https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 27 (September2024); 302-309

Systematic Review

RP-HPLC Method Development and Validation for the Simultaneous Estimation of Bilastine and Montelukast Sodium in Tablet Dosage Form

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ABSTRACT
A new, simple, specific, accurate, precise and reproducible analytical method has been developed and validated for the simultaneous estimation of Bilastine and Montelukast sodium in Tablet Dosage Form. Bilastine is a selective histamine H1 receptor and montelukast is a leukotriene antagonist receptor. The aim of experiment was to develop and validate RP-HPLC method for the estimation of Bilastine and Montelukast sodium in tablet dosage from. Ofloxacin was used as an internal standard. The HPLC system used for analysis was JASCO, PU 2075plus, UV 2080 plus with a column Qualisil 5 BDS-C18 (250 mm x 4.6 m, 5 µm). Acetonitrile: potassium dihydrogen phosphate buffer (pH adjusted to 6.2 with TEA) in the ratio of 38:82 was used as mobile phase. The optimized conditions were: flow rate (1.2 ml/min), wavelength (254 nm), injection volume (20 µl), working time was 10 minutes.
The retention times of Bilastine, Ofloxacin, and Montelukast sodium were found to be 3.017min, 4.367min, and 7.342min respectively. % Assay was found to be 101.13% for Bilastine and 99.67% for Montelukast sodium. The linearity range of Bilastine and Montelukast sodium were found to be 2-14 µg/ml and 4-28 µg/ml with a correlation coefficient (r^2) of 0.9994 and 0.9998 respectively. % Recovery was obtained as 99.91%-100.56% and 99.45%-100.38% for Bilastine and Montelukast sodium respectively. LOD and LOQ was found to be 0.107µg/ml and 0.325µg/ml for Bilastine and 0.412µg/ml and 1.248µg/ml for Montelukast sodium. The analytical method was validated as per ICH guidelines. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in flow rate and mobile phase composition separately and analysis being performed by different analysts.

Keywords: Validation, RP-HPLC, Bilastine, Ofloxacin, Montelukast

Received: 02/07/2024 Accepted: 05/08/2024

DOI: https://doi.org/10.53555/AJBR.v27i3.1361

INTRODUCTION

Montelukast Sodium (MTL) is white or almost white hygroscopic powder. Chemically ''1-[[[(1R)-1-[3-(1E)-2-(7-chloro-2-quinolinyl) ethyl) phenyl)-3-[2-(1-hydroxy-1methylethyl) phenyl) propyl)] thio) methyl]- cyclo-propane acetic acid" [1-4]. Montelukast sodium is one of the receptor antagonists used in the treatment of severe respiratory diseases such as asthma and related allergic [5]. Bilastine (BLS) is a white crystalline powder. Chemically "2-[4-(2-{4-[1-(2-Etleukotrienehoxyethyl)-1H-benzimidazol-2-yl]-1-piperidinyl} ethyl) phenyl]-2-methylpropanoic acid" [5,6]. Bilastine is a new, well-tolerated, non-sedating H-1 antihistamine recently approved to treat symptomatic treatment of allergic rhinitis and chronic urticaria in India [7]. Bilastine is as effective as other

non-sedating antihistamines in allergic rhinoconjuctivitis and chronic urticaria in 12 and18 years old individuals respectively [8].

Literature survey reveals that few analytical methods such as UV Spectroscopy [8], RP-HPLC [9-11], QbD [12], UPLC [13,14], HPTLC [15] etc. are available. Some analytical methods are available for estimation of Bilastine alone and in combination with other drugs like Desloratadine [16], Levocetirizine [17]. For Montelukast sodium, methods are available for single drug and in combination with Ebastine [18], Fexofenadine hydrochloride [19], Desloratadine [20], Levocetirizine hydrochloride [21], Bambuterol hydrochloride [22]. Also, few analytical methods are available for the simultaneous estimation of Bilastine and Montelukast sodium. The Internal standard method corrects for different sources of volume errors, including injection-to-injection variation, volume errors in sample preparations in the chromatographic system. Various HPLC quantification methods are available for the analysis of pharmaceutical dosage form such as Normalized peak area method, External standard method, Internal standard method, Method of standard addition, etc. The internal standard is used to improve the precision of quantitative analysis. An internal standard is a known concentration of a chemical that is added in a sample to quantify the components of the sample [23] but, no analytical method is reported for the simultaneous estimation of Bilastine and Montelukast sodium using Internal Standard. Hence, there is need to develop a new and validated HPLC method for the simultaneous estimation of Bilastine and Montelukast sodium in tablet dosage form.

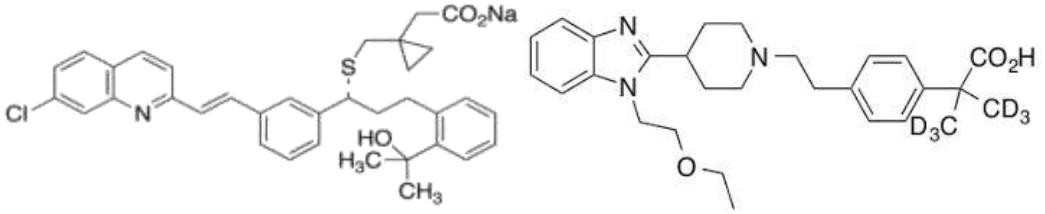

Fig. A [10] Fig. B [2]

Figure 1. Structure of drugs: A) Montelukast Sodium B) Bilastine

EXPERIMENTAL:

Materials and Reagents

BLS and MTL sample were received as a gift from Shree Icon Pharmaceutical Laboratories, Vijayawada, Andhra Pradesh. Reagents like acetonitrile & methanol (HPLC grade) were secured from Merck Life Science Pvt. Ltd. Mumbai, India. Triethylamine (TEA)was obtained from Fine Chem. Industries Mumbai, India. BILAFORD20 (BLS 20mg & MTL10mg) used as a Marketed Formulation.

Instrumentation

The HPLC system consist of solvent delivery module JASCO with UV Detector with 10µl pump loop, controlled by Browin software. Qualisil 5 BDS- C18 column (250×4.6mm; 5µm) was used for separation. The weighing was carried out using CONTECH balance. LABMAN LMPH-9 L22459 digital pH meter was used to determine the pH. The Citizen Digital Ultrasonic Cleaner CD4820 sonicator was used for sonication.

Preparation of Stock Solution

Preparation of Buffer (10mM)

Weighed accurately about 0.260gm of Potassium Dihydrogen Phosphate and transferred into volumetric flask containing 1000ml HPLC grade water. Then pH was adjusted to 6.2 with Tri-ethylamine and then buffer solution was filtered through 0.45µm membrane filter.

Preparation of Mobile Phase and Diluents:

The HPLC grade acetonitrile was sonicated for 30 min using digital Ultrasonicator to degas it. The pH of phosphate buffer was attained to 6.2 with Tri-ethylamine and sonicated for 30 min to degas it. The final mobile phase composed of Potassium dihydrogen phosphate (10mM): ACN in the ratio of 38:82 %v/v.

Preparation of Standard Stock Solution:

Weighed accurately about 20mg of Bilastine and 10mg of Montelukast sodium and transferred it into separate 100ml volumetric flask containing 20ml of solvent (Acetonitrile: Methanol) (1:1) sonicated for 10 min. The content was dissolved by sonication for 10 min with intermediate shaking and made volume upto 100 ml with diluents. This solution further diluted 20µg/ml and 10µg/ml of Bilastine and Montelukast sodium respectively.

Preparation of Internal Standard Stock Solution:

Weighed accurately about 10mg of Ofloxacin and transferred to a 100ml volumetric flask with containing 50ml of diluent. The content was dissolved by using sonication for 10 min. The volume was made up to 100ml with diluent, and further diluted to a concentration of 10µg/ml. Also mixed standard were prepared to get 20µg/ml of Bilastine and 10µg/ml Montelukast sodium.

Preparation of Sample Solution and Assay of Marketed Formulation:

Twenty tablets were weighed and average weight was determined. crushed them into fine powder. Weighed about 0.308gm equivalent to (20mg of Bilastine and 10mg of Montelukast Sodium) and transferred it to 100ml volumetric flask separately containing (acetonitrile: methanol) (1:1) sonicated for 10min and volume was made up to mark with diluent and filtered. Resulting working standard solution containing 200 & 100µg/ml of Bilastine and Montelukast

Sodium respectively. Further dilution was prepared from working standard solution.

Determination of Wavelength

The individual and overlain spectra were recorded using in the range of 200-400nm. The absorption maxima were found to be 214.0 nm of Bilastine, 282.0 nm of Montelukast sodium. From the overlain spectrum of drugs, the iso-absorptive point was found to be 254 nm and was used in the present study.

METHOD VALIDATION [25]

Assay of marketed formulation

The prepared sample solution was injected in six replicates into the HPLC system. Peak area was determined and % RSD were calculated.

Analytical Method validation

System suitability: System suitability were evaluated by injecting six replicates of 20 µg/ml BIL and 10 µg/ml MTL. A chromatogram was reported. The system suitability parameter such as tailing factor, resolution, theoretical plates, retention time and peak area were estimated. The %RSD of this parameters were within the limit i.e. (<2).

Linearity: The mixed solutions were injected into the HPLC system. A calibration curve was constructed by the plotting Peak area of analyte versus concentration of drugs. Calculated correlation coefficient & regression line equation.

Accuracy: The accuracy was determined by the standard spike method of reanalysis sample at 50%, 100% and 150% levels, and the mixed solution was reanalyzed. At each level, three replicates injected and calculated amount, amount found and percent recovery were added. Each level should had a recovery of 98-102%.

Precision

a) System precision: System precision was performed by injecting six replicates of a mixed standard solutions of 20µg/ml BIL and 10µg/ml MTL into HPLC. Peak area was determined and % RSD was calculated.

b) Method precision: The six replicate of sample solution containing 20µg/ml BIL and 10µg/ml MTL have been analyzed for method precision. The % assay and % RSD was calculated.

c) Intermediate precision

i. **Intraday precision:** In the intraday studies, three injections of each concentration i.e., 8,12,16µg/ml BIL and 4,6,8 µg/ml MTL were injected into HPLC system in different time interval on a same day. %RSD was determined.

ii. **Interday precision:** Interday precision was performed by injecting three replicates of each sample concentration i.e., 8,12,16 µg/ml BIL and 4,6,8µg/ml MTL were injected into chromatographic system on different day and calculated the %RSD.

Specificity: Specificity of method can be termed as absence of any interference at retention times of samples. Specificity was performed by injecting blank, sample and standard solution into HPLC system. Chromatograms were reported. Retention times from sample & standard solutions were compared for identification of analytes.

Robustness: Robustness of analytical procedure means measure of its capacity to remain unaffected by small but deliberate change in method parameters. Robustness was carried by varying two parameters from the optimized chromatographic condition The flow rate varied by ±0.2ml and mobile phase composition varies by ±2. Calculated % RSD.

Ruggedness: The degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories, different analyst etc. Ruggedness was determined by injecting sample into HPLC system and evaluated by analyst 1 & analyst 2 on a same day. Calculated %RSD.

Limit of Detection and Limit of Quantitation: LOD is low level of concentration of analyte in sample which can be determined and LOQ is low level of concentration of analyte in sample which can be estimated. LOD and LOQ of drug were derived by calculating signal-to-noise ratio i.e., 3.3 for LOD and 10 for LOQ.

$LOD = 3.3\sigma/slope$

$LOQ = 10\sigma/slope$

Where, σ = Standard deviation of the response

S = Slope of calibration curve

Result and Discussion

Method development: Chromatographic conditions (mobile phase composition, its pH) were optimized through several trials to achieve the better sensitivity and a good peak shape for both drugs. Different combination ratios were tested. The best chromatographic separation was achieved with a Qualisil 5 BDS C18 column (250×4.6mm ID, 5µm particle size). A mobile phase consisting of acetonitrile and phosphate buffer pH-6.2 adjusted with triethylamine (TEA) (38:82%v/v) with a flow rate of 1.2 ml/min, an injection volume of 20µl and detected wavelength at 254 nm using UV detector with 10 min run time. Under the chromatographic conditions, Bilastine and Montelukast sodium were detected using the internal standard ofloxacin with retention time 3.017min, 7.342min and 4.367min respectively. The optimized method was validated according to ICH guidelines. A typical chromatogram of Bilastine and Montelukast sodium and ofloxacin is shown in the fig 3.

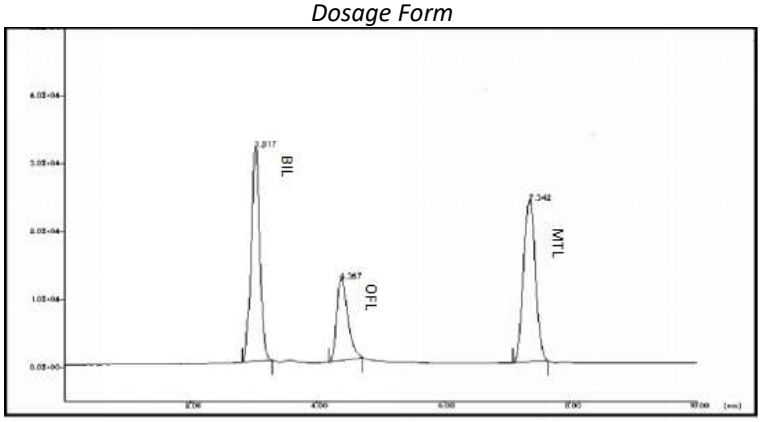

Fig 3- Chromatograms of Bilastine, Ofloxacin and Montelukast Sodium

Assay of marketed formulation

Peak area ratio was determined and % RSD were calculated. The percentage assay of Bilastine and Montelukast sodium were found to be 101.14 % w/w and 99.60 % w/w respectively.

Formula for Assay:

$$\%\ \boldsymbol{Assay} = \frac{Peak\ Area\ Ratio\ of\ test}{Peak\ Area\ Ratio\ of\ std.} \times \frac{Std.wt.(mg/g)}{Dilution\ factor} \times \frac{Dilution\ factor}{Wt.of\ sample\ (mg/g)} \times \frac{Percent\ potency\ of\ Std.drug}{100} \times \frac{Avg.wt}{Label\ Claim} \times 100$$

….Equation (1)

Table 1: Assay of Marketed Formulation

Marketed Formulation	Drugs	Avg sample area	Std. wt. (µg/ml)	Label amount (mg)	Amount found (mg)	% Assay
BILAFORD-20 (BIL-20mg MTL-10mg)	Bilastine	163835	20	20	0.60	101.13
	Montelukast sodium	83325.2	10	10	0.30	99.67

Analytical Method validation

Specificity: The specificity of method was determined by injecting 20µg/ml of Bilastine and 10µg/ml of Montelukast sodium into chromatographic system in triplicates. The chromatograph of blank, sample and standard were recorded. No interference of mobile phase constituents and excipients at the retention time of standard and sample which shows that the method was specific.

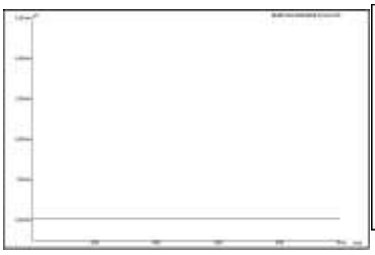

Fig.4 Chromatogram of Blank Solution

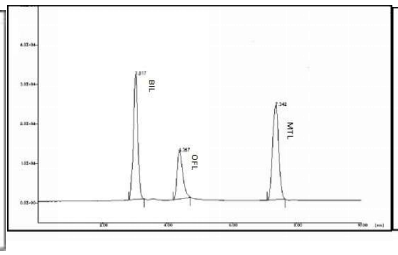

Fig.5 Chromatogram of Standard Solution

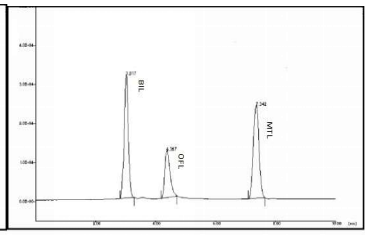

Fig.6 Chromatogram of Sample Solution

System suitability: System suitability were evaluated by injecting six replicates of 20 µg/ml BIL and 10 µg/ml MTL.A chromatogram was reported. In the chromatogram of standard mixture, the retention time for Bilastine and Montelukast sodium was found to be 3.026 min and 7.356 min. The system suitability parameter such as tailing factor, resolution, theoretical plates, retention time and peak area were estimated. The %RSD of these parameters are within the limit i.e. (<2)

Table 2: Summary of system suitability parameters

Parameter	BIL	OFL	MTL	Acceptance criteria
Tailing factor	0.828	1.418	0.236	≤ 2
Retention time	3.017min	4.367min	7.342min	≥ 2
Theoretical plates	3828.6	2971.3	8967.3	≥ 2000
RSD % of peak area	1.171	1.681	1.248	≤ 2

Ratio	1.484	-	0.236	≥ 2
Resolution	-	-	0.743	≥ 2

Linearity: The Linearity range of BIL and MTL were found to be 4-28 µg/ml and 2-14 µg/ml respectively. The mixed solutions were injected into the HPLC chromatogram. A calibration curve was constructed by the plotting Peak area of analyte versus concentration of drugs. The correlation coefficient (r^2) value of Bilastine and Montelukast sodium 0.9994 and 0.9998 respectively. Calculated correlation coefficient & regression line equation.

Table 3: Linearity values of BIL and MTL

Parameter	**BIL**	**MTL**
Linearity range	4-28 µg/ml	2-14 µg/ml
Regression equation	Y=0.0691x+0.01	Y=0.0729x-0.0103
Slope	0.0691	0.0729
Intercept	0.01	0.0103
r^2	0.9994	0.9998

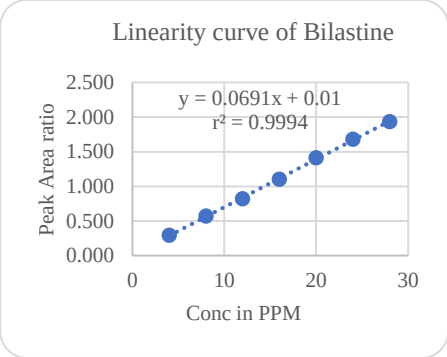

Fig 7. Linearity plot of Bilastine

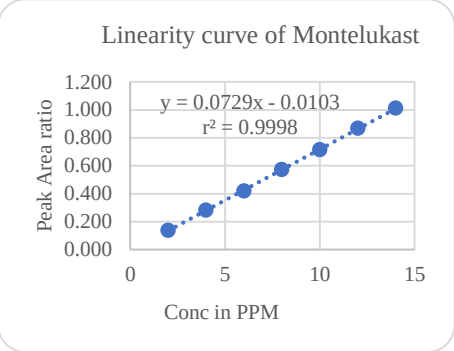

Fig 8. Linearity plot of Montelukast sodium

Accuracy: The accuracy was determined by the standard spike method of preanalysis sample. At 50%,100%,150% Bilastine and Montelukast sodium shows 99.91 % - 100.56% and 99.45% - 100.38 % mean recovery respectively. It was found that the percentage recovery for both medications was within the range of 98.00-102.00 % at all rates, which was found to be well within acceptance criteria limits.

Table 4: Recovery values of BIL and MTL

% Accuracy level	**50%**		**100%**		**150%**	
	BIL	**MTL**	**BIL**	**MTL**	**BIL**	**MTL**
Amount Present(mg)	0.1540 (10)	0.1540 (5)	0.1540 (10)	0.1540 (5)	0.1540 (10)	0.1540 (5)
	0.1540 (10)	0.1540 (5)	0.1540 (10)	0.1540 (5)	0.1540 (10)	0.1540 (5)
	0.1540 (10)	0.1540 (5)	0.1540 (10)	0.1540 (5)	0.1540 (10)	0.1540 (5)
Amount of Std. added (mg)	5.2	2.5	10.1	5.1	15.1	7.51
	5	2.6	10.12	4.98	15.2	7.49
	5.1	2.5	9.97	5.12	14.9	7.52
Amount Recovered (mg)	5.24	2.49	10.12	5.05	15.06	7.51
	5.00	2.61	10.06	4.94	15.20	7.50
	5.14	2.52	10.04	5.12	14.90	7.59
% Recovery	100.79	99.46	100.21	99.09	99.72	100.00
	100.03	100.34	99.39	99.24	99.99	100.16
	100.87	100.77	100.73	100.00	100.02	100.99
Mean Recovery	**100.56**	**100.19**	**100.11**	**99.45**	**99.91**	**100.38**
%RSD	**0.46**	**0.67**	**0.68**	**0.49**	**0.16**	**0.53**

Precision

d) System precision: System precision was evaluated by injecting six replicates of standard solutions of Bilastine and Montelukast sodium into chromatographic system. System precision was assessed by calculating the Bilastine and Montelukast sodium standard peak areas. The results were expressed in percentage of %RSD and were found to be 0.021 and 0.950 for Bilastine and Montelukast sodium respectively which were within the acceptable limit of < 2 percent.

e) Method precision: The six replicate of sample solution containing 20µg/ml BIL and 10µg/ml MTL have been analyzed for method precision. the % RSD and the percent assay were found to be 0.461 and 0.411 of Bilastine and Montelukast sodium respectively. Which were below the acceptable < 2 percent limit.

f) Intermediate precision

iii. **Intraday precision:** In the intraday studies, three injections of each concentration i.e., 8,12,16µg/ml BIL and 4,6,8 µg/ml MTL were injected into HPLC system in different time interval on a same day. The % RSD in Intraday precision for Bilastine (8,12 and 16 µg/ml) were found to be 0.273, 0.367, 0.578 % and for Montelukast sodium (4,6 and 8 µg/ml) were found to be 0.212, 0.363, 0.687% respectively. % RSD in intraday studies were found well within the appropriate limits.

iv. Interday precision: Interday precision is performed by injecting three replicates of each sample concentration i.e., 8,12,16 µg/ml BIL and 4,6,8µg/ml MTL were injected into chromatographic system on different day and % RSD for Bilastine (8,12 and 16 µg/ml) were found to be 0.273, 0.367, 0.578 % and for Montelukast sodium (4,6 and 8 µg/ml) were found to be 0.23, 0.52, 0.48% respectively% RSD in inter day studies were found well within the appropriate limits.

Table 5: Intraday precision

Bilastine			**Montelukast sodium**		
Conc.(µg/ml)	±SD	%RSD	Conc.(µg/ml)	±SD	%RSD
8	0.274	0.273	4	0.212	0.212
12	0.368	0.367	6	0.364	0.363
14	0.579	0.578	8	0.686	0.687

Table 6: Interday precision

Day	**Bilastine**			**Montelukast sodium**		
	Conc (µg/ml)	±SD	%RSD	Conc (µg/ml	±SD	%RSD
Day 1	8	0.212	0.212	4	0.301	0.301
Day 2						
Day 3						
Day 1	12	0.364	0.363	6	0.691	0.692
Day 2						
Day 3						
Day 1	14	0.686	0.687	8	1.005	1.007
Day 2						
Day 3						

Robustness: The robustness of the developed method was determined by decreasing the flow rate to 1 ml/min and increasing to 1.4ml/min from 1.2 ml/min (Actual), % RSD for retention time, USP tailing, peak area was found to be 2.56, 0.64, 1.29 %, and 6.64, 0.74, 1.13% for Bilastine and Montelukast sodium respectively. The device suitability parameters were reported well within reasonable limits (less than 2.0 percent RSD percentage).

Table 7: Robustness of BIL and MTL

Parameter	**Bilastine**				**Montelukast sodium**			
Flow rate	Rt	Tailing Factor	Peak Area	Ratio	Rt	Tailing Factor	Peak Area	Ratio
1ml/min	3.08	1.11	157412	0.75	7.73	1.18	80985	0.39
	3.21	1.10	158234	0.77	9.46	1.17	80261	0.39
	3.25	1.10	159452	0.77	9.10	1.19	81145	0.39
1.2ml/min	2.56	1.11	158231	0.78	6.64	1.18	81362	0.40
	2.57	1.11	159323	0.78	6.63	1.18	80236	0.39
	2.55	1.11	156420	0.76	6.61	1.16	80203	0.39
1.4ml/mi	2.42	1.10	156478	0.76	6.16	1.18	81952	0.40

n	2.41	1.12	155261	0.76	5.93	1.18	80254	0.39
	2.35	1.10	156255	0.77	5.53	1.17	80695	0.40
Avg.		1.11	157601	0.77		1.18	80799.7	0.39
SD		0.01	1456.32	0.01		0.01	619.38	0.00
%RSD		**0.64**	**0.92**	**1.29**		**0.74**	**0.77**	**1.13**

Ruggedness: The method Ruggedness was accomplished by analysts 1 and analysts 2 injecting the analyzed sample. The % RSD for analyst 1 was found to be 1.32, 1.752% for Bilastine and Montelukast sodium respectively. The % RSD for analyst 2 was found to be 1.464, 1.40 % for Bilastine and Montelukast sodium respectively. The %RSD was found well within acceptable limits (less than 2.0 percent RSD).

Table 8: Ruggedness of BIL and MTL

Drug	Parameter	Conc.(µg/ml)	±SD	%RSD
Bilastine	Analyst 1	12	0.0100	1.32
	Analyst 2		0.0112	1.46
Montelukast	Analyst 1	6	0.0068	1.75
	Analyst 2		0.0055	1.40

Limit of Detection and Limit of Quantitation: The LOD and LOQ for Bilastine are found to be 0.107µg/ml, and 0.325µg/ml and 0.412µg/ml and 1.248µg/ml for Montelukast sodium respectively. These values show the method is suitable for determining the lower concentration and confirmed the sensitivity of the proposed method for their determination.

Table 9: LOD and LOQ of BIL and MTL

Drug	LOD(µg/ml)	LOQ(µg/ml)
Bilastine	0.107	0.325
Montelukast sodium	0.412	1.248

CONCLUSION

An RP-HPLC method was successfully developed and validated for the simultaneous estimation of bilastine and montelukast sodium in a tablet dosage form. From the results of validation parameters, the proposed method was found to be sensitive, accurate, precise, robust, rugged and specific. The proposed method can thus be applied for routine quality control analysis of Bilastine and Montelukast Sodium in tablet dosage form using Ofloxacin as a Internal Standard.

ACKNOWLEDGEMENT

The authors would like to thank, Shree Icon Pharmaceutical Laboratories, Vijayawada. (Andhra Pradesh), for providing the Bilastine and Montelukast Sodium and Ofloxacin API as a gift sample.

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