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Method Development, Validation and Simultaneous Estimation of Bilastine and Montelukast using RP-HPLC

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A B S T R A C T

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Montelukast and Bilastine, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Zorbax C18 (4.6x150mm, 5µm) column using a mixture of Methanol: Phosphate Buffer pH 3.9 (55:45v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 255nm. The retention time of the Montelukast and Bilastine was 2.061, 2.462±0.02min respectively. The method produce linear responses in the concentration range of 1-5µg/ml of Montelukast and 100-500µg/ml of Bilastine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Montelukast, Bilastine, RP-HPLC, validation.

A R T I C L E I N F O

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1. Introduction

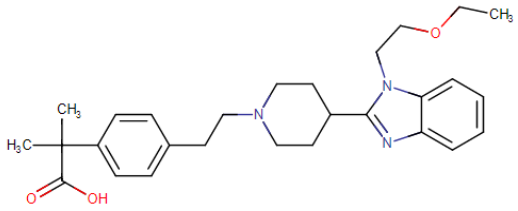

Fig.1: Bilastine

Chemical name/ Nomenclature / IUPAC Name : 2-[4-(2-{4-[1-(2-Ethoxyethyl)-1H-benzimidazol-2-yl]-1-piperidinyl}ethyl)phenyl]-2-methylpropanoic acid
Molecular Formula : $C_{28}H_{37}N_3O_3$
Molecular Weight : 463.622 g·mol−1

Physicochemical properties:
Description (Physical State): Solid
Solubility: water solubility 0.00203 mg/mL
Melting point: 200.3 (°C)
pKa (strongest acidic): 4.06
Log P: 5.02

Pharmacokinetic properties:
Bioavailability : 1
Half-life : 14.5 hours
Absorption : Bilastine has a Tmax of 1.13 h Label. The absolute bioavailability is 61%. No accumulation observed with daily dosing of 20-100 mg after 14 days. Cmax decreased by 25 % and 33% when taken with a low fat and high fat meal compared to fasted state. Administration with grapefruit juice decreased Cmax by 30%.

Protein binding : Bilastine is 84-90% bound to human plasma proteins

Metabolism: Bilastine does not interact with the cytochrome P450 system and does not undergo significant metabolism in humans

Excretion : Bilastine is mainly excreted in the feces (66.5%) with some excreted in the urine (28.3%) Label. Nearly all is excreted as the parent compound.

Pharmacodynamics:

Mechanism of action: Bilastine is a selective histamine H1 receptor antagonist (Ki = 64nM). 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.

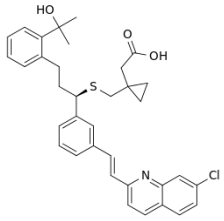

Fig.2: Montelukast

IUPAC Name: (E,Z)-2-(1-((1-(3-(2-(7-Chloroquinolin-2-yl)vinyl)phenyl)-3-(2-(2-hydroxypropan-2-yl)phenyl)propylthio)methyl)cyclopropyl)acetic acid

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

Molecular Weight : 586.184 gm/mole.

Official Pharmacopoeia : USP, EP

Physicochemical properties:

Description (Physical State): Solid

Solubility: Water Solubility8.2e-06 mg/mL

Storage Conditions: Store montelukast sodium tablets at 20^o to 25^oC (68^o to 77^oF), excursions permitted to 15^o to 30^oC (59^o to 86^oF)

Dosage: Tablet

pKa(strongest acidic): 4.4

Log P: 7.25

Pharmacokinetic properties:

Bioavailability : 63–73%

Half-life : 2.7–5.5 hours

Absorption : Rapidly absorbed following oral administration (bioavailability is 64%)

Volume of Distribution : 8 to 11 L

Protein binding : 99%

Excretion : Coupled with estimates of montelukast oral bioavailability, this indicates that montelukast and its metabolites are excreted almost exclusively via the bile.

Adverse effects/Side effects**:** Feeling confused, Not able to focus, Bad dreams, Not able to sleep, Sleepwalking.

Pharmacodynamics:

Montelukast, like zafirlukast, is a leukotriene receptor antagonist used as an alternative to anti-inflammatory medications in the management and chronic treatment of asthma and exercise-induced bronchospasm (EIB). Unlike zafirlukast, montelukast does not inhibit CYP2C9 or CYP3A4 and is, therefore, not expected to affect the hepatic clearance of drugs metabolized by these enzymes.

2. Materials and Methods

Table 1: Instruments used

S.N	Instruments	Model
1	HPLC	WATERS, Alliance 2695 separation module. Software: Empower 2,996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Bilastine and Montelukast working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.03ml of Bilastine and 3.0ml of Montelukast from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer pH 3.9 in proportion 55:45 v/v respectively.

Optimization of Column: The method was performed with various columns like C18 column, Symmetry and X-Bridge. Zorbax C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.

Temperature : 35ºC

Column : Zorbax C18 (4.6×150mm, 5µ)

Mobile phase : Methanol: Phosphate Buffer pH 3.9 (55:45v/v)

Flow rate : 1ml/min

Wavelength : 255nm

Injection volume : 10 µl

Run time : 8 min

Preparation of buffer and mobile phase:

Preparation of Phosphate buffer pH 3.9:

Accurately weighed 6.8 grams of KH2PO4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.9.

Preparation of mobile phase:

Accurately measured 550 ml (55%) of Methanol and 450ml of Buffer (45%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

Validation Parameters

Specificity study of drug:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Bilastine and 10mg of Montelukast working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.03ml of Bilastine and 3.0ml of Montelukast from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Preparation of Sample Solution:

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Bilastine and Montelukast sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.03ml of Bilastine and 3.0ml of Montelukast from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of drug solutions for linearity:

Accurately weigh and transfer 10 mg of Bilastine and 10mg of Montelukast working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

1µg/ml of Bilastine & 100µg/ml of Montelukast:

Pipette out 0.01ml of Bilastine and 1.0ml of Montelukast stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

2µg/ml of Bilastine & 200µg/ml of Montelukast:

Pipette out 0.02ml of Bilastine and 2.0 ml of Montelukast stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

3µg/ml of Bilastine & 300µg/ml of Montelukast:

Pipette out 0.03ml of Bilastine and 3.0ml of Montelukast stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

4µg/ml of Bilastine & 400µg/ml of Montelukast:

Pipette out 0.04ml of Bilastine and 4.0ml of Montelukast stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (5µg/ml of Bilastine & 500µg/ml of Montelukast):

Pipette out 0.05ml of Bilastine and 5.0ml of Montelukast stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure:

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.

Precision

Preparation of Bilastine and Montelukast Product Solution for Precision:

Accurately weigh and transfer 10 mg of Bilastine and 10mg of Montelukast working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.03ml of Bilastine and 3.0ml of Montelukast from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

Day 1: 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 to be within the specified limits.

Day 2:

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 to be within the specified limits.

Accuracy:

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10 mg of Bilastine and 10mg of Montelukast working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.015ml of Bilastine and 1.5ml of Montelukast from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% Standard stock solution:

Accurately weigh and transfer 10 mg of Bilastine and 10mg of Montelukast working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.03ml of Bilastine and 3.0ml of Montelukast from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% Standard stock solution:

Accurately weigh and transfer 10 mg of Bilastine and 10mg of Montelukast working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.045ml of Bilastine and 4.5ml of Montelukast from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and

measured the peak responses. Calculate the Amount found and Amount added for Bilastine and Montelukast and calculate the individual recovery and mean recovery values.

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution:

Accurately weigh and transfer 10 mg of Bilastine and 10mg of Montelukast working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.03ml of Bilastine and 3.0ml of Montelukast from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of flow conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded

Effect of Variation of mobile phase organic composition: The sample was analyzed by variation of mobile phase i.e. Methanol: Buffer was taken in the ratio and 50:50, 60:40 instead (55:45), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

3. Results and Discussion

Optimized Chromatogram (Standard)

Mobile phase : Methanol: Phosphate Buffer pH 3.9 (55:45v/v)
Column : Zorbax C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 255 nm
Column temp : 35ºC
Injection Volume : 10 µl
Run time : 8minutes

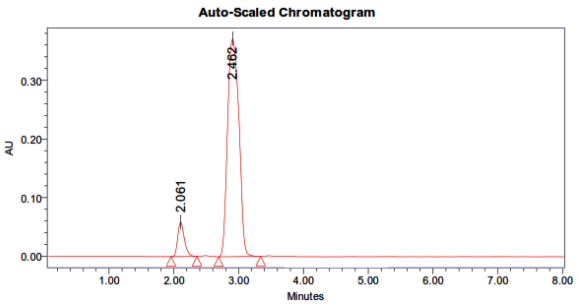

Fig.3: Optimized Chromatogram (Standard)

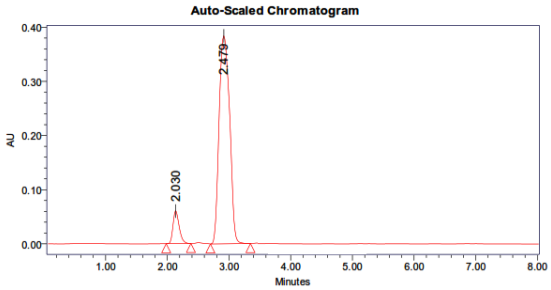

Fig.4: Optimized Chromatogram (Sample)

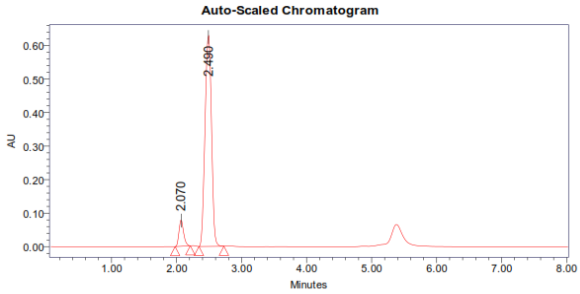

Fig.5: Specificity Chromatogram

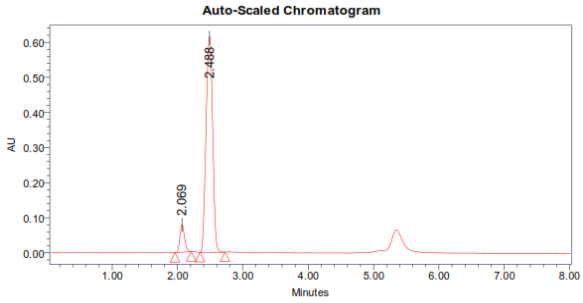

Fig.6: Chromatogram showing assay of standard

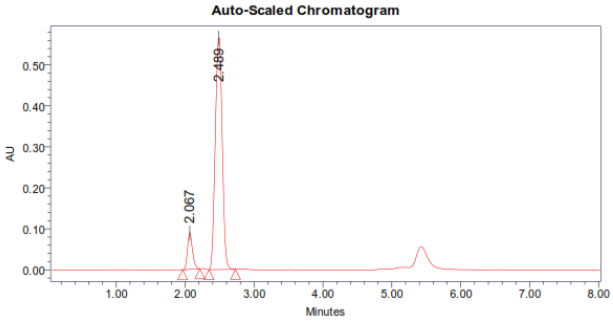

Fig.7: Chromatogram showing assay of sample

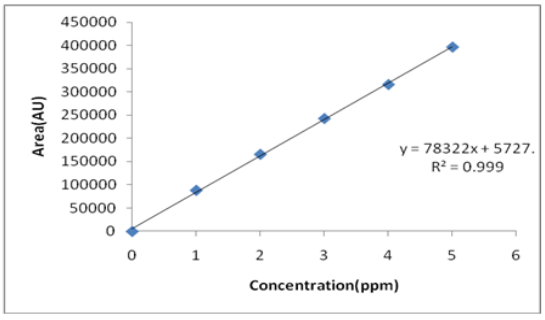

Fig.8: Calibration graph for Montelukast

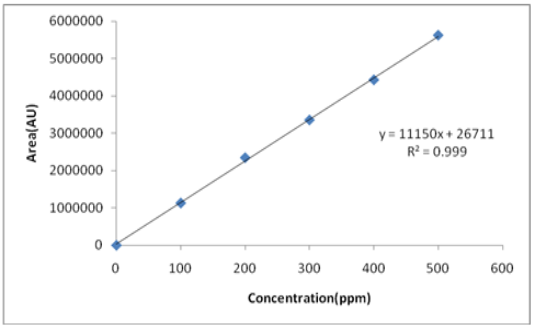

Fig.9: Calibration graph for Bilastine

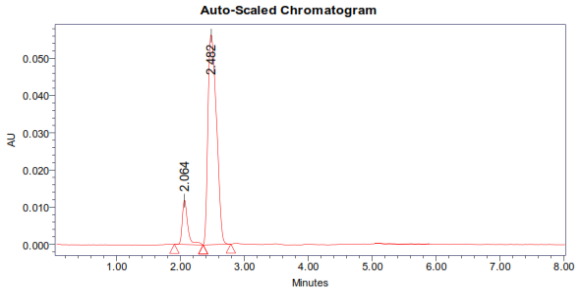

Fig.10: Chromatogram showing precision

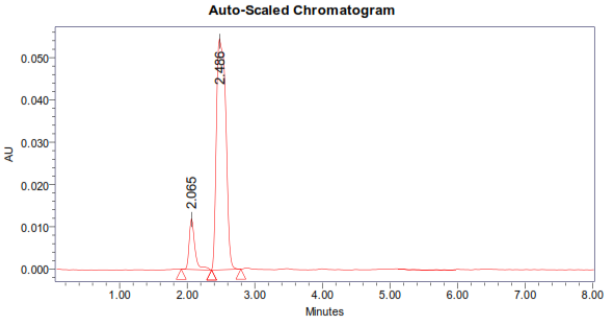

Fig.11: Intermediate precision (Day-1)

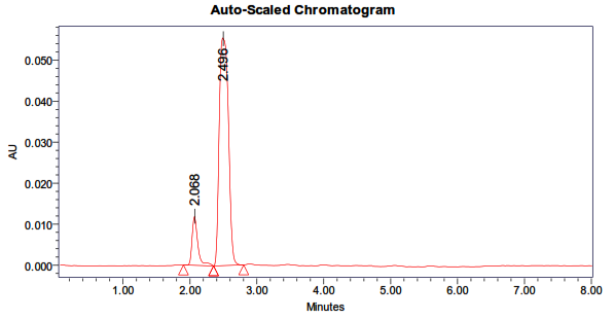

Fig.12: Intermediate precision (Day-2)

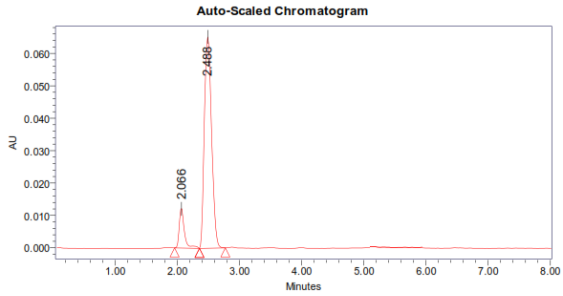

Fig.13: Accuracy-50% injection

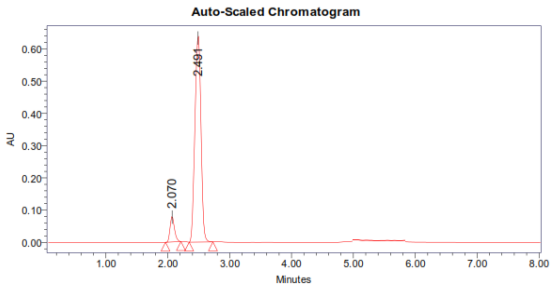

Fig.14: Accuracy-100% injection

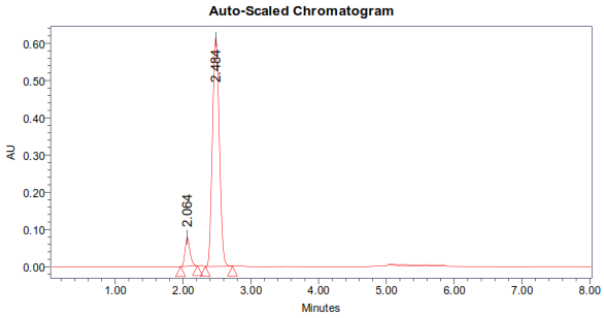

Fig.15: Accuracy-150% injection

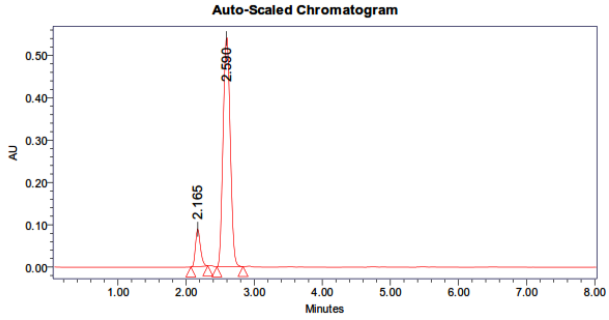

Fig.16: Less organic composition

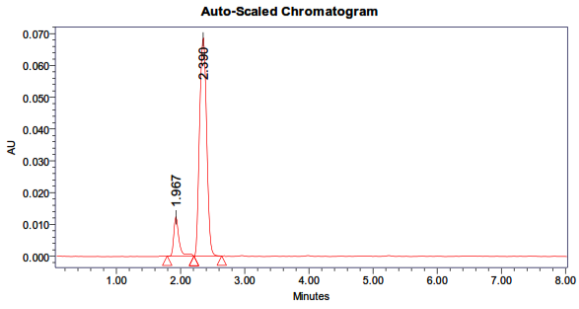

Fig.17: More organic composition

Table 2: Results of system suitability for Montelukast

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Montelukast	2.048	246713	73455	11318	1.1
2	Montelukast	2.074	245617	78152	7105	1.2
3	Montelukast	2.071	245830	78146	8974	1.2
4	Montelukast	2.069	240552	78242	7087	1.2
5	Montelukast	2.070	245725	77705	5124	1.2
Mean			244887.4			
Std. Dev			2462.26			
% RSD			1.005466			

Table 3: Results of system suitability for Bilastine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Bilastine	2.446	3363754	636862	8484	1.1
2	Bilastine	2.490	3326434	641486	7889	1.0
3	Bilastine	2.489	3345949	638081	7846	0.9
4	Bilastine	2.488	3336621	617725	6772	0.9
5	Bilastine	2.490	3355244	631710	6884	0.9
Mean			3345600			
Std. Dev			14753.43			
% RSD			0.44098			

Table 4: Peak results for Assay sample

S.No	Name	Rt	Area	Height	USP Tailing	USP plate count
1	Montelukast	2.068	244102	89282	1.2	5949
2	Bilastine	2.489	3357566	576562	1.0	6866
3	Montelukast	2.070	240052	88021	1.2	5861
4	Bilastine	2.491	3371663	576999	1.0	6808
5	Montelukast	2.067	243230	88882	1.2	5879
6	Bilastine	2.489	3364001	570315	1.0	6823

Table 5: Results of Intermediate precision for Montelukast

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Montelukast	2.066	242721	11323	5272	1.21
2	Montelukast	2.066	240155	11564	5168	1.16
3	Montelukast	2.066	240945	11887	5310	1.14
4	Montelukast	2.065	240385	11938	5275	1.19
5	Montelukast	2.069	249920	11652	5078	1.10
6	Montelukast	2.067	240820	11750	5225	1.17
Mean			243991			
Std. Dev			4641.97			
% RSD			1.5			

Table 6: Results of Intermediate precision for Bilastine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Bilastine	2.477	3325309	54143	6149	1.25
2	Bilastine	2.478	3323780	53740	6127	1.21
3	Bilastine	2.483	3328190	54791	6607	1.28
4	Bilastine	2.486	3329035	55098	6769	1.28
5	Bilastine	2.489	3325968	52379	6709	1.30
6	Bilastine	2.483	3327725	54779	6756	1.36
Mean			3326668			
Std. Dev			1985.641			
% RSD			0.059689			

Table 7: Robustness Results for Montelukast

Sample analysis	**Peak Area**	**Retention Time**	**Theoretical plates**	**Tailing factor**
Actual Flow rate of 1.0 mL/min	247392	2.061	7243	1.2
Less Flow rate of 0.9 mL/min	69214	2.267	4713	1.3
More Flow rate of 1.1 mL/min	388838	1.864	4740	1.2
Less organic phase	445628	2.165	4709	1.2
More organic phase	69404	1.967	5590	1.4

Table 8: Robustness Results for Bilastine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	3530866	2.462	3389	1.1
Less Flow rate of 0.9 mL/min	527373	2.690	5275	1.0
More Flow rate of 1.1 mL/min	4363129	2.284	5611	1.0
Less organic phase	3965572	2.590	5550	1.0
More organic phase	527708	2.390	6273	1.0

4. Conclusion

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Montelukast and Bilastine in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Montelukast and Bilastine was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: Phosphate Buffer pH 3.9 (55:45v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Montelukast and Bilastine in bulk drug and in Pharmaceutical dosage forms.

5. References

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