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Research Article

Development and Validation of Reverse Phase High Performance Liquid Chromatography Method for *In-vitro* Dissolution Testing of Bilastine and Montelukast Sodium Tablets

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ABSTRACT

The objective of this experimental work is to develop and validate a reverse-phase high-performance liquid chromatographic (RP-HPLC) method, which is capable of quantitative estimation of Bilastine (BLS) and Montelukast (MTK) content present in dissolution media accurately and precisely. A simple, fast, specific, rugged, and reproducible analytical method was developed and validated on a Shimadzu High-performance liquid chromatography (HPLC) system. A Hypersil BDS C18-Column (100 mm x 4.6mm, 3 µm) and 0.1%v/v Triethylamine buffer (pH-3.0): Acetonitrile used as mobile phase with flow rate 1.0 mL/min in gradient mode. The column oven temperature and sample cooler temperature were set to 40°C and 25°C, respectively. The injection volume was 10 µL, and chromatograms were recorded at 220 nm. The run time for 1 sample analysis was 13 minutes. The optimized conditions for dissolution testing include USP type-II apparatus at 75 rpm with 900 mL, 0.5% w/v sodium lauryl sulfate media at 37.0 ± 0.5°C and collected after 45 minutes. Under the above conditions, the retention time (RT) of Bilastine and Montelukast was 1.84 and 7.23 minutes, respectively, without any interference of dissolution media or any other degradant at the RT of both drugs. The calibration curve was linear with R^2 value of 0.99984 and 0.99988 for BLS and MTK, respectively. The sample was found stable in dissolution media beyond 12 hours. The analytical method was validated as per International Council for Harmonisation (ICH) guidelines with respect to specificity, precision, linearity, accuracy, stability in aqueous solution, and robustness. The proposed method was found useful for the routine analysis of dissolution samples for quality control purposes.

Introduction

Dissolution testing is a regulatory requirement for all types of solid oral dosage forms, and it is continuously in use throughout the process of drug development, drug release, and routine stability testing to maintain quality control of drug products.[1] Dissolution is the rate-limiting step for drug absorption from a pharmaceutical dosage form meant for oral administration.[2] Hence, it is an important in-vitro tool that provides information about drug release patterns throughout the batches of the formulation.[3]

Bilastine is the latest H1-antihistaminic drug that is recently got approval to treat allergic rhinitis and chronic urticaria as a symptomatic treatment in India. It binds with Histamine H1 receptor and preventing its activation hence reduces the development of allergic symptoms.[4] Montelukast is a leukotriene receptor antagonist used to treat asthma and recommended for preventing and treating asthma in patients with age group 2 and more.[5] Montelukast can control asthma in adults if symptoms continue using sporadic short-acting β-agonists and the patient cannot use an inhaled corticosteroid (ICS).[6-8] A combination therapy of BLS and MTK may provide additive benefits in reducing SARC symptoms and allergic rhinitis-associated asthma.

Chemically BLS is 2-[4-(2-{4-[1-(2-ethoxyethyl)-1H-1,3-benzodiazol-2-yl]piperidin-1-yl}ethyl)phenyl]-2-methylpropanoic acid having molecular weight 463.622. It

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is sparingly soluble in 0.1 N HCL with pKa value is around 4.18.[9,10]

Chemically MTK is 2-[1-({[(1R)-1-{3-[(E)-2-(7-chloroquinolin-2-yl)-ethenyl]phenyl}-3-[2-(2-hydroxypropan-2-yl)phenyl]propyl]sulfanyl}methyl)cyclopropyl] acetic acid having molecular weight 586.184 g/mol. It is freely soluble in methanol, ethanol and water with estimated pKa of 2.7 and 5.8.[11]

An extensive literature review revealed that different dissolution medium at different pH was used to dissolve BLS and MTK drug product for quality control testing.[12-14] It is also revealed that several UV/HPLC methods are reported either for a particular drug or in combinations with other drugs.[15-16] However, no analytical method for In-Vitro dissolution testing for simultaneous estimation of BLS and MTK by HPLC has been documented so far.

This investigation presents optimization of a single dissolution media for extraction of both drugs and the development of a reverse phase HPLC method for simultaneous estimation of BLS and MTK content from dissolution samples. The optimized method was further validated regarding specificity, system precision, method precision, intermediate precision, ruggedness, linearity, recovery, stability in aqueous solution, and robustness according to International Council for Harmonisation (ICH) guidelines.[17]

Material And Methods

Chemicals and Reagents

BLS and MTK Sodium working standards were received as gift samples from M/s. Synokem Pharmaceuticals Limited, Haridwar, Uttarakhand, India. Fixed-dose combination tablets of BLS and MTK Sodium were prepared with a label claim of 20 mg and 10 mg. Methanol and Acetonitrile were of HPLC grade from Sisco research laboratories limited. The other reagents like triethylamine, orthophosphoric acid, sodium lauryl sulfate were of analytical grade. Water used to prepare buffers and other solutions was obtained from the Milli-Q water system from the analytical laboratory of M/s. Kusum Healthcare Pvt. Ltd., Rajasthan, India.

Instrumentation

The HPLC with a diode-array detector (Make: Shimadzu Model: LC-2010 CHT), Dissolution apparatus type-II (Paddle) with six vessels, Analytical weighing balance, pH meter, sonicator was used during the experiment.

Dissolution Method Optimization

The selection of dissolution media was based on solubility, and pKa values of BLS and MTS identified through literature survey. Dissolution test monograph for MTK was already published in the Indian Pharmacopoeia using dissolution apparatus with 900 mL 0.5% w/v Sodium lauryl sulfate, 50 RPM for 30 minutes. However, the monograph for BLS was not published either individually or in combination. Hence, solubility screening studies of BLS and MTK was conducted with four different types of dissolution media, i.e., 0.1 N HCL, phosphate buffer with pH 4.5, 7.2, and 0.5% w/v Sodium lauryl sulfate, and it is observed that tablets were dispersed entirely within 30 minutes in 0.1% w/v SLS media which was confirmed by UV spectrophotometric analysis. Hence, USP Apparatus Type-II (Paddle) at 75 RPM, 37.0 ± 0.5°C temperature with 900 mL 0.1% w/v SLS dissolution medium was selected for carrying dissolution profile of both drugs with single sampling time at 45 minutes to avoid any discourse.

Preparation of Dissolution Medium

Accurately weighed and transferred approximately 40 gm of SLS in 8000 mL of purified water steadily with continuous mixing of media (0.5% w/v SLS).

Dissolution Methodology

Apparatus: USP Apparatus Type-II (Paddle)
Speed: 75 RPM
Temperature: 37.0 ± 0.5°C
Sampling time: 45 min
Dissolution medium: 900 mL 0.5% w/v SLS

Chromatographic Method Optimization

The most common practice for developing an HPLC analytical method is the hit and trial technique. Blank solution and standard solution were used for analyte peak detection, and different trials on mobile phase flow, column, oven temperature, pH of mobile phase were made. The final optimized conditions consist of a Hypersil BDS C18 column (100 x 4.6 mm, 3µm) as stationary phase and 0.1%v/v Triethylamine buffer pH-3.0:Acetonitrile used as mobile phase at 1.0 mL/min flow in gradient mode of separation. A 0.45 µm Whatman filter paper was used for filtration of buffer and degassed by sonication. The column oven temperature and sample cooler temperatures were 40°C and 25°C, respectively. The injection volume was 10 µL, and chromatograms were recorded at 220 nm. Under these conditions, the run time for 1 sample analysis was 13 minutes. The gradient program used for peak separation was summarized in Table 1.

Preparation of Diluent

Acetonitrile: Methanol: Water, 40:40:20.

Table 1: Gradient program used in HPLC

Time (minute)	*Flow (mL/min)*	*Buffer (%)*	*Acetonitrile (%)*
0.01	1.0	60	40
3.00	1.0	60	40
5.00	1.0	80	20
8.00	1.0	80	20
10.00	1.0	60	40
13.00	1.0	60	40

Preparation of Buffer for Mobile Phase

Accurately 2 mL of triethylamine added into 2000 mL of Milli-Q water, mixed well and pH adjusted to 3.00 with orthophosphoric acid and filtered through 0.22 µm membrane filter.

Preparation of Stock and Standard Solution of Bilastine and Montelukast

The stock solution of BLS and MTK was prepared by transferring 87.84 mg of BLS and 44.52 mg of Montelukast Sodium (equivalent to 42.91 mg of MTK) into a 200 mL volumetric flask. About 120 mL diluent was added to the flask and sonicated to dissolved and made up the volume with diluents (Stock solution). Further 10 mL of this stock solution was transferred into 200 mL of volumetric flask and final volume was made up with dissolution medium to get final concentration of 21.75 µg/mL of BLS and 10.65 µg/mL of MTK (after potency correction, Bilastine: 99.06% and Montelukast: 99.23%). Filtered this stock solution using a 0.45 µm nylon syringe filter and used as a standard solution.

Preparation of Placebo Solution

About 200 mg standard placebo equivalent to one tablet (excluding active substance, the average weight of 1 tablet is 230 mg) was added into a 900 mL dissolution medium preheated at temperature 37.0 ± 0.5°C, and dissolution testing was performed per dissolution methodology. After 45 minutes, 10 mL aliquots drawn and filtered through a 0.45 µm nylon-syringe filter and used as a placebo sample solution.

Preparation of Sample Solution

Placed one intact tablet into each six-vessel containing 900 mL of dissolution medium preheated at temperature 37.0 ± 0.5°C, and dissolution testing was performed as per dissolution methodology. After 45 minutes, 10 mL aliquots drawn and filtered through 0.45 µm nylon syringe filter and used as a sample solution.

Result

System Suitability

A system suitability parameter was accomplished by injecting six replicate injections of standard solution, and chromatograms were recorded. The number of theoretical plates, tailing factor, resolution in the first injection, and %RSD of six injections were calculated, and obtained results were presented in Table 2.

Specificity

After establishing system suitability as per criteria, blank, common placebo, standard, and sample solutions were injecting in single, and chromatograms were recorded. The representative chromatograms of blank (Fig. 1) and placebo solution (Fig. 2) show no interference at the RT of BLS and MTK. Standard solution chromatographs (Fig. 3) were identical to sample solution chromatograms (Fig. 4) regarding RT.

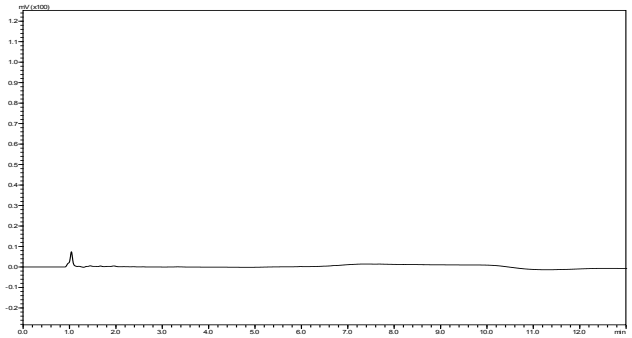

Fig. 1: Blank chromatograms

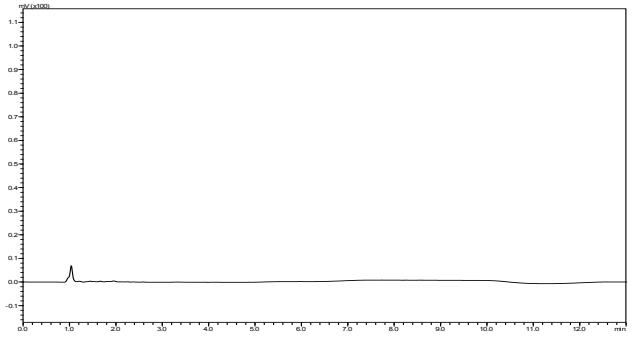

Fig. 2: Placebo chromatograms

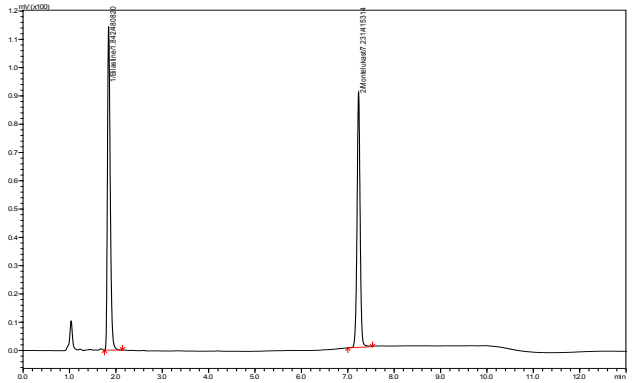

Fig. 3: Standard chromatogram of simultaneous montelukast and blastine

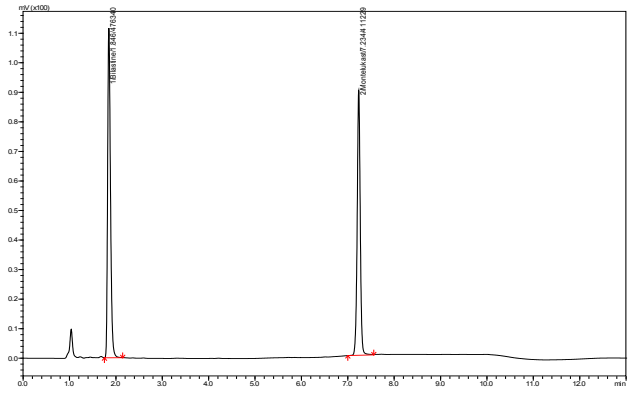

Fig. 4: Chromatogram of montelukast and blastine peak in sample

System Precision

Area count from six replicate injections of standard solution injected for system suitability check was used to calculate system precision and the %RSD. It was found to be 0.1, and 0.5% for BLS and MTK, respectively.

Method Precision

Six method precision (MP) sample solutions were prepared independently by placing one intact tablet in every six vessels of dissolution apparatus, and dissolution was performed as per dissolution methodology. After establishing system suitability, each six method precision sample solution was injected one by one, and chromatograms were recorded. The obtained results were presented in Table 3.

Intermediate Precision

Intermediate precision (IP) was carried on another HPLC system on a different day by the different analysts. Six sample solutions were prepared independently by placing one intact tablet in every six vessels of the dissolution apparatus, and dissolution was performed as per dissolution methodology. After establishing system suitability as per criteria, each six intermediate precision sample solutions was injected one by one, and chromatograms were recorded. The obtained results were presented in Table 3.

Ruggedness

A method can reproduce the finalized results in different conditions without unexpected differences in the results. Method precision and intermediate precision data were used to calculate ruggedness, and obtained results were expressed as Overall RSD (%) and presented in Table 3.

Linearity and Range

Calibration standards of various concentrations 20, 50, 80, 90, 100, and 120% were prepared from the standard stock solution and diluted with dissolution media to get the concentration of 4.351–26.104 µg/mL and 2.179–13.072 µg/mL of BLS and MTK respectively. All calibration curve standards were injected in a single after establishing system suitability as per criteria, and chromatograms were recorded, and obtained results show an excellent linear relationship between area vs. concentration (Figs. 5 and 6) for both BLS and MTK. The obtained slope, intercept, and correlation Coefficient was presented in Table 4.

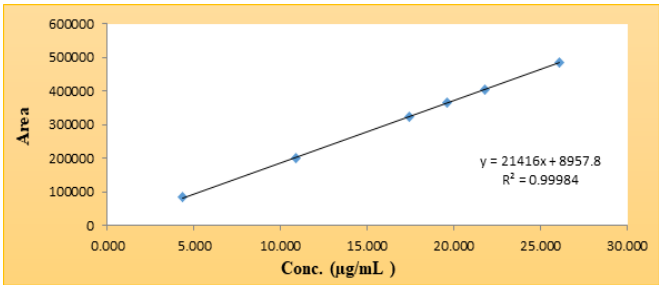

Fig. 5: Calibration curve of Bilastine

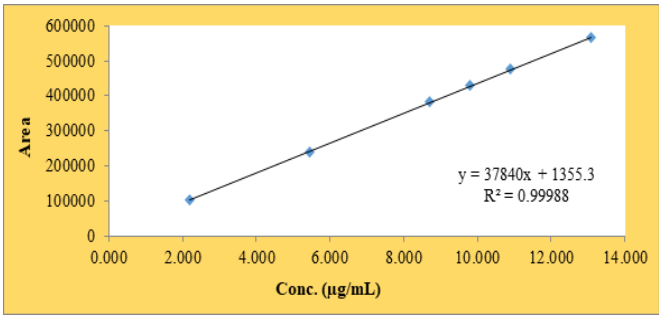

Fig. 6: Calibration curve of Montelukast

Table 2: Result of system suitability

System Suitability Parameters	*Bilastine*	*Montelukast*
Retention Time	1.842	7.231
Resolution	46.3	-
Tailing factor	1.4	1.0
Theoretical plate count	4307	57325
% RSD	0.1%	0.5%

Table 3: Result of method precision, intermediate precision and ruggedness

	Ruggedness of Bilastine		*Ruggedness of Montelukast*	
Sample MP/IP	*% Release MP*	*% Release IP*	*% Release MP*	*% Release IP*
1	98	99	103	99
2	98	98	104	98
3	98	98	99	98
4	97	97	95	96
5	97	97	95	95
6	98	98	95	96
Mean ± SD	98 ± 0.52	98 ± 0.75	99 ± 4.18	97 ± 1.55
%RSD	0.5	0.8	4.2	1.6
Overall mean ± SD	98 ± 0.62		98 ± 3.11	
Overall RSD (%)	0.6		3.2	

Table 4: Result of linearity, LoD and LoQ

			Bilastine		*Montelukast*	
% level of calibration standards (%)	*Stock vol. taken(mL)*	*Dilution (mL)*	*Concentration (µg/mL)*	*Area*	*Concentration (µg/mL)*	*Area*
Linearity 20	2.0	200	4.351	102124	2.129	82998
Linearity 50	5.0	200	10.877	239525	5.322	200211
Linearity 80	4.0	100	17.403	383857	8.516	324295
Linearity 90	4.5	100	19.578	430648	9.580	365892
Linearity 100	5.0	100	21.754	475192	10.645	403176
Linearity 120	6.0	100	26.104	565415	12.774	484435
Intercept			8957.8		1355.3	
Slope			21416		37840	
Regression coefficient (R^2)			0.99984		0.99988	
LoD (µg/mL)			0.37		0.16	
LoQ (µg/mL)			1.12		0.48	

Table 5: Result of accuracy (Recovery)

		% Recovery Bilastine			*% Recovery Montelukast*			
Recovery sample name	*BLS amount added (mg)*	*BLS amount recovered (mg)*	*% Recovery*	*Mean*	*MTK amount added (mg)*	*BLS amount recovered (mg)*	*% Recovery*	*Mean*
Rec.- 50% -1	10.20	9.95	97.5		5.17	5.20	100.6	
Rec.- 50% -2	9.78	9.68	99.1	98.1	5.20	5.20	100.1	100.2
Rec.- 50% -3	10.11	9.87	97.6		5.11	5.11	100.0	
Rec.- 100% -1	19.83	19.27	97.2		9.49	9.39	99.0	
Rec.- 100% -2	20.02	19.34	96.6	95.5	9.69	9.41	97.1	99.1
Rec.- 100% -3	21.25	19.70	92.7		9.95	10.07	101.2	
Rec.- 150% -1	29.97	29.30	97.8		14.90	14.79	99.2	
Rec.- 150% -2	30.82	29.75	96.5	97.0	14.75	14.69	99.7	99.8
Rec.- 150% -3	30.43	29.38	96.6		14.57	14.65	100.5	
		Mean±SD	96.8±1.752			Mean± SD	99.7±1.20	
		%RSD	1.8			%RSD	1.2	

Limit of Detection (LoD) and Quantitation (LoQ)

The limit of detection (LoD) and limit of quantitation (LoQ) were calculated using a signal-to-noise ratio, and the obtained results were presented in Table 4.

Accuracy (Recovery)

Accuracy sample solutions were prepared in triplicate at three levels (50, 100, and 150%) of the standard concentration. After establishing system suitability as per criteria, each accurate sample solution was injected once, and chromatograms were recorded. The obtained results of the accuracy study were presented in Table 5.

Stability in Analytical Solution

After establishing system suitability as per criteria, method precision sample solution injected initially was again injected at specific time intervals to monitor the % change in response with time. The representative chromatograms observed that only 0.4 and 0.1% difference in the concentration of BLS and MTK was observed up to 12 hours.

Robustness

The robustness of the analytical procedure was measured by doing some deliberate variations in method parameters, i.e., Flow rate ± 10%, Column Oven Temperature ±5°C, and Wavelength ±5 nm impart an indication of its reliance during normal usage, and obtained results were presented in Table 6A and Table 6B for BLS and MTK respectively.

Discussion

A new, simple, fast, specific, rugged, and reproducible HPLC method was developed and validated for simultaneous estimation of BLS and MTK in 0.5% w/v Sodium lauryl sulfate dissolution medium. The method was validated in line with ICH guidelines, and the % drug release of BLS and MTK were found within the specification limit (95–105%). Run time is relatively short (13 minutes) for

Table 6A: Result of Robustness of Bilastine

% Assay							
S. No.	*MP*[a]	*FP*[b]	*FM*[b]	*CTP*[b]	*CTM*[b b,*]	*WP*[b]	*WM*[b]
1	98	99	99	99	99	99	99
2	98	98	98	98	98	98	98
3	98	98	98	98	98	98	98
4	97						
5	97						
6	98						
Mean	98[#]	98*	98*	98*	98*	98*	98*
SD	0.5[#]	0.6*	0.6*	0.6*	0.6*	0.6*	0.6*
% RSD	0.5	0.6	0.6	0.6	0.6	0.6	0.6
Mean of All % RSD	0.6						

[a]=(n = 6),[b]=(n = 3),([#]=[a]), (*=[a+b]), MP: Method precision, FP: Flow plus, FM: Flow minus, CTM: Column temperature minus, CTP: Column temperature plus, WM: Wavelength minus, WP: Wavelength plus

Table 6B: Result of Robustness of Montelukast

% Assay							
S. No.	*MP*[a]	*FP*[b]	*FM*[b]	*CTP*[b]	*CTM*[b b,*]	*WP*[b]	*WM*[b]
1	103	98	98	98	98	98	98
2	104	98	98	97	97	97	97
3	99	97	97	97	97	97	97
4	95						
5	95						
6	95						
Mean	99[#]	98*	98*	98*	98*	98*	98*
SD	4.2[#]	3.3*	3.3*	3.4*	3.4*	3.4*	3.4*
% RSD	4.2	3.4	3.4	3.4	3.4	3.4	3.4
Mean of All % RSD	3.5						

[a]=(n = 6),[b]=(n = 3),([#]=[a]), (*=[a+b]),MP: Method precision, FP: Flow plus, FM: Flow minus, CTM: Column temperature minus, CTP: Column temperature plus, WM: Wavelength minus, WP: Wavelength plus

both drugs that help rapid quantification of %drug release in dissolution samples in routine analysis. Therefore, we can conclude that the proposed dissolution method and chromatographic method can be applied successfully to quantify BLS and MTK from quality control samples for dissolution testing.

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