CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.8171001

Available online at: http://www.iajps.com *Research Article*

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

P. Jyothi *, V. Soumya[1]

[1]Department of Pharmaceutical Analysis, Surabhi Dayakar Rao College of Pharmacy, Gajwel, Siddipet, Telangana 502312.

Abstract:
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 (4.6 x 150mm, 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.*

Corresponding author:
P. Jyothi,
Department of Pharmaceutical Analysis,
Surabhi Dayakar Rao College Of Pharmacy,
Siddipet, Telengana.
Email Id- surapharmalabs@gmail.com

QR code

Please cite this article in press P. Jyothi et al, ***Development And Validation Of RP-HPLC Method For Simultaneous Estimation Of Bilastine And Montelukast In Combined Dosage Form,*** *Indo Am. J. P. Sci, 2023; 10 (06).*

1. INTRODUCTION:

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the *mobile phase,* which carries it through a structure holding another material called the *stationary phase.* The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.

Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase. The factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and affinity or differences among their molecular weights[1, 2]. Because of these differences, some components of the mixture stay longer in the stationary phase, and they move slowly in the chromatography system, while others pass rapidly into mobile phase, and leave the system faster .

Based on this approach three components form the basis of the chromatography technique.

- Stationary phase: This phase is always composed of a “solid” phase or “a layer of a liquid adsorbed on the surface a solid support”.
- Mobile phase: This phase is always composed of “liquid” or a “gaseous component.”
- Separated molecules

The type of interaction between stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on separation of molecules from each other. Chromatography methods based on partition are very effective on separation, and identification of small molecules as amino acids, carbohydrates, and fatty acids. However, affinity chromatographies (ie. ion-exchange chromatography) are more effective in the separation of macromolecules as nucleic acids, and proteins. Paper chromatography is used in the separation of proteins, and in studies related to protein synthesis; gas-liquid chromatography is utilized in the separation of alcohol, esther, lipid, and amino groups, and observation of enzymatic interactions, while molecular-sieve chromatography is employed especially for the determination of molecular weights of proteins. Agarose-gel chromatography is used for the purification of RNA, DNA particles, and viruses.

Stationary phase in chromatography, is a solid phase or a liquid phase coated on the surface of a solid phase. Mobile phase flowing over the stationary phase is a gaseous or liquid phase. If mobile phase is liquid it is termed as liquid chromatography (LC), and if it is gas then it is called gas chromatography (GC). Gas chromatography is applied for gases, and mixtures of volatile liquids, and solid material. Liquid chromatography is used especially for thermal unstable, and non-volatile samples.

The purpose of applying chromatography which is used as a method of quantitative analysis apart from its separation, is to achive a satisfactory separation within a suitable timeinterval. Various chromatography methods have been developed to that end. Some of them include column chromatography, thin-layer chromatography (TLC), paper chromatography, gas chromatography, ion exchange chromatography, gel permeation chromatography, high-pressure liquid chromatography, and affinity chromatography.

- Column chromatography
- Ion-exchange chromatography
- Gel-permeation (molecular sieve) chromatography
- Affinity chromatography
- Paper chromatography
- Thin-layer chromatography
- Gas chromatography
- Dye-ligand chromatography
- Hydrophobic interaction chromatography
- Pseudoaffinity chromatography
- High-pressure liquid chromatography (HPLC)

High-prssure liquid chromatography (HPLC)

Using this chromatography technique it is possible to perform structural, and functional analysis, and purification of many molecules within a short time, This technique yields perfect results in the separation, and identification of amino acids, carbohydrates, lipids, nucleic acids, proteins, steroids, and other biologically active molecules, In HPLC, mobile phase passes through columns under 10–400 atmospheric pressure, and with a high (0.1–5 cm//sec) flow rate. In

this technique, use of small particles, and application of high presure on the rate of solvent flow increases separation power, of HPLC and the analysis is completed within a short time.
Essential components of a HPLC device are solvent depot, high- pressure pump, commercially prepared column, detector, and recorder. Duration of separation is controlled

Essential components of a HPLC device are solvent depot, high- pressure pump, commercially prepared column, detector, and recorder. Duration of separation is controlled with the aid of a computerized system, and material is accrued.

Types of HPLC
There are following variants of HPLC, depending upon the phase system (stationary) in the process:
1. Normal Phase HPLC
This method separates analytes on the basis of polarity. NP-HPLC uses polar stationary phase and non-polar mobile phase. Therefore, the stationary phase is usually silica and typical mobile phases are hexane, methylene chloride, chloroform, diethyl ether, and mixtures of these.
Polar samples are thus retained on the polar surface of the column packing longer than less polar materials.
2. Reverse Phase HPLC
The stationary phase is nonpolar (hydrophobic) in nature, while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. It works on the principle of hydrophobic interactions hence the more nonpolar the material is, the longer it will be retained.
3. Size-exclusion HPLC
The column is filled with material having precisely controlled pore sizes, and the particles are separated according to its their molecular size. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later.
4. Ion-Exchange HPLC
The stationary phase has an ionically charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionizable samples.

The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time.

MATERIALS AND METHODS
Instruments And Glasswares Model- HPLCWATERS, Alliance 2695 separation module. Software: Empower 2,996 PDA detector, pH meter- LabIndia, Weighing machine- Sartorius, Volumetric flasks- Borosil, Pipettes and Burettes- Borosil, Beakers- Borosil, Digital ultra sonicator- Labman.

HPLC METHOD DEVELOPMENT:
TRAILS
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

VALIDATION
PREPARATION OF BUFFER AND MOBILE PHASE:
Preparation of Phosphate buffer pH 3.9:
Accurately weighed 6.8 grams of KH_2PO_4 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.

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

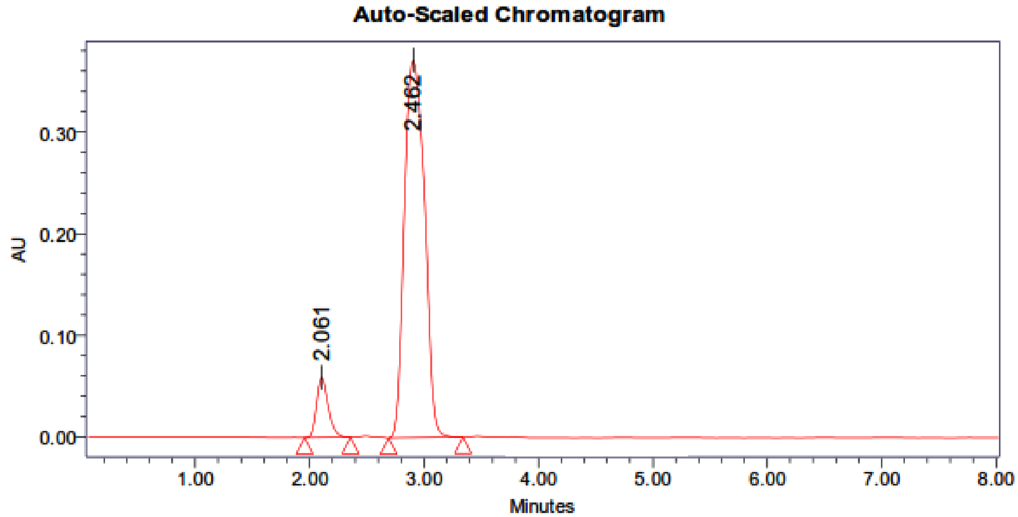

Figure 1:Optimized Chromatogram

Table 1: - peak results for optimized

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Montelukast	2.061	247392	58952	1.2	7243
2	Bilastine	2.462	3530866	371748	1.1	3389

Observation: From the above chromatogram it was observed that the Montelukast and Bilastine peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized chromatogram.

Optimized Chromatogram (Sample)

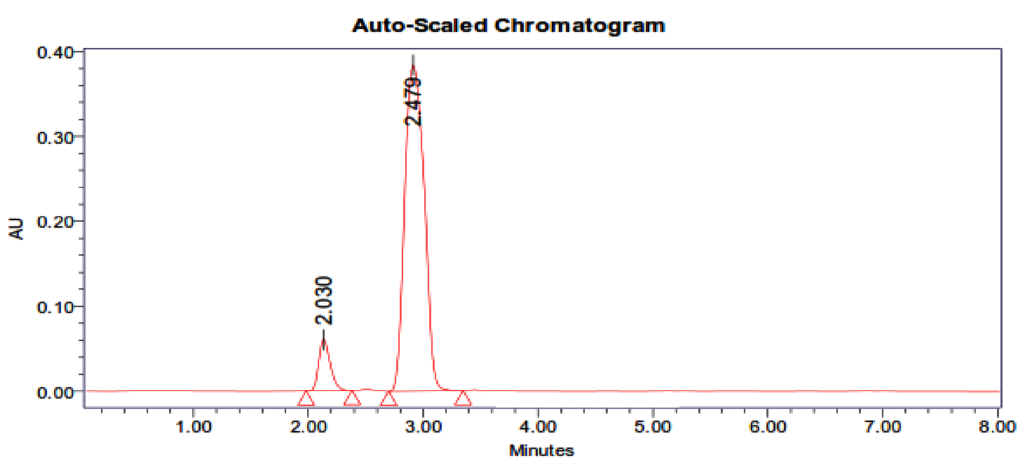

Figure 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Montelukast	2.030	240019	60878	1.2	7246
2	Bilastine	2.479	3544380	384304	1.1	3375

Acceptance criteria:

- Theoretical plates must be not less than 2000
- Tailing factor must be not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Assay (Standard):

Table 3: 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			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table 4: 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			

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Assay (Sample):

Table 5: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

%ASSAY =

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

The % purity of Montelukast and Bilastine in pharmaceutical dosage form was found to be 100.2 %.

LINEARITY

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Montelukast:

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	1	88442
66.6	2	165724
100	3	242754
133.3	4	315906
166.6	5	396371

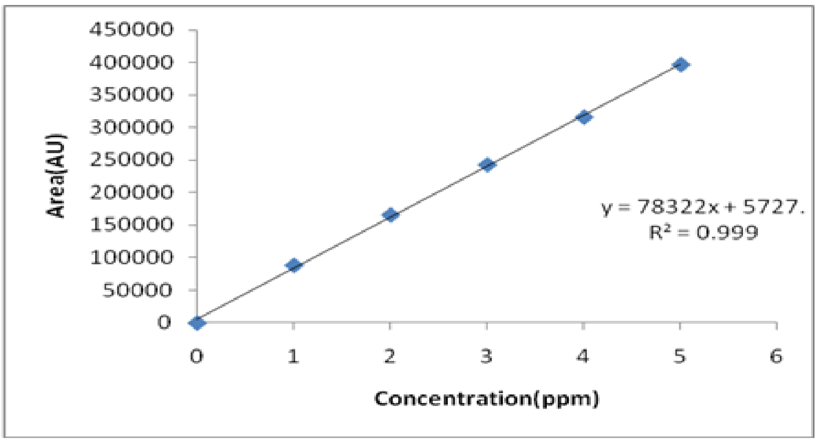

Figure 3 : calibration graph for Montelukast

Table 6: Bilastine

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	100	1131032
66	200	2345302
100	300	3355282
133	400	4429382
166	500	5623754

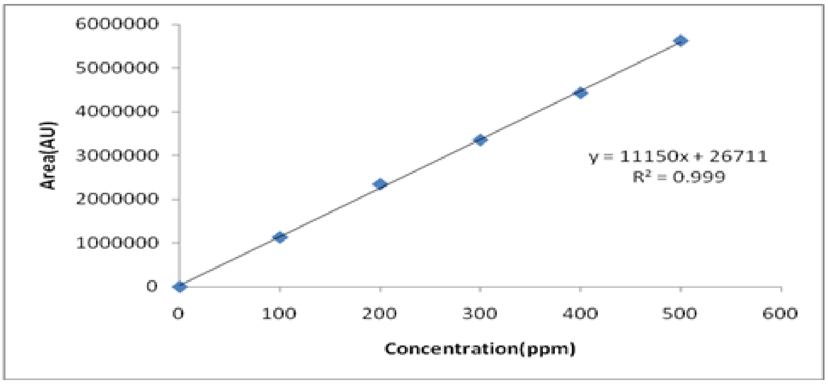

Figure4: calibration graph for Bilastine

REPEATABILITY

Results of repeatability for Montelukast:

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Montelukast	2.065	249684	12079	5343	1.0
2	Montelukast	2.064	249696	12068	5473	1.2
3	Montelukast	2.064	246325	11949	5473	1.1
4	Montelukast	2.065	249816	11811	5389	1.1
5	Montelukast	2.067	249892	11735	5180	1.0
Mean			249082.6			
Std. Dev			1543.964			
% RSD			0.61986			

Acceptance criteria:

- %RSD for sample should be NMT 2

Table 7: Results of method precession for Bilastine:

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Bilastine	2.486	3233700	59095	6654	1.2
2	Bilastine	2.484	3241323	57552	6524	1.3
3	Bilastine	2.482	3245927	57213	6440	1.3
4	Bilastine	2.483	3245927	57096	6411	1.4
5	Bilastine	2.483	3222194	54363	6260	1.4
Mean			3237814			
Std. Dev			10060.62			
% RSD			0.310722			

Acceptance criteria:

- %RSD for sample should be NMT 2

Intermediate precision:

Table 8: 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			

Acceptance criteria: %RSD of six different sample solutions should not more than 2

Table 9: 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			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Table 10: Results of Intermediate precision Day 2 for Montelukast

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Montelukast	2.067	249499	11594	5240	1.2
2	Montelukast	2.069	240991	11357	5130	1.2
3	Montelukast	2.068	240431	11878	5136	1.2
4	Montelukast	2.069	241330	11748	5267	1.2
5	Montelukast	2.067	240519	11830	5222	1.2
6	Montelukast	2.067	240470	11475	5982	1.2
Mean			242206.7			
Std. Dev			3590.034			
% RSD			1.48222			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2

Table 11 : Results of Intermediate precision for Bilastine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Bilastine	2.485	3426979	53353	6700	1.3
2	Bilastine	2.484	3446641	54454	6563	1.3

3	Bilastine	2.496	3430606	53532	6855	1.3
4	Bilastine	2.484	3430952	55157	6864	1.3
5	Bilastine	2.490	3431676	56223	6942	1.3
6	Bilastine	2.490	3429187	58578	6644	1.3
Mean			3433812			
Std. Dev			7041.409			
% RSD			0.205061			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

ACCURACY:

The accuracy results for Montelukast

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	124675.7	15	15.1	101%	100.4%
100%	242006.3	30	30.1	100.5%	
150%	357449	45	44.9	99.7%	

The accuracy results for Bilastine

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	1696259	18.75	18.71	99.8%	99.2%
100%	3351661	37.5	37.2	99.4%	
150%	4975094	56.25	55.47	98.6%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table12: RESULTS FOR ROBUSTNESS

MONTELUKAST:

Parameter used for 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

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

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

Acceptance criteria

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

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.

ACKNOWLEDGEMENT

The Authors are thankful to the Management and Principal, Department of Pharmacy, Surabhi dayakar rao college of pharmacy, rimmanaguda,telengana for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

REFERENCES:

1. Shethi PD. HPLC- Quantitative analysis of pharmaceutical formulations. 1st Ed. New Delhi: CBS Publishers & Distributors; 2001: 8-10, 101-103.
2. Kasture AV, Mahadik KR, Wadodkar SG, More HN. Pharmaceutical Analysis: Vol-II. 8th Ed. Pune: Nirali Prakashan; 2002: 48-57.
3. Prajapati GA. Method development and validation for simultaneous estimation of Hypertensive drugs by RP-HPLC. M.Pharm Thesis, Maliba Pharmacy College, Gujarat Technological University, Gujarat, India, 2011: 7-28.
4. Gabor S. HPLC in pharmaceutical Analysis: Vol. I. 1st Ed. London: CRC Press; 1990:101-173.
5. Jeffery GH, Bassett J. Vogel's textbook of Quantitative Chemical Analysis. 5th Ed. NewYork : John Wiley & Sons Inc; 1991: 217-235.
6. Hobart HW, Merritt LL, John AD. Instrumental Methods of Analysis. 7th Ed. New Delhi: CBS Publishers; 1988: 580-610.
7. Sharma BK. Instrumental Method of Chemical Analysis. 20th Ed. Meerut: Goel Publishing House; 2001: 54-83.
8. Ashutoshkar. Pharmaceutical Drug Analysis. 2nd Ed. New Delhi: New Age International Publisher; 2005: 455-466.
9. Ahuja S, Michael WD. Hand book of Pharmaceutical Analysis by HPLC. 1st Ed.London: Elsevier Academic Press; 2005: 44-54.
10. Snyder LR, Kirkland JL, Glajch JL. Practical HPLC Method Development. 3rd Ed. New York: Wiley; 1988: 227.
11. Skoog DA, West DM. Principles of Instrumental Analysis. 2nd Ed. Saunders Golden Sunburst Series. Philadelphia; 1980: 674-675, 690-696.
12. Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), PP 1-7.
13. A.BraithWait and F.J.Smith, Chromatographic Methods, 5th edition, Kluwer Academic Publisher, (1996), PP 1-2.
14. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.

15. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 15-23.
16. Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.