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Method development and Validation for Estimation of Montelukast and Bilastine by Using RP-HPLC.

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ABSTRACT

For the simultaneous estimation of the dosage forms of tablets and bulk bilastine and montelukast, a straightforward, accurate, and precise approach was created. The Ascentis 150 x 4.6 mm, 5m chromatogram was used. 0.9 ml/min of mobile phase, which contained Buffer 0.01N Kh2Po4: Acetonitrile in a 75:25 ratio, was pumped through the column. This approach uses 0.01N Kh2Po4 as its buffer. Additionally, the temperature was kept at 30°C.The chosen optimized wavelength is 214.0 nm. Bilastine and Montelukast were found to have retention times of 2.150 and 2.792 minutes, respectively. It was discovered that the Montelukast and Bilastine % RSDs were 0.4% and 0.8%, respectively. For bilastine and montelukast, the recovery percentages were 99.74% and 100.24%, respectively. Values for LOD and LOQ were derived by regression.

Key Words: Montelukast, Bilastine, RP-HPLC

INTRODUCTION

Montelukast is a leukotriene receptor antagonist (LTRA) used to treat asthma and relieve seasonal allergy symptoms. It is taught orally. Montelukast inhibits the action of leukotriene D4 by binding to the cysteinyl leukotriene receptor CysLT1 in the lungs and bronchi. The molecular weight of montelukast is 419.37 and the chemical formula of montelukast is C20H19F2N3O5. The drug is sold in the United States and other countries by Merck and Co under the brand name Singular.

Bilastine is a histamine H1 receptor inhibitor (Ki = 64 nm). During an allergic reaction, mast cells become inflamed and release histamine and other chemicals. Bilastine binds to H1 receptors and prevents their activation, thus reducing the development of allergic symptoms caused by the release of histamine from mast cells. The molecular weight of Bilastine is 463.62 and the chemical formula of Bilastine is C28H37N3O3. Bilastine does not interact with H1 receptors. Cytochrome P450 is not extensively metabolized in humans.

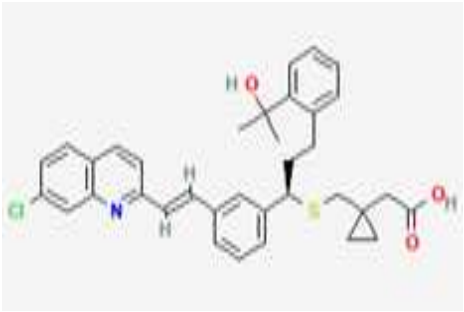

Fig.1.Montelukast Chemical Structure

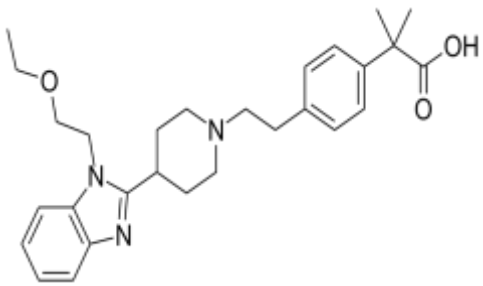

Fig.2. Bilastine Chemical Structure

MATERIALS AND METHODS

Standard:-Bilastine and Montelukast pure drugs (API),

Chemicals required:-Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. Alltheabovechemicals and solvents are from Rankem.

Apparatus and Insrtuments used:

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. ,UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Bilastine and Montelukast solutions.

Methodology :

Solubility of Bilastine and Montelukast: The solubility of the standards indicates that they are soluble in a 60:40 ratio of acetonitrile to water, and this solvent is chosen to be used as a diluent when making stock solution.

Bilastine and Montelukast Standard stock solutionsPreparation: Weighed around 10 mg of montelukast and 20 mg of bilastine, then put them in a 50 ml volumetric flask. We then added about 30 ml of diluent to the flask and sonicated it in a water bath for 10 minutes. Subsequently, add diluent until the mark is reached, and mark the volumetric flask with the standard stock solution (400µg/ml bilastine and 200µg/ml of montelukast).

Bilastine and MontelukastStandard working solutions Preparation(100%):1 milliliter of the standard stock solutions for montelukast and bilastine was pipetted out, transferred, and filled with diluent to the appropriate level in a volumetric flask with a capacity of 10 ml. And after that, the volumetric flask containing the produced solution was labeled with 20µg/ml of Montelukast and 40µg/ml of Bilastine.

Sample stock solutions Preparation: The average weight of ten tablets was determined.A 100 ml volumetric flask containing 10 tablets or the equivalent weight of 10 tablets is weighed. Next, about 50 ml of diluent is added to the flask, and it is sonicated for 25 minutes under a water bath. Finally, more diluent is added to the flask until the mark is reached. After passing through 0.45µm filters, the produced solution was labeled with the following information: 100µg/ml of Montelukast and 200µg/ml of Bilastine.

Sample working solutionPreparation (100%): One milliliter of the sample stock solution was pipetted out, put into a ten-milliliter volumetric flask, and diluted to the appropriate level. following which it bears the label (20µg/ml of Montelukast and 40µg/ml of Bilastine).

Buffer solution preparation:

Buffer: (0.01N KH_2PO_4)

Orthophosphoric acid solution was made by weighing 1.42 grams of potassium dihydrogen ortho phosphate in a 1000 milliliter volumetric flask, adding 900 milliliters of milli-Q water to it, degassing it to sonicate it, and then adding water to make up the volume. The PH was then adjusted to 4.0 using dil.

0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

Optimization of chromatography conditions

The first trial used an Agilent 150 column with a mobile phase of acetonitrile and potassium dihydrogen orthophosphate at a flow rate of 1 ml/min. The column was heated to 30 °C, and the photo diode array (PDA) was detected at 214 nm.

A second trial was conducted because although peaks were eluted in this trial, they were seen to be eluted in void volume. There were insufficient amounts of tailing, area, resolution, and USP plate count.

Using a Kromosil 150 column and a mobile phase consisting of acetonitrile and potassium dihydrogen orthophosphate at a flow rate of 1 ml/min, the second trial was conducted at 30 °C. The photo diode array (PDA) was detected at 214 nm.

The peaks in this trail were eluted by altering the stationary phase, but the area, resolution, tailing, and USP plate count that were noted were insufficient.

Results and Discussions:

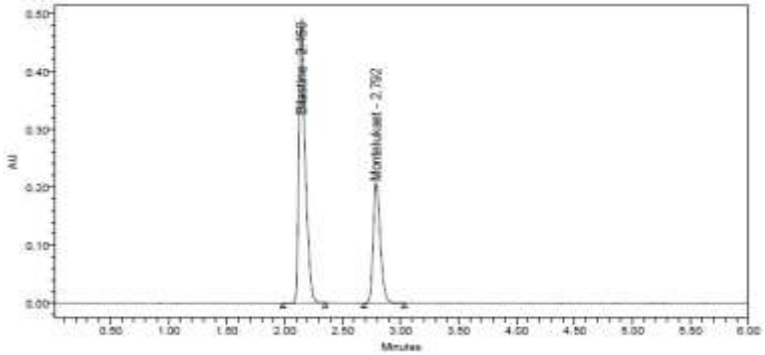

Fig.3.Optimizedchromatogram

Following a number of runs, the Ascentis C18(4.6×250mm,5µm) column with acetonitrile and potassium dihydrogen orthophosphate as the mobile phase at a flow rate of 1 ml/min, column temperature of 30 °C, and Photo Diode Array (PDA) detection at 214 nm were found to be the optimal chromatographic conditions. Peak area, resolution, tailing, and USP plate count were all perfect under optimal conditions.

Method Validation:

The RP-HPLC method was validated according to the ICH guidelines. And method validation parameters are precision, linearity, accuracy, robustness, limit of detection (LOD), limit of quantification (LOQ).

1. System suitability parameters:

By making standard solutions of 40 ppm Bilastine and 20 ppm Montelukast, then injecting the solutions six times, the parameters such as peak tailing, resolution, and USP plate count were ascertained, hence determining the system appropriateness parameters.

It is recommended that the percentage RSD for the results of six standard injections not exceed 2%.

Table.1. System suitability data of Montelukast and Bilastine.

S no	Bilastine			Montelukast			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	RS
1	2.141	7824	1.33	2.784	10633	1.2	5.7
2	2.149	7808	1.36	2.789	10966	1.25	5.9
3	2.150	7711	1.37	2.792	10496	1.26	5.9
4	2.154	7891	1.37	2.798	10316	1.24	5.9
5	2.154	7706	1.39	2.799	10439	1.21	5.9

Discussion :

The ICH Guidelines state that a plate count of more than 2000, a tailing factor of less than 2, and a resolution of more than 2 are requirements for compliance. Therefore, every system-suitable parameter was met and exceeded.

2. Specificity:

Examining any disturbance with the optimum procedure. Using this strategy, we won't be able to discover the conflicting peaks in the blank and placebo during the medication retention times. Thus, this approach was described as particular.

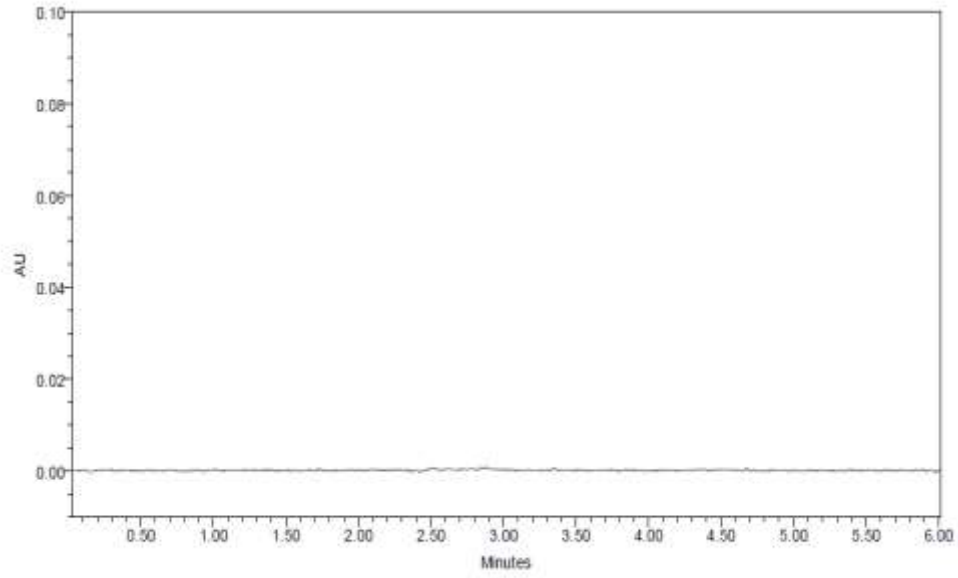

Fig.4.Chromatogram of blank

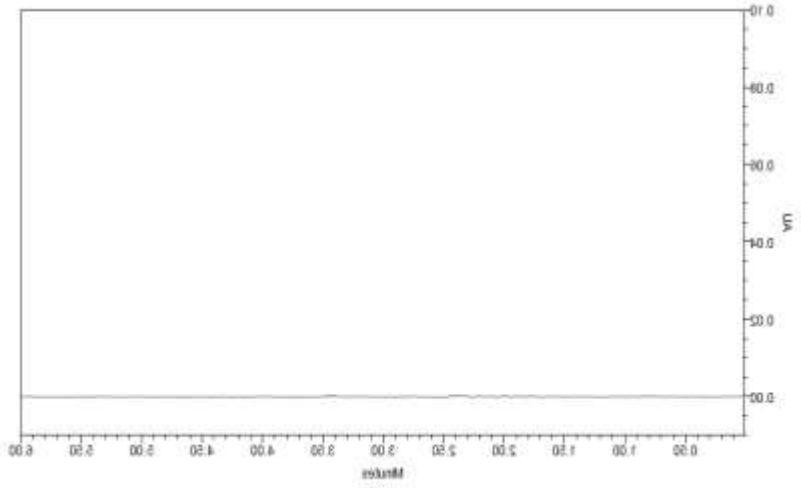

Fig.5.Chromatogram of Placebo

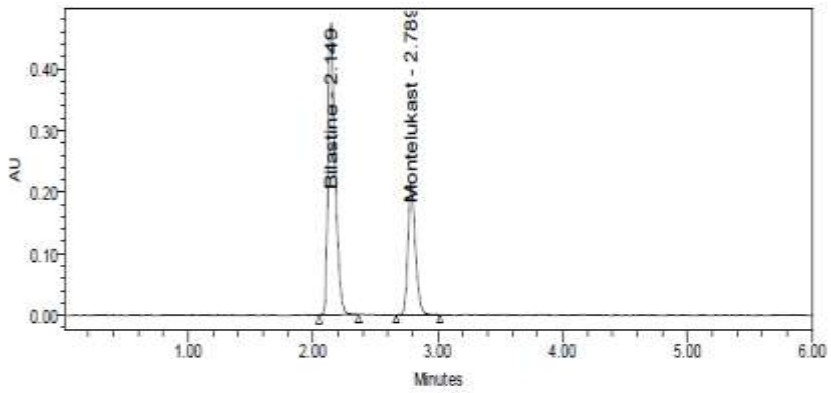

Fig.6.Typical Chromatogram

Discussion:

Retention time of Bilastine and Montelukast were 2.149 minutes and 2.798 minutes respectively. We won't find any interfering peaks in blank and placebo. So, this method is said to be specific.

3. Precision:

It is defined as the degree of agreement between the individual test findings obtained from repeatedly applying the process to several homogeneous sample samplings. to six times inject the standard and blank into HPLC systems at concentrations of 100 ppm. Both intraday (analysis on the same day) and interday (analysis on any other day) precision are used to determine the system precision.

Table.2. Precision data of Montelukast and Bilastine

S. No	**Area of Bilastine**	**Area of Montelukast**
1.	1843646	884382
2.	1856792	879671
3.	1846399	885245
4.	1831625	880391
5.	1846213	887390
6.	1874697	889129
Mean	1849895	884368
S.D	14569.4	3753.6
%RSD	**0.8**	**0.4**

Discussion:

Six injections were made from a single volumetric flask containing the working standard solution, and the areas that were obtained were as previously described. For two medications, the average area, standard deviation, and percentage RSD were computed. % RSD for Montelukast and Bilastine was found to be 0.4% and 0.8%, respectively. The system precision was passed using this method because the precision limit was less than "2".

4. Linearity:

Peak area was determined after the material was introduced into the chromatographic apparatus at each level. Plot the peak area versus concentration graph, then get the R2 value. By injecting the prepared concentration of 1µg/ml to 60µg/ml from the working standard, the linearity was ascertained

Table.3. Linearity of Montelukast and Bilastine

Bilastine		Montelukast	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
10	473483	5	222505
20	925130	10	444331
30	1369028	15	662946
40	1878090	20	885223
50	2345912	25	1115401
60	2734416	30	1305764

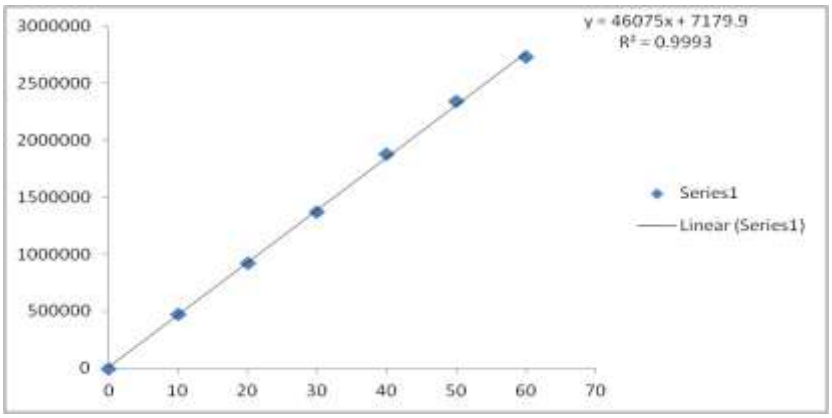

Fig.7.Calibration curve of Bilastine

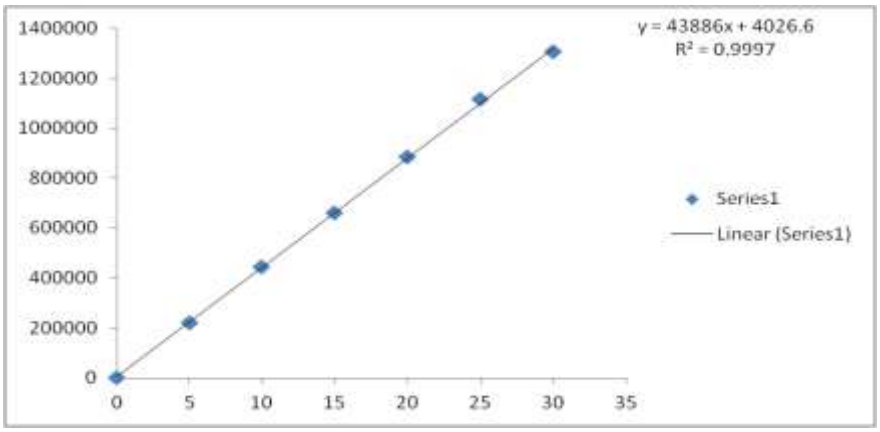

Fig.8.Calibration curve of Montelukast

Discussion:

Six identical linear dosages of bilastine (10–60µg/ml) and montelukast (5–30µg/ml) were administered. Based on the previously published average areas, the linearity equations for Montelukast and Bilastine were y = 46075x + 7179.9 and y = 43886x + 4026.6, respectively. A correlation value of 0.999 was discovered for the two drugs..

5. Accuracy: Accuracy is the degree to which test results agree with the true value, or how closely procedure-derived results resemble the true value.The samples were made by 50–150% spikes of a known amount of the analyte.

Table.4. Accuracy data of Bilastine

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	20	19.9	99.7	99.74%
	20	20.0	99.9	
	20	20.2	101.0	
100%	40	40.0	99.9	
	40	39.9	99.7	
	40	39.7	99.2	
150%	60	59.5	99.2	
	60	59.7	99.5	
	60	59.8	99.6	

Table.5. Accuracy data of Montelukast

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	10	10.12	101.22	100.24%
	10	10.07	100.73	
	10	10.03	100.32	
100%	20	20.14	100.70	
	20	19.94	99.71	
	20	19.91	99.56	
150%	30	29.78	99.26	
	30	29.98	99.94	
	30	30.20	100.68	

Discussion:

% of Recovery was obtained as 99.74% and 100.244% for Bilastine and Montelukast respectively.

6. Robustness:

Samples were injected in duplicate under minor, purposeful conditions including f-(0.9 ml/min), f+(1.1 ml/min), 50:50 MP–60:40 MP+, temp-(25°C) and temp+ (35°C). Additionally, SST was met and beyond the limits.

Table.6. Robustness data of Montelukast and Bilastine

S.no	Condition	%RSD of Bilastine	%RSD of Montelukast.
1	Flow rate (-) 0.8ml/min	0.7	0.4
2	Flow rate (+) 1.0ml/min	1.5	1.7
3	Mobile phase (-) 70B:30A	0.8	0.3
4	Mobile phase (+) 80B:20A	1.0	0.6
5	Temperature (-) 25°C	0.8	0.6
6	Temperature (+) 35°C	0.4	0.4

7. LOD and LOQ: For the determination of limit of detection(LOD) and limit of quantification(LOQ),the limits obtained for estimation of bilastine is 0.06,0.18.and montelukast is 0.03,0.08.

Table.7. Data of Limit of Detection(LOD) and Limit of Quantification(LOQ)

Molecule	LOD	LOQ
Bilastine	0.06	0.18
Montelukast	0.03	0.08

Conclusion:

Using a simple, precise, and accurate method, the simultaneous estimate of the bilastine and montelukast was completed with corresponding retention durations of 2.150 and 2.792 minutes. It was discovered that the percentage RSDs for Montelukast and Bilastine were 0.4% and 0.8%, respectively. The percentages of recovery for bilastine and montelukast were 99.74% and 100.24%, respectively. The Montelukast and Bilastine regression models yielded LOD and LOQ values of 0.06, 0.18, and 0.03, 0.09, respectively. For both Montelukast and Bilastine, the assay findings were 99.84% and 99.89%, respectively. Bilastine's regression equation is y = 46075x + 7179.9, but Montelukast's is y = 43886x + 4026.6.This approach was designed with reduced retention and run times, making it an easy-to-use and cost-effective solution that may be implemented in industries for routine quality control testing.

As a result, it can be said that the assay technique is reliable, precise, accurate, and specific, making it appropriate for both routine analysis and stability analysis.

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