Chetan Vilasrao Patil *et al*, International Journal of Pharmaceutical Sciences & Medicine (IJPSM), Vol.8 Issue. 3, March- 2023, pg. 132-149

ISSN: 2519-9889
Impact Factor: 5.9

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

Chetan Vilasrao Patil[1]*; Shabnam Khan; Ramakant Sharma[2]; Jeevan Patel[2]; Dr. Rakesh Patel[3]

[1]PG Scholar, School of Pharmacy, Dr. A.P. J. Abdul Kalam University, Indore
[2]Assistant Professor, School of Pharmacy, Dr. A.P. J. Abdul Kalam University, Indore
[3]Professor and Principal, School of Pharmacy, Dr. A.P. J. Abdul Kalam University, Indore

DOI: 10.47760/ijpsm.2023.v08i03.010

ABSTRACT:

In the present research work, a successful attempt was made for determination of Bilastine and Montelukast in Bulk and dosage form by high performance liquid chromatography. The method was developed by experimentation, based on literature survey. The simplicity, rapidity, reproducibility and economy of the proposed method completely fulfill the objective of this research work. The HPLC method was developed and validated for simultaneous estimation of Bilastine and Montelukast. The mobile phase was consisting of pH 5.5 Acetate: methanol. The linearity range of Bilastine was found to be 50-250µg/ml and Montelukast 25-125 µg/ml. The calibration curve was plotted and regression equation of Bilastine was found to be y = 9153.17x + 1658869.30 with correlation coefficient (r2) of 0.9996 and Montelukast y = 22933.18x + 2251569.30 with correlation coefficient (r2) of 0.9991. Detection was done at 225 nm and the retention time of Bilastine was found to be 4.3 min and Montelukast 11.3 min with the flow rate of 1.0 ml/min. From accuracy study % recovery of Bilastine was found in the range of 99.57- 100.07% and Montelukast is 99.667-100.45 % which is in the limits accordingly the ICH guidelines.

Keywords: Validation, Bilastin, Montelukast, Tablet Dosage Form

Chetan Vilasrao Patil *et al*, International Journal of Pharmaceutical Sciences & Medicine (IJPSM), Vol.8 Issue. 3, March- 2023, pg. 132-149

ISSN: 2519-9889
Impact Factor: 5.9

INTRODUCTION

High-Performance Liquid Chromatography (HPLC)?

High-performance liquid chromatography (HPLC) is an advanced form of liquid chromatography used in separating the complex mixture of molecules encountered in chemical and biological systems, in order to understand better the role of individual molecules. Among different chromatographic methods, high performance liquid chromatography (HPLC) offers a greater variety of stationary phases, which there by allows selective interactions and more possibilities for separation. In HPLC the separation is about 100 times faster than the conventional liquid chromatography due to packing of particles in the range of 3-10 m. In HPLC mobile phase composition is changed in a programmed fashion to increase the efficiency of separation. Depending on the unique affinity of each component (referred to as the analyte) between the mobile phase and the stationary phase, each analyte migrates along the column at different speeds and emerges from the column at different times, thus establishing a separation of the mixture. Analytes with higher affinity for the mobile phase migrate faster down the column, whereas those with higher affinity for the stationary phase migrate slower. This migration time (referred to as retention time) is unique for each analyte and can be used in its identification. With the appropriate use of a detection method after the column, each analyte can also be quantified for analysis.Smaller column particle size can improve chromatographic resolution.

Principle of Separation in HPLC!

The sample is introduced in a small volume into the stream of mobile phase percolating through the column which is to be separated and analyzed. The components of the sample move through the column at different velocities, which are functions of specific physical or chemical interactions with the stationary phase. The velocity of component depends on its chemical nature, the nature of the stationary phase (column) and on the composition of the

ISSN: 2519-9889
Impact Factor: 5.9

mobile phase. The time at which a specific analyte emerges from the column (elutes) is called the retention time. The retention time measured under particular conditions is considered as an identifying characteristic of a given analyte. The use of smaller particle size packing materials.

MATERIALS AND METHODOLOGY

Identification of drug:

Organoleptic properties of drug

The sample of Bilastine and Montelukast was checked for organoleptic properties such as colour and odour.

Melting point determination

Identification of Bilastine and Montelukast was done by checking its melting point using melting point apparatus.

Solubility analysis

Solubility of Bilastine and Montelukast was checked by dissolving it in number of solvents. It was found that Bilastine and Montelukast was soluble in Ethanol, Methanol, Isopropyl Alcohol, insoluble water.

Fourier Transform Infra-red Spectroscopy (FTIR)

The IR study of pure drug was carried out by using Fourier transform infrared spectrophotometer (BRUKER). Infrared absorption spectrum of Bilastine and Montelukast was recorded and interpreted over the wave number 4000 to 600 cm-1 using Fourier Transform spectrophotometer (Bruker, ECO- ATR)

High Performance Liquid Chromatographic Method

Optimization of Detection Wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is one that gives good response for the drugs that are to be detected. For good response, optimization of wavelength was done at different wavelengths by UV detector. In the present study, drug solutions of 50 µg/ml of Bilastine and

ISSN: 2519-9889
Impact Factor: 5.9

25 µg/ml Montelukast were prepared in methanol. After observing UV spectra of the drug, wavelength by Overlain spctra was found at 225 nm and selected for further study.

Selection of Chromatographic Conditions

The selection of HPLC method depends upon the nature of the sample, its molecular weight and solubility. RP-HPLC method was selected for the initial separations because of its simplicity and suitability. The chromatographic variables such as mobile phase ratio and flow rate were studied. The condition that gave the best resolution, symmetry and selectivity was selected.

Optimization of Chromatographic Parameters: Optimizations in HPLC is the process of finding a set of conditions that sufficiently enable the quantification of the analyte with acceptable accuracy, precision, sensitivity, specificity, cost, ease and speed

Preparation of standard stock solutions

Accurately Weighed and transferred 20 mg of Bilastine and 10 mg of Montelukast working Standards into a 10ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 2.5 ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10 ml and the final concentration of Bilastine is 500 µg/mL and 250 µg/mL is of Montelukast. The working standard solutions of these drugs were obtained by appropriate dilution of the respective stock solution with mobile phase.

Optimization of Mobile Phase Strength

Based on drug solubility, stability and suitability of drug in different solvents, various mobile phases and compositions were tried to get a good resolution and sharp peak. The standard solution containing drugs were run in different mobile phases.

Preparation of Mobile Phase A(pH 5.5 Acetate buffer) :

Dissolve 3.85g of ammonium acetate in 1000 ml water, add 1 ml of Triethylamine, and adjust pH 5.5 with diluted glacial acetic acid. Mobile phase was filtered through 0.45µm membrane filter and degassed by sonication for 20 min.

ISSN: 2519-9889
Impact Factor: 5.9

Selection of mobile phase

Standard solutions of Bilastine (50µg/mL) and Montelukast (25µg/mL) were injected into the RP-HPLC system and run in different solvent systems. Different mobile phases systems like Acetate buffer and Methanol were initially tried in the isocratic mode in order to determine the best conditions.

LOD and LOQ:

LOD and LOQ determined by the following formula by taking the standard deviation of y-intercept and slope from the linearity curves.

LOD= 3.3σ

RESULTS AND DISCUSSION

Identification of drug

Organoleptic properties of drugs

Sr. No.	Organoleptic Property	Bilastine	Montelukast Sodium
1	Colour	White to off- white powder	White to off- white powder
2	Odor	Odorless	Odorless

Melting point of drug

Sr. No.	Name of drug	M.P. (˚C)
1	Bilastine	201-203 ^{o}C
2.	Montelukast	85-90 ^{o}C

Solubility Study:

Solubility of Bilastine and montelukast was observed by dissolving them in differentsolvents and the observed results are given in the table no.16

IJPSM

Chetan Vilasrao Patil *et al*, International Journal of Pharmaceutical Sciences & Medicine (IJPSM), Vol.8 Issue. 3, March- 2023, pg. 132-149

ISSN: 2519-9889
Impact Factor: 5.9

Solubility Study

Sr. No	Solvents	Solubility	
		Bilastine	Montelukast
1	Water	Soluble	Soluble
2	Methanol	Freely soluble	Freely soluble
3	Acetate Buffer pH 5.5	Freely soluble	Freely soluble
4	ACN	Soluble	Soluble

FTIR spectrum of Bilastine

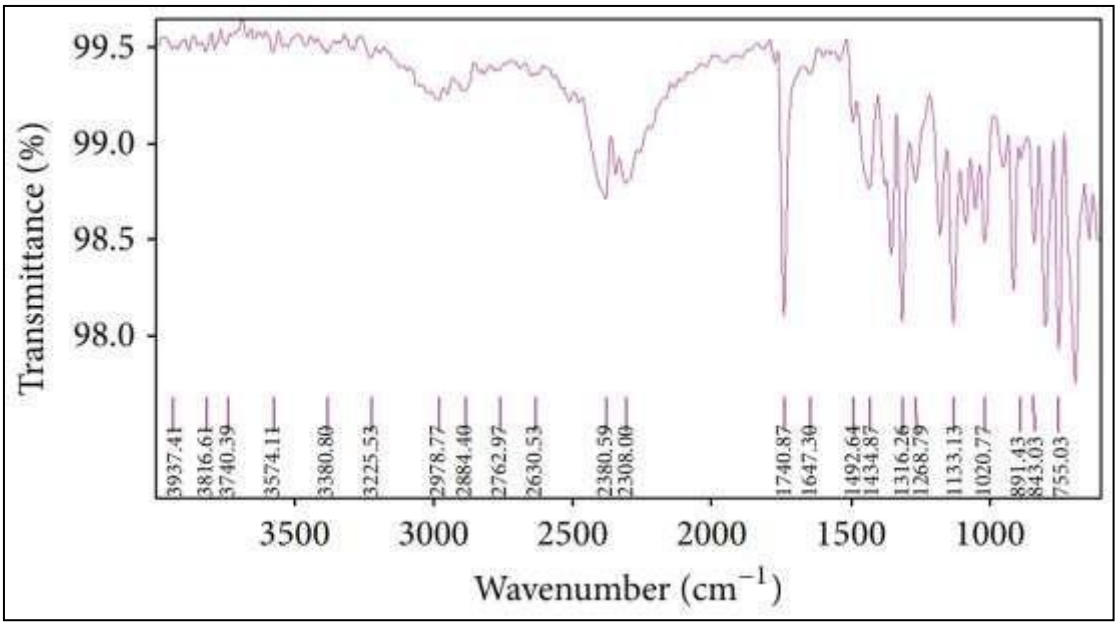


Fig.No.9: IR Spectrum of Bilastine

IJPSM

Chetan Vilasrao Patil *et al*, International Journal of Pharmaceutical Sciences & Medicine (IJPSM), Vol.8 Issue. 3, March- 2023, pg. 132-149

ISSN: 2519-9889
Impact Factor: 5.9

Interpretation of FTIR Spectrum of Bilastine

Sr. No.	Functional group	dard range(cm-1)	rved range(cm-1)
1	N-H stretching	2300-2500	2380.59
2	C=O Stretch	1700-1800	1740.87
3	C-O-C Stretch	1200-1100	1133.13
4	C-H Bending	1400-1300	1326.16

FTIR spectrum of Montelukast sodium

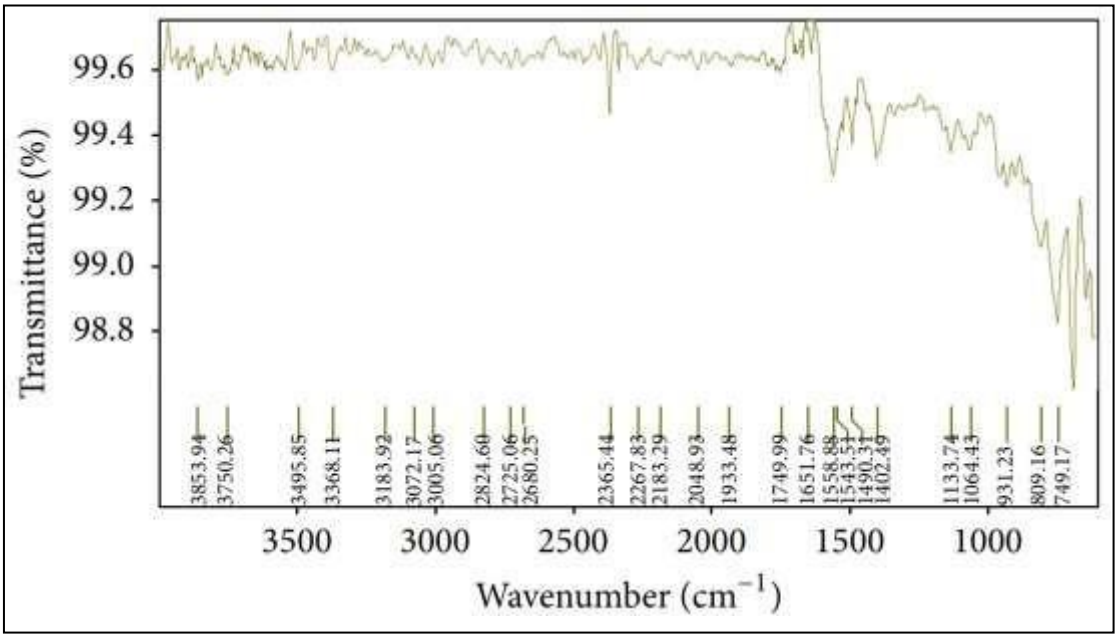


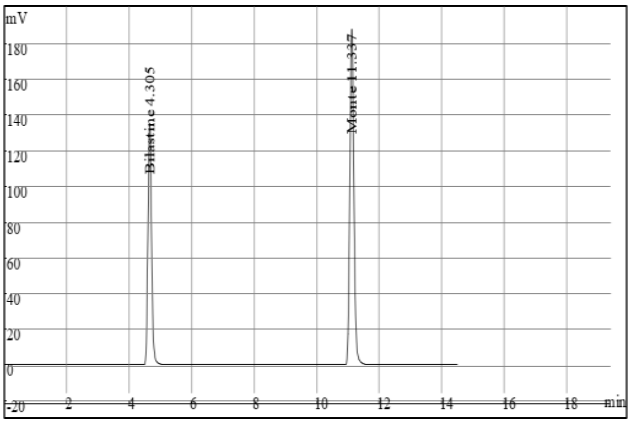
Fig.No.10: IR Spectrum of Montelukast Sodium

ISSN: 2519-9889
Impact Factor: 5.9

Interpretation of FTIR Spectrum of Montelukast

Sr. No.	Functional group	dard range(cm-1)	rved range(cm-1)
1	N-H stretching	2300-2500	2364.44
2	C=O Stretch	1600-1500	1558.88
3	C-CL Stretch	700-800	749.17
4	C-H Bending	1400-1300	1402.49

Development of simultaneous HPLC method for Bilastine and Montelukast. High performance liquid chromatographic method was developed and validated for determination of Bilastine and montelukast in bulk and dosage form.



Accuracy

Accuracy was studied by standard addition method and % recovery found was within acceptable limit. Results of recovery study are shown in Table no.21 & 23 and statistical validation is shown in Table no. 22 & 24.

ISSN: 2519-9889
Impact Factor: 5.9

Statistical validation of Bilastine by HPLC method

Level of addition	% Mean recovery*	SD	% RSD
50%	100.074	0.55574	0.555327
100%	99.5754	1.02255	1.02691
150%	99.9563	0.25926	0.259375

***Average of three determination

Optimized chromatogram of Bilastine and Montelukast LinearityData of calibration curve of Bilastine and Montelukast by HPLCmethod

Sr. No.	Bilastine		Montelukast	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	50	2130445	25	2821037
2	100	2554222	50	3385864
3	150	3028280	75	4013136

ISSN: 2519-9889
Impact Factor: 5.9

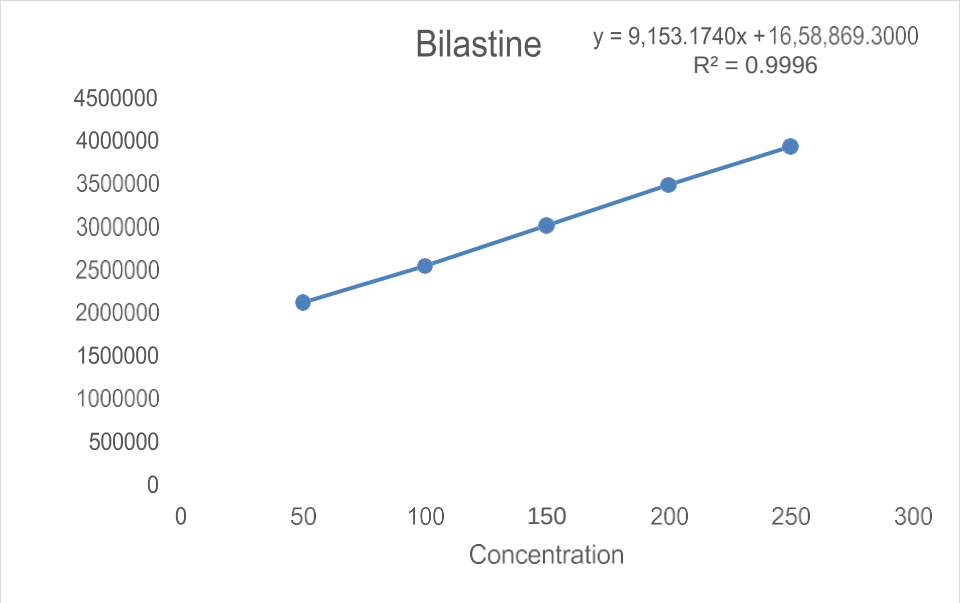


Fig. No. 12: Calibration curve for Bilastine

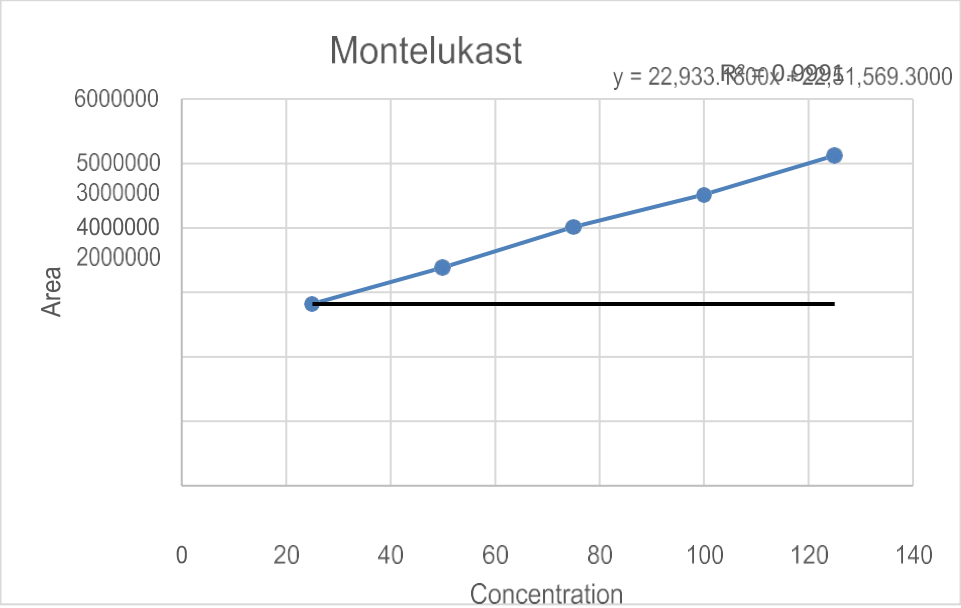


Fig. No. 13: Calibration curve for Montelukast

ISSN: 2519-9889
Impact Factor: 5.9

Optical characteristics

Optical characteristics and statistical data of linearity for Bilastine and Montelukastby HPLC method are summarized in Table no. 20

Accuracy

Accuracy was studied by standard addition method and % recovery found was within acceptable limit. Results of recovery study are shown in Table no.21 & 23 and statistical validation is shown in Table no. 22 & 24.

Table No.22: Statistical validation of Bilastine by HPLC method

Level of addition	% Mean recovery*	SD	% RSD
50%	100.074	0.55574	0.555327
100%	99.5754	1.02255	1.02691
150%	99.9563	0.25926	0.259375

*****Average of three determination

Table No.23: Data for recovery study of Montelukast by HPLC method

Level of addition	tandard added (µg/ml)	conc. (µg/ml)	Total conc. (µg/ml)	Area obtained*	Std Area	Drug recovered (µg/ml)	%Recovery
50%	25	50	75	4014581	4013136	75.027005	100.0360068
	25	50	75	4024581		75.213891	100.2851884
	25	50	75	4001548		74.783436	99.71124826
100%	50	50	100	4531546	4514271	100.38268	100.3826753
	50	50	100	4501564		99.718515	99.71851491
	50	50	100	4465846		98.927291	98.92729081
150%	75	50	125	5136481	5123481	125.31717	100.2537337
	75	50	125	5102497		124.48804	99.59043471
	75	50	125	5201467		126.90266	101.5221292

ISSN: 2519-9889
Impact Factor: 5.9

Table : Statistical validation of Montelukast by HPLC method

Level of addition	% Mean recovery*	SD	% RSD
50%	100.011	0.2878	0.287767
100%	99.6762	0.72862	0.730983
150%	100.455	0.98152	0.977066

***Average of three determination

Precision Study for Bilastine

The % RSD found below 2, Hence results complies as per guidelines

Data for intraday precision of Bilastine by HPLC method

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	50	2130481	2129512.33	3532.067	0.165863
2	50	2132459			
3	50	2125597			
4	100	2556594	2560926.33	15268.19	0.596198
5	100	2556444			
6	100	2569741			
7	150	3028487	3022953.33	6705.036	0.221804
8	150	3024876			
9	150	3015497			

% Assay of Marketed formulation

The % Assay of BLHIST-M (20mg+10mg) marketed formulation of Blaze remedies was calculated and given in table No. 33

ISSN: 2519-9889
Impact Factor: 5.9

Table Data of % Assay of marketed formulation

Sr. NO.	Drug	Area ofSample	Area of Standard	Drug Recovered	% Assay
1	**Bilastine (20 mg)**	3014849	3028280	19.91	**99.56**
2	**Montelukast (10 mg)**	3957895	4013136	9.86	**98.62**

Table No Data for interday precision of Bilastine by HPLC method

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	50	2139974	2134778.67	8580.28277	0.40192845
2	50	2124875			
3	50	2139487			
4	100	2554879	2563724.67	10172.8711	0.39680045
5	100	2574841			
6	100	2561454			
7	150	3021844	3018627	5267.55987	0.17450185
8	150	3021489			
9	150	3012548			

Precision Study for Motelukast

ISSN: 2519-9889
Impact Factor: 5.9

Table No. Data for intraday precision of Montelukast by HPLC method

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	25	2814597	2810273.33	7558.198	0.268949
2	25	2801546			
3	25	2814677			
4	50	3389487	3395301.67	12084.15	0.355908
5	50	3394870			
6	50	3401548			
7	75	4003648	4014451.33	9548.932	0.237864
8	75	4017943			
9	75	4021763			

Table No.28: Data for interday precision of Montelukast by HPLC method

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	25	2801464	2785786	13919.8559	0.49967427
2	25	2781015			
3	25	2774879			
4	50	3421545	3423187.67	2563.04298	0.07487299
5	50	3421877			
6	50	3426141			
7	75	4001544			

ISSN: 2519-9889
Impact Factor: 5.9

8	75	4057894	4027074	28545.0293	0.70882803
9	75	4021784			

Limit of detection and limit of Quantitation

The results of LOD and LOQ are presented in Table No. 29

Table No. 29: Results of LOD and LOQ values of Bilastine and Montelukast

Drugs	**LOD (µg/ml)**	**LOQ (µg/ml)**
Bilastine	1.273394	3.8587707
Montelukast	0.368806	1.1175947

Robustness

Table No.30: Data for Robustness study of Bilastine and Montelukast

Sr. No	**Parameter**	**Condition**	**Bilastine (150 µg/ml)**				**Montelukast (75 µg/ml)**			
			Area	**Mean**	**SD**	**%RSD**	**Area**	**Mean**	**SD**	**% RSD**
1	Change in Flow rate (ml/min)	0.9	3024876	3021424.33	5974.13	0.1977	4021546	4019525	3502.78	0.08714
2		1	3014526				4015480			
3		1.1	3024871				4021548			
1	Change in Wavelength (nm)	223	3001548	3018414.67	15538.77	0.5148	4010215	4015758	5681.74	0.14149
2		225	3032148				4015490			
3		227	3021548				4021569			

*****Average of three determination Ruggedness

ISSN: 2519-9889
Impact Factor: 5.9

Table No.31: Data for ruggedness study of Bilastine and Montelukast

Sr.No	Analyst	Bilastine (150 µg/ml)				Montelukast (75 µg/ml)			
		Area	Mean area*	SD	% RSD	Area	Mean area*	SD	% RSD
1	Analyst-I	4216542	4217835	3279.96	0.07776	4216542	4217835	3279.96	0.07776
		4215398				4215398			
		4221564				4221564			
2	Analyst-II	4203651	4219948	22508.7	0.53339	4203651	4219948	22508.7	0.53339
		4210563				4210563			
		4245631				4245631			

*Average of three determination

Specificity

Excipients and impurities were not interacting with the standard drugs. Hencemethod is specific. Results of specificity are shown in Table no. 32Table No.32: Data for specificity study of Bilastine and Montelukast

Drug	Drug conc. (µg/ml)	Excipients (µg/ml)	Total conc. (µg/ml)	Area	Mean	SD	%RSD
Bilastine	50	100	150	2137849	2131599	9669.80	0.45
	50	100	150	2120461			
	50	100	150	2136487			
	100	100	200	2561497	2560641	9219.85	0.36
	100	100	200	2551023			
	100	100	200	2569403			
	150	100	250	3021497	3020655.3	1106.50	0.03
	150	100	250	3019402			
	150	100	250	3021067			

ISSN: 2519-9889
Impact Factor: 5.9

Montelukast	25	50	75	2816497	2821344	4992.89	0.17
	25	50	75	2821064			
	25	50	75	2826471			
	50	50	100	3389401	3391342.3	5559.31	0.16
	50	50	100	3397612			
	50	50	100	3387014			
	75	50	125	4019731	4021486	1724.36	0.04
	75	50	125	4023178			
	75	50	125	4021549			

System Suitability:

System suitability parameters were measured to verify the system, method and column performance. Standard solution of Bilastine and montelukast was injected into the system for five times and system suitability parameters were checked.

Table No. Data for System suitability study

Sr. No.	**Bilastine**			**Montelukast**		
	Retention Time (min)	**eoreticalplates**	**ymmetryFactor**	**etention Time (min)**	**eoreticalplates**	**ymmetryFactor**
1	4.32	5164	1.15	11.32	8487	1.25
2	4.3	5746	1.12	11.36	8552	1.24
3	4.28	5019	1.13	11.31	8462	1.25
4	4.38	5845	1.13	11.37	8359	1.23
5	4.34	5316	1.12	11.32	8252	1.24
6	4.29	5946	1.14	11.34	8539	1.25
Mean	4.3183333	5506	1.1316667	11.33667	8441.8333	1.2433333
SD	0.0371035	388.93547	0.0116905	0.024221	115.69169	0.008165
%RSD	0.8592078	7.06384799	1.0330296	0.213654	1.3704569	0.6566997

ISSN: 2519-9889
Impact Factor: 5.9

REFERENCES

[1]. Chatwal G. R., Anand S. K., Instrumental Methods of Chemical Analysis, Fifth Edition, 2008, Himalaya Publishing House, 2.108-2.124.
[2]. Kasture A.V., Mahadik K. R., Wadodkar S. G., More H. N., Pharmaceutical Analysis, Vol.II, Seventh Edition, 2007, Nirali Publication, 28-30
[3]. Validation of Compendial Procedures, United State Pharmacopeia, USP 36 NF.
[4]. Kenkel J., Analytical Chemistry for Technicians, Third Edition, 2009, Published by CRC Publication, 2-4.
[5]. V. Gupta, A.D. K. Jain, N.S. Gill, K. Gupta, Development and validation of HPLC method - a review , Int. Res J Pharm. App Sci., 2(4) (2012) 17-25
[6]. M.S. Azim, M. Mitra, P.S. Bhasin, HPLC method development and validation: A review, Int. Res. J. Pharm. 4(4) (2013) 39-46..
[7]. Jeffery G. H., Bassett J., Mendham J., Denny R. C., Vogel's Textbook of Quantitative chemical Analysis, Fifth Edition, 1991, Longman Scientific and Technical Publishers, 3-13.
[8]. Pavia D. L., Lampman G. M., Kriz G. S., Introduction to Spectroscopy, Third Edition, Thomson Learning publication, 356, 797-817.
[9]. M.W. Dong, Modern Hplc for practicing scientists, John Wiley & Sons, New Jersey, 2006.
[10]. Beckett A. H., Stenlake J. B., Practical Pharmaceutical Chemistry, Fourth Edition Part II, 2004, CBS Publishers and distributors, 284-300,162-163.
[11]. Furnis B. S., Vogel's Textbook of practical organic chemistry. Longman group UK Ltd 5th Edition Edition, 1989, UK, 384-386.
[12].B. Prathap, G.H.S. Rao, G. Devdass, A. Dey, N. Harikrishnan , Review on Stability Indicating HPLC Method Development, International Journal of Innovative Pharmaceutical Research.3(3) (2012) 229237.

