Solution:

Standard Solution Preparation:

Prepare Montelukast and bilastine working standard by dissolving ten milligrams of Montelukast and twenty milligrams of bilastine in ten milliliter clean and dry volumetric flask. P pour the flask up to the mark with the same solvent, then add about seven milliliters of diluent and sonicate until clear. (Stock program)Later on, use a pipette to transfer 0.6 ml of each stock solution into a 10-milliliter volumetric flask respectively. Pour in half of the diluent.

Preparation of Sample Solution:

Weigh 10 milligrams of Montelukast and 20 milligrams of Bilastine, or an equivalent quantity of powdered tablet and transfer into a clean dry 10-milliliter volumetric flask. To dissolve, sonicate about 7 ml of diluent. Again pour the solvent to the mark up to the neck of the flask. (Stock program)Subsequently, dilute 0.6 ml of each stock solution to 10 ml by pipetting the volume into a volumetric flask. Pour in half of the diluent.

Mobile Phase Optimization:

We tested the mobile phase with a range of pH and quantity combinations, including acetonitrile: methanol, methanol: phosphate, and orthophosphoric acid buffer, among others. The last thing to do was to change the mobile phase by adding 70% acetonitrile by volume and 0.1% OPA buffer (pH 3.0).

. Optimization of Column:

In the process, many types of the column were employed which included C18, Phenomenex, YMC and Inertsil ODS columns. Spursil C18ODS (4.6 x 150mm, 3µm) showed higher efficiency as this column gave best peak shape and resolution at flow rate 0.9 ml/min.

Method Validation:

System suitability: A Standard solution was prepared by using Curcumin and Piperineworking standards as per the test method and was injected in replicates five times into the HPLC system.The system suitability parameters like theoretical plates, tailing factor, resolution were evaluated from standard chromatograms.

The standard and sample solutions were injected five times and peak areas of injections were measured in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.The results were given in table-3.

Three replicate injections of standard and sample solutions were injected and the assay was calculated by using the formula:

$$\% \text{ ASSAY} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity: Take each solution and load it to the chromatographic apparatus in order to determine its peak area.The correlation coefficient should be established when one describes the plot with two quantities, concentration on the X-axis and the peak area on the Y-axis with reference to time.

Accuracy: The accuracy of the newly developed method was evaluated by recovery studies at three different levels equivalent to 50,100&150%. At each level, the target concentration was spiked in triplicates and the amount recovered was calculated the percentage recovery at each level was calculated and reported in table-5.

Precision:

Repeatability:

The standard solution was injected five times and the peak area for all five injections was measured. The %RSD for the area of five replicate injections was found to be within the specified limits. The results were given in table -6.

Intermediate precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining the same conditions. The standard solution was injected six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits. The results were given in table –7.

Limit of Detection and Limit of Quantification:

From the linearity data, the limit of detection and quantification were calculated using the following formulae.

$$LOD = \frac{3.3\ \sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte

$$LOQ = \frac{10\ \sigma}{S}$$

The values were given in table -

Robustness: A study was conducted to determine the effect of variation in flow rate, change in mobile phase composition, and detection of wavelength. A standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and1.2ml/min. The same studies were also performed by varying mobile phase composition and detection wavelength. The system suitability parameters were evaluated .

RESULTS AND DISCUSSION

Method development:

Optimized Chromatogram (Standard)

Instrument used	:	Waters HPLC with auto sampler and PDA detector.
Temperature	:	Ambient (25□ C)
Mode of separation	:	Isocratic mode
Column	:	Spursil C_{18}ODS (4.6 x 150mm, 3□m)

pH	:	3.0
Mobile phase	:	30% 0.1 % OPA buffer 70% Acetonitrile
Flow rate	:	0.9 ml per min
Wavelength	:	276 nm
Injection volume	:	20 □l
Run time	:	10 min

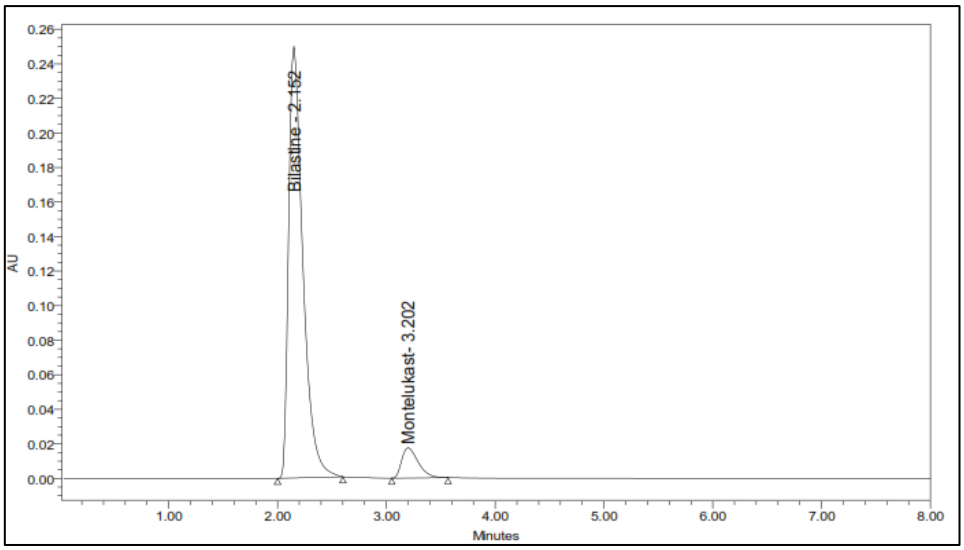

Figure-2: Optimized Chromatogram (Standard)

Table-2: Peak results for Optimized Chromatogram (Standard)

S.No.	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Montelukast	10min	856985	69854	1.25	8547

Method Validation:

System suitability: The theoretical plates are more than 2000 and the tailing factor is less than 2 in each injection for both the analytes. The values were within the acceptance criteria.

Table-3: Results of system suitability for Molnupiravir

S.No.	PeakName	RT	Area (µV*sec)	Height(µV)	USP Plate Count	USPTailing
1	Molnupiravir	3.146	856985	69854	8569	1.26
2	Molnupiravir	3.123	856857	68954	8547	1.25
3	Molnupiravir	3.192	857894	68975	8596	1.25
4	Molnupiravir	3.164	857468	69854	8541	1.26
5	Molnupiravir	3.181	854785	69856	8616	1.25
Mean			856797.8			
Std.Dev.			1197.992			
%RSD			0.139822			

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Linearity: A graph was plotted with peak areas vs concentration and the correlation coefficient was calculated. The r^2 values were found to be 0.999 which was within the limits confirms the linearity of the method.

Table-4: Data for Linearity of Montelukast and Bilastine

S. No	Bilastine		Montelukast	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	40	711207	20	70870
2	80	1523949	40	149418
3	120	2424393	60	225206
4	160	3195929	80	300255
5	200	4030298	100	369941

Accuracy: Three target concentrations 50%, 100%, 150% were prepared concerning target assay and injected into the HPLC system in triplicates. At each spike level, the mean recovery values are between 98 to 102 % which were in agreement with the acceptance criteria. The recovery values indicate the method is accurate.

Table 5: Accuracy (recovery) data for Bilastine

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1227603	10	10.19	100.39	99.73
100%	2418303	20	19.88	99.40	
150%	3665386	30	39.87	99.91	

*Average of three determinations

Table 6: Accuracy (recovery) data for Montelukast

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	111536	5	4.87	99.34	99.52
100%	223577	10	9.95	99.56	
150%	335727	15	14.90	99.67	

*Average of three determinations

Precision: Repeatability was performed in five replicate injections and the % RSD of the peak areas was calculated. The % RSD for the peak areas of five standard injections was found to be0.183536which was within the limits.

Intermediate precision was also performed on two different days the % RSD for the peak areas of six standard injections was found to be 0.119311which was in agreement with acceptance criteria.

Table 7: Results of Intermediate precision for Bilastine

Injection	Area
Injection-1	2410627
Injection-2	2412992
Injection-3	2406358
Injection-4	2407422
Injection-5	2460678
Injection-6	2419407
Average	2419580.7
Standard Deviation	20665.3
%RSD	0.9

Table 8: Results of Intermediate precision for Montelukast

Injection	Area
Injection-1	223945
Injection-2	223191
Injection-3	223728
Injection-4	223705
Injection-5	223792
Injection-6	223337
Average	223616.3
Standard Deviation	289.2
%RSD	0.1

Specificity: There is no interference observed in the blank. The chromatograms of Standard and Sample were identical with the same retention time.

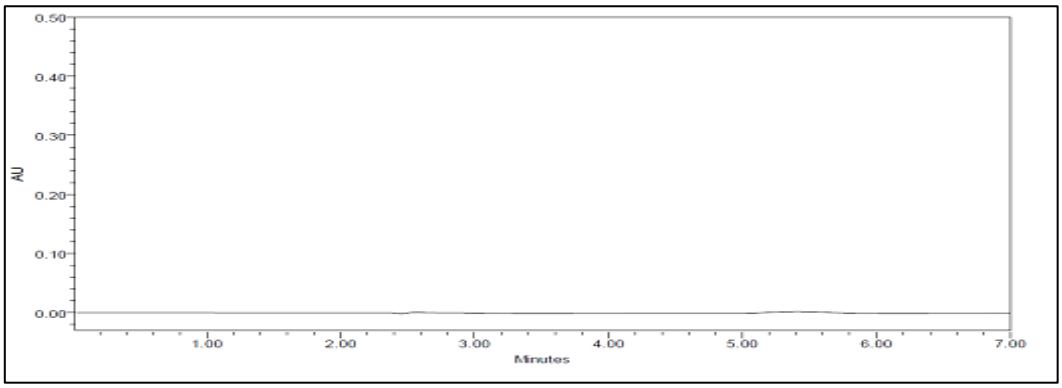

Figure No. 3: Chromatogram of blank

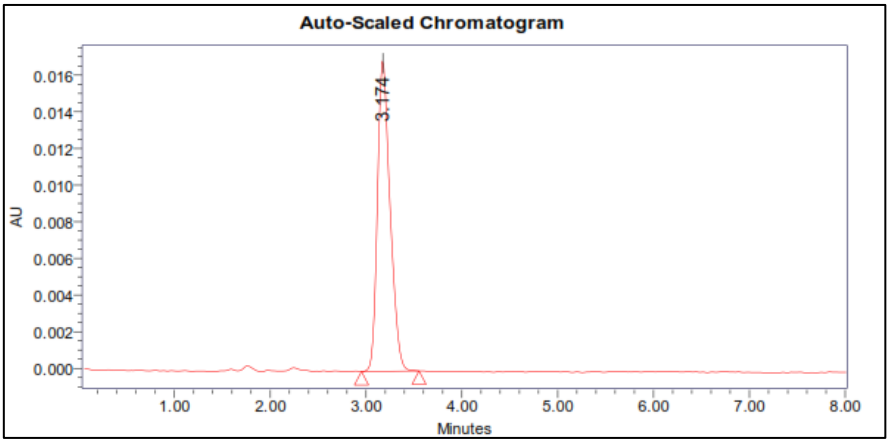

Figure-4: Chromatogram of Standard

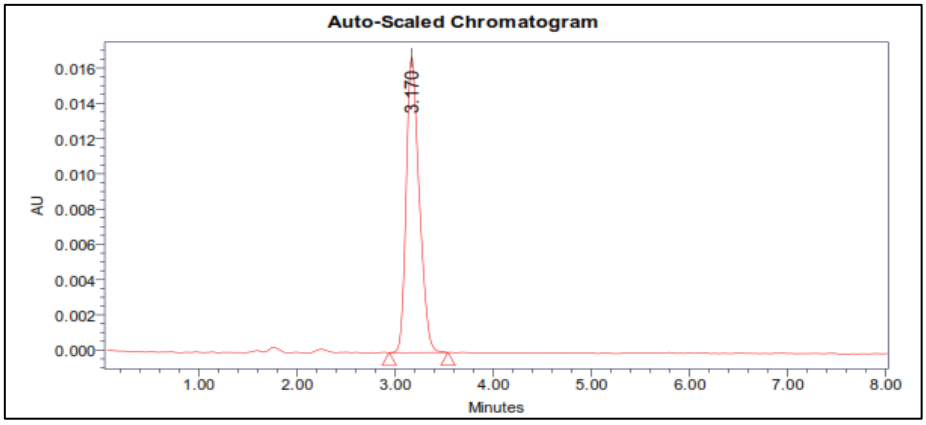

Figure-5: Chromatogram of Sample

Limit of Detection and Quantitation (LOD and LOQ): The LOD and LOQ of montelukast were found to be 1.54µg/ml and 4.56µg/ml respectively. The results indicate that the method was sensitive.

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.

a) The flow rate was varied at 0.8 ml/min to 1.0 ml/min.

Standard solution 60& 120 µg/ml of Montelukast & Bilastine prepared and analysed using the varied flow rates along with method flow rate.

b) The Organic composition in the Mobile phase was varied ±10%

Standard solution 60 & 120 µg/ml of Montelukast & Bilastine was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

Table-9: Robustness data of Montelukast & Bilastine was

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	856985	3.174	8547	1.25
Less Flow rate of 0.9 mL/min	841542	3.488	8256	1.23
More Flow rate of 1.1 mL/min	812546	2.877	8146	1.20
Less organic phase	802654	4.705	8365	1.16
More organic phase	826549	2.090	8154	1.14

CONCLUSION

Bilastine and Montelukast were quantified using RP-HPLC.

The approach is useful for regular analysis since the test of Bilastine and Montelukast was done using tablets yielded percentages of 100.26 and 99.29, respectively.

The approach can provide high sensitivity because the linearity of Bilastine and Montelukast was determined to be linear with correlation coefficients of 0.999 and 0.999, respectively.

The approach demonstrates a precision of 0.3 for Bilastine and 0.1 for Montelukast, indicating that it meets the acceptance standards of accuracy, which is an RSD of no more than 2.0%.

The approach demonstrates repeatability when done on various days, with a precision of 0.9 for Bilastine and 0.1 for Montelukast, demonstrating that the RSD should not exceed 2.0%, the acceptability requirement for intermediate precision.

A percentage recovery rate between 97.0 and 103.1 percent is considered to be within the acceptable range for accuracy. Bilastine had a complete recovery rate of 99.73% and Montelukast of 99.52%. It is clear from the validation results that the devised approach can demonstrate high accuracy and consistency, as the results fall well within the limit.

Three and ten, respectively, are the cutoffs for LOD and LOQ. For Bilastine, the LOQ and LOD were determined to be 3.00 and 9.98, respectively. For Montelukast, the LOQ and LOD were 2.98 and 10.00, respectively.

There is little to no deterioration in the data, and the robustness limit for variations in the mobile phase and flow rate is well within the limit. This proves that, under certain circumstances, the approach is both accurate and well-suited to the system.

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