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A New HPLC Stability Indicating Method and Validation for Simultaneous Quantitation of Bilastine and Montelukast Sodium in API and Marketed Formulation by QbD Approach

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A new HPLC stability indicating method was developed and optimized by using a statistical tool, design expert 13.0.1.0 (Stat-Ease) for simultaneous quantitation of bilastine and montelucast sodium in marketed tablet dosage form. Randomized response surface methodology with two factor central composite design was utilized for mobile phase optimization and the effect of the independent variables, flow rate and volume of organic phase on critical quality attributes, resolution and retention time was studied. The analytes were resolved on a inertsil C18 (150 × 4.6 mm) column with 5 µm particle size. Within the design space the optimum chromatographic condition chosen was 0.1% orthophosphoric acid with acetonitrile at 60:40 (%v/v) having a flow rate of 1 min/mL for 10 min. The retention time (R_t) for bilastine and montelucast sodium was 2.445 and 3.787 min, respectively. The method was completely validated as per the current ICH guidelines. Forced degradation was carried out in acidic, basic, photolytic, neutral, oxidation, thermal conditions to prove the stability indicating property of HPLC method. The method's applicability was studied by determining bilastine and montelucast sodium in the marketed tablet dosage form. This validated RP-HPLC stability indicating method can be suggested for routine quality control analysis in industries and research laboratories for the fixed dose marketed tablet formulation.

Keywords: Bilastine, Montelucast sodium, DoE, Response surface methodology, Quality by design.

INTRODUCTION

Seasonal allergic rhinoconjunctivitis, urticaria and asthma are caused by potent inflammatory mediators histamine and cysteinyl leukotrienes [1]. Allergic rhinitis is a symptomatic nasal illness characterized by allergen induced inflammation of the nasal membranes mediated by immunoglobulin E (IgE). Three nasal reactions occurred in allergy are nasal obstruction, sneezing and mucosal discharge [2]. Both asthma and allergic rhinitis are systemic inflammatory disorders and are frequently comorbidities in patients from all ages, ethnic group and countries are affected by allergic rhino conjunctivitis. It affects quality of life because of moderate to severe symptoms [3]. A combination therapy of bilastine (BIL) and montelucast (MNT) was reported to be effective [1].

Bilastine is a benzimidazole-piperidinyl derivative and chemically 2-[4-[2-[4-[1-(2-ethoxyethyl) benzimidazol-2-yl]-piperidin-1-yl]ethyl] phenyl]-2-methyl propionic acid. It is a highly selective, long acting H_1 receptor antagonist with rapid onset of action [4,5]. Montelucast sodium is a styryl quinoline derivative and the chemical name is 2-[1-[1(*R*)-[3-[2(*E*)-(7-chloroquinolin-2-yl)vinyl]phenyl]-3[2-(1-hydroxy-1-methyl-ethyl)phenyl]propylsulfanylmethyl]cyclopropyl] acetic acid sodium salt. Montelucast act by blocking cysteinyl leukotriene receptor [6]. Fixed dose combination of bilastine and montelucast sodium is recently approved in March 2020 by Central Drugs Standard Control Organization, India for use in the therapy of allergic rhinitis and asthma [7].

Several analytical methods have been reported for the analysis of bilastine individually *viz.,* chromatographic [8-13], UV-spectroscopic [14,15] and electroanalytical [16]. Similarly, for montelucast sodium chromatographic [17-22], UV spectroscopic [22-24], electrochemical methods [25-27] and bioanalytical assay [28] were reported individually or in combi-

nation with other drugs by UV-spectroscopic [29-32], chromatographic [31-34], densitometric [34], LC-MS/MS [35], spectrofluorimetric and potentiometric methods [36,37]. Recently one spectroscopic method [38] and two HPTLC methods [39,40] are also reported for the analysis of newly approved FDC combination of bilastine and montelucast sodium in tablet dosage form in the market. However, there was no reported HPLC methods for the simultaneous analysis of newly approved FDC combination of bilastine and montelucast sodium in tablet dosage form. So a simple, precise and economic HPLC stability indicating method development for simultaneous quantitation of bilastine and montelucast sodium in presence of their degradants is the aim of the present work. The developed method was validated according to current ICH guidelines and can be used to determine assay of bilastine and montelucast sodium in tablets.

EXPERIMENTAL

A WATERS HPLC 2695 System equipped with a quaternary pump, PDA detector (2996) and auto sampler integrated with Empower 2 Software was utilized to obtain and process chromatographic data.

The separation of the analytes was achieved on Inertsil C_{18} (150 × 4.6 mm, 5 µ) column. The mobile phase consisting of 0.1% orthophosphoric acid at 60% and acetonitrile at 40% ratio. The column was equilibrated with premixed mobile phase before injection. The column temperature was kept at 30 ºC and injection volume was 10.0 µL. The flow rate was maintained at 1mL/min.

Bilastine (BIL) and montelucast sodium (MNT) API was kind gift of Microlabs, Bangalore, India. Tablets were purchased from the local market, brand name Billargic M. Orthophosphoric acid (HPLC grade), acetonitrile (HPLC grade), milli-Q water, hydrogen peroxide, hydrochloric acid, sodium hydroxide were purchased from Merck (India) Ltd., Mumbai, India.

0.1% Orthophosphoric acid buffer preparation: Orthophosphoric acid solution (1 mL) was taken in 1 L volumetric flask, about 100 mL of milli-Q water was added and final volume make up to 1000 mL with milli-Q water.

Diluent preparation: Acetonitrile (500 mL) was transferred into a 1000 mL volumetric flask and 500 mL milli-Q water was added into the flask. The solution was sonicated for 15 min and then filtered.

Standard and sample solution preparation: Accurately weighed 10 mg BIL and 5 mg MNT working standards and transferred into a 50 mL clean dry volumetric flasks then 10 mL of diluent was added, sonicated for 10 min and final volume was made with diluent to get the concentrations of 200 µg/mL BIL and 100 µg/mL of MNT.

Twenty tablets (brand name Billargic M) were finely powdered with a mortar and pestle. A weight of the powder equivalent to 20 mg of BIL and 10 mg of MNT (label claim) was accurately weighed and transferred into a 100 mL volumetric flask, 50 mL of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by milli-Q filters (200 µg/mL BIL and 100 µg/mL of MNT). Filtered sample stock solution (1 mL) was transferred to 10 mL volumetric flask and made up with diluent to prepare 20 µg/mL BIL and 10 µg/mL of MNT.

Method validation: The proposed HPLC method for the simultaneous quantitation of BIL and MNT in existence of their impurities and excipients was validated according to ICH guidelines. Linearity, precision, accuracy, robustness, LOD, LOQ were confirmed to illustrate the method's intended purpose.

Calibration curve: From the standard stock solution 0.25, 0.5, 0.75, 1, 1.25, 1.5 mL each BIL and MNT was pipetted separately to 10 mL volumetric flasks to obtain 5, 10, 15, 20, 25, 30 µg/mL of BIL and 2.5, 5, 7.5, 10, 12.5, 15 µg/mL of MNT, respectively. The solutions were injected in the optimized chromatographic conditions previously mentioned keeping the injection volume constant in three replicates. For each drug plotted the calibration curve by taking average peak area on *y*-axis against concentration on *x*-axis and the regression coefficient were calculated.

Degradation studies: The degradation analysis was carried out to ensure that the method could distinguish BIL and MNT from the likely degradation products produced during the forced degradation experiment. Forced degradation is a process in which drug products and drug substances are degraded under conditions that are more severe than accelerated conditions, resulting in degradation products that may be analyzed to determine the molecule's stability [41]. The following degradation studies were performed and studied by utilising the proposed HPLC method.

Oxidation: To a 1 mL of stock solution of BIL and MNT 1 mL of 20% H_2O_2 was added separately. The solutions were kept for 30 min at 60 ºC. The resultant solution was diluted to obtain 20 ppm and 10 ppm of BIL and MNT respectively in the solution and 10 µL was injected into the system, recorded the chromatograms to assess the sample stability and also to determine if there is any interference exists between the drug and its related impurities.

Acid, base and neutral degradation: To a 1 mL of sample solution of BIL and MNT, 1 mL of 2 N HCl and 2 N NaOH were added separately and refluxed at 60 ºC for 30 min for acid and base forced degradation, respectively. Hydrolysis was studied by refluxing the drug for 6 h in water at temperature of 60 ºC. The resultant solution was diluted to obtain 20 ppm and 10 ppm of BIL and MNT respectively in the solution. This solution (10 µL) was injected into a HPLC system and recorded the chromatograms to assess the sample stability.

Dry heat and photodegradation: To perform the dry heat degradation study, sample solution was placed in hot air oven at 105 ºC for 6 h. Similarly, for photodegradation the sample solution was exposed to UV radiation for 7 days in UV chamber. Then the resultant solution was diluted to obtain 20 ppm and 10 ppm of BIL and MNT, respectively and 10 µL was injected into HPLC system and recorded the chromatograms to assess the sample stability.

RESULTS AND DISCUSSION

Method development and its optimization: From the literature review and preliminary experimental screening, it was observed that acetonitrile in the concentration range from

30-50% produce resolution of peaks. With lesser organic phase tailing was observed for both BIL and MNT peak. Different reagents such as potassium dihydrogen phosphate, orthophosphoric acid and acetic acid were tried as buffers having different composition to get the resolution and peak symmetry. Only orthophosphoric acid showed satisfactory response in terms of peak symmetry and resolution. By changing the buffer from orthophosphoric acid to potassium dihydrogen phosphate peak broadening for both BIL and MNT was observed. Hence, orthophosphoric acid was optimized as buffer. By decreasing orthophosphoric acid concentration in buffer solution from 0.1% while maintaining the other parameters constant the plate count in MNT was decreased and the peak broadening occurs. Hence 0.1% orthophosphoric acid was optimized as buffer composition. Similarly, by changing the flow rate in the range from 0.6 mL/min to 1.4 mL/min it was observed, less than 1 mL/min flow rate prolongs the retention time and more than 1 mL/min decrease the resolution. Similarly, the effect of temperature was observed on resolution of drugs. Acetonitrile concentration and flow rate was chosen as two independent factors for method optimization. Other factors like orthophosphoric acid concentration, temperature were kept constant. Different columns like Agilent C_{18} (150 × 4.6 mm, 5 µ), Krom C_{18} (150 × 4.6 mm, 5 µ), Symmetry C_{18} (150 × 4.6 mm, 5 µ), Zorbax C_{18} (150 × 4.6 mm, 5 µ) and Inertsil C_{18} (150 × 4.6 mm, 5 µ) columns were tried considering resolution, peak symmetry and selectivity, Finally Inertsil C18 (150 × 4.6 mm, 5 µ) column was chosen for method optimization. The flow rate was set at 1 mL/min and the temperature was kept constant at 30 ºC. The optimized chromatogram BIL and MNT is shown in Fig. 1.

Orthophosphoric acid (0.1%) along with acetonitrile at the ratio of 60:40 having flow rate 1 mL/min at 30 ºC column temperature was found most suitable for both BIL and MNT with a resolution of 6.6 between them, which shows there is no interference by the degradants and optimum retention time in presence of their degradation products. The outcomes of optimization process is shown in Table-1.

Application of quality by design (QbD) for method optimization: The present work discuss about the application

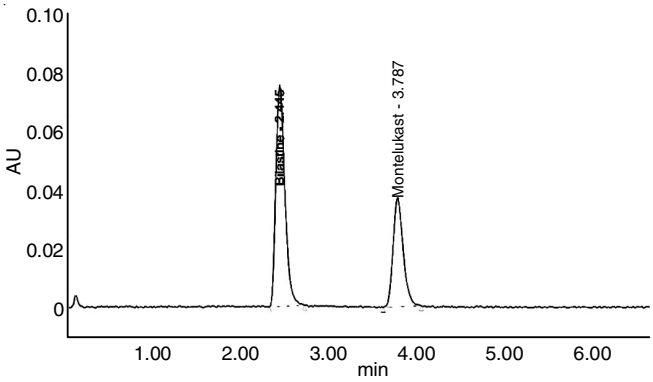

Fig. 1. Optimized chromatogram

TABLE-1
SYSTEM SUITABILITY PARAMETERS

Parameters	BIL	MNT	Ref. [42]
Retention time	2.445	3.787	–
Tailing factor (T)	1.29	1.24	< 2
No. of theoretical plates (N)	2684	5035	> 2000
Resolution (Rs)	6.6	6.6	> 1.5

of statistical tools to design a particular and reliable analytical HPLC method for the determination of BIL and MNT in presence of their potential degradation products. Design-Expert 13.0.1.0 (Stat-Ease) was used as a statistical tool for obtaining statistical calculations, model generation, P value, F value, perturbation curve, contour plot, surface plot, *etc*. so that optimum combined effect of independent variables such as flow rate and organic phase concentration (v/v) on responses like retention time and resolution of BIL and MNT can be determined.

For optimization of mobile phase randomized response surface methodology with two factor central composite design was applied. Two independent critical factors were selected from previous experience, which are having significant effect on resolution of peaks. A total of 13 experimental runs were performed and analyzed for two responses *i.e.* retention time and resolution for optimization of the proposed method (Table-2). Multiple regression analysis was performed on the design matrix and the obtained responses. The independent variables and responses were then correlated using the second order

TABLE-2
LAY OUT OF DESIGN SUMMARY

Std	Run	Factor 1	Factor 2	Response 1	Response 2	Response 3
		A: Flow rate (mL/min)	B: Organic phase (%V/V)	Retention time BIL (min)	Retention time MNT (min)	Resolution
13	1	1.0	40	2.487	3.798	6.602
6	2	1.4	40	2.454	4.244	8.601
12	3	1.0	40	2.467	3.986	6.876
1	4	0.6	30	4.309	18.765	23.015
5	5	0.6	40	4.678	16.021	15.102
8	6	1.0	50	2.554	7.456	13.052
3	7	0.6	50	3.116	8.277	12.132
7	8	1.0	30	1.767	4.789	5.098
4	9	1.4	50	1.897	6.897	14.024
9	10	1.0	40	2.502	3.673	6.698
10	11	1.0	40	1.876	5.998	9.765
2	12	1.4	30	1.234	1.098	1.903
11	13	1.0	40	2.376	3.687	6.687

polynomial function. The two independent variables taken were flow rate and volume of organic phase modifier (acetonitrile). The responses were retention time of BIL, retention time of MNT and resolution. The retention time for BIL varied from 1.234 to 4.678 and for MNT it ranged from 1.098 to 18.765. Similarly, the resolution ranged from 1.903 to 23.015. The chromatographic condition was optimized and utilized to check drug degradation in all forced degradation conditions.

The significance of the experimental quadratic models were tested using ANOVA, the results of which are reported on Table-3. The F values for BIL, MNT and resolution models are found to be 13.74, 18.94 and 15.92, respectively which are significant. P values for BIL, MNT and resolution models are 0.0017, 0.0006 and 0.0011, respectively. The P values for all the six models are less than 0.05 which are desirable. Lack-of-fit tests are used to determine how well each model fits the data. All the three models exhibits not significant lack-of-fit. From the corresponding F and P values all the other terms are also significant. The signal-to-noise ratio which should be greater than 4, can be used to determine adequate precision [43]. In present study, high adequate precision values were obtained indicating a good signal. For each model the polynomial equation in coded form are summarized as follows:

$$R_t \text{ of BIL} = +2.44 - 1.09 \times A + 0.0428 \times B + 0.4640 \times AB + 0.8643 \times A^2 - 0.5412 \times B^2$$

$$R_t \text{ of MNT} = +4.68 - 5.14 \times A - 0.3370 \times B + 4.07 \times AB + 4.33 \times A^2 + 0.3168 \times B^2$$

$$R_s = +7.44 - 4.29 \times A + 1.53 \times B + 5.75 \times AB + 4.12 \times A^2 + 1.35 \times B^2$$

where A is the flow rate, B is organic phase (%v/v acetonitrile). Both A and B represent the main effect terms whereas AB represent their interaction effect. The effect of critical quality attributes (CQA) on response variables was graphically assessed (Fig. 2). Based on the results from design of experiments, 1 mL/min flow rate and 40% v/v of acetonitrile were selected. The proposed method was validated according to ICH guidelines in terms of linearity, specificity, precision, accuracy, LOD, LOQ, robustness, *etc.*

Counter and surface plots are examples of response surface plots that can be used to generate desired response values and operating conditions. The researcher can use both contour and surface plots to better understand the nature of relationship between the two variables (flow rate and mobile phase concentration) and the response (retention time and resolution).The response surface is viewed as a two-dimensional plane in a contour plot with all points with the same response joined to form counter lines of constant responses. A surface plot shows a three-dimensional picture of the response which can help to see it more clearly [44].

Fig. 2 represents the perturbation curves in which the effect of flow rate and organic phase concentration (independent variables) was assessed on the retention time and resolution of the drugs (response). From Fig. 2a-b, it was evident that by decreasing flow rate resolution decreases whereas by increasing organic mobile phase concentration the resolution between

TABLE-3
ANOVA FOR QUADRATIC MODEL

Response	Source	Sum of squares	df	Mean square	F value	P value
Response 1 R_t (BIL)	Model	10.16	5	2.03	13.74	0.0017 significant
	A-Flow rate	7.08	1	7.08	47.88	0.0002
	B-Organic phase	0.0110	1	0.0110	0.0744	0.7929
	AB	0.8612	1	0.8612	5.82	0.0466
	A^2	2.06	1	2.06	13.95	0.0073
	B^2	0.8089	1	0.8089	5.47	0.0519
	Residual	1.07	7	0.1479		
	Lack of Fit	0.7546	3	0.2515	3.59	0.1246 not significant
	Pure Error	0.2806	4	0.0701		
	Cor Total	11.20	12			
Response 2 R_t (MNT)	Model	289.53	5	57.91	18.94	0.0006 significant
	A-Flow rate	158.35	1	158.35	51.79	0.0002
	B-Organic phase	0.6814	1	0.6814	0.2229	0.6512
	AB	66.32	1	66.32	21.69	0.0023
	A^2	51.71	1	51.71	16.91	0.0045
	B^2	0.2771	1	0.2771	0.0906	0.7721
	Residual	21.40	7	3.06		
	Lack of Fit	17.43	3	5.81	5.84	0.0606 not significant
	Pure Error	3.98	4	0.9943		
	Cor Total	310.94	12			
Response 3 resolution	Model	331.13	5	66.23	15.92	0.0011 significant
	A-Flow rate	110.26	1	110.26	26.51	0.0013
	B-Organic phase	14.08	1	14.08	3.39	0.1083
	AB	132.30	1	132.30	31.81	0.0008
	A^2	46.97	1	46.97	11.29	0.0121
	B^2	5.02	1	5.02	1.21	0.3085
	Residual	29.11	7	4.16		
	Lack of Fit	21.63	3	7.21	3.86	0.1126 not significant
	Pure Error	7.48	4	1.87		
	Cor Total	360.25	12			

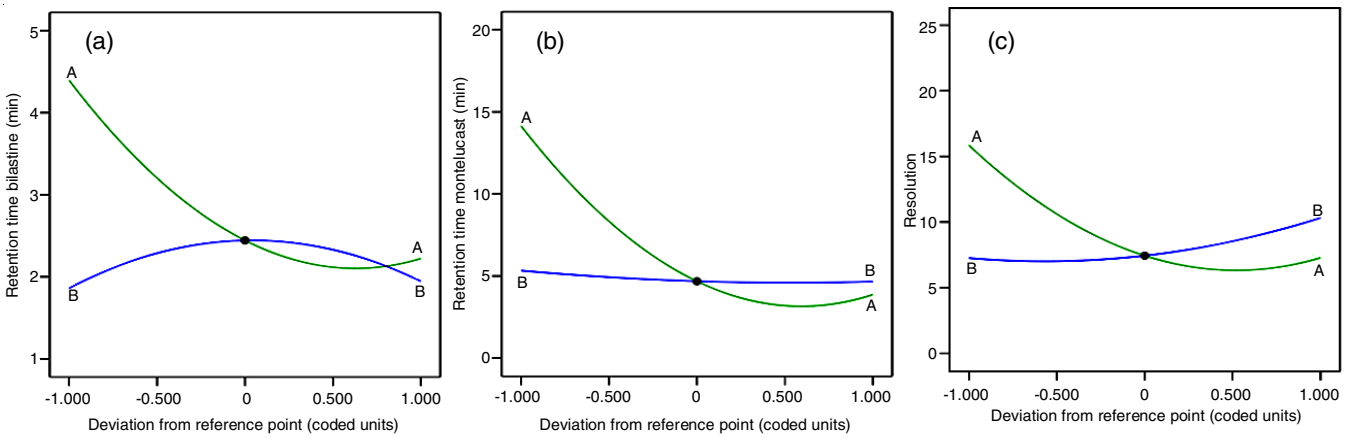

Fig. 2. Perturbation plots (a) R_t bilastine, (b) R_t montelucast, (c) resolution (R_s), where A is flow rate and B is volume of acetonitrile (v/v)

BIL and MNT increases (Fig. 2c). The concentration of acetonitrile has the most prominent effect on resolution (Fig. 2c) followed by retention time of MNT (Fig. 2b) and BIL (Fig. 2a). Counter plots and surface plots for RT and resolution of BIL and MNT are illustrated in Figs. 3 and 4, respectively. The concentration of organic phase was plotted against flow rate and both the counter and surface plots were analyzed and found that by increasing flow rate retention time of both drugs decreases. Flow rate effect will be more on R_t of BIL and MNT than resolution. Factor B (concentration of acetonitrile) had a more prominent effect than factor A (flow rate) on resolution.

Method validation

Linearity and range: A working standard solution (10 µL) from each concentration was injected into the chromatograph three times and under previously mentioned chromatographic conditions. Then from the chromatograms plotted the calibration curve by taking the average peak area on Y-axis

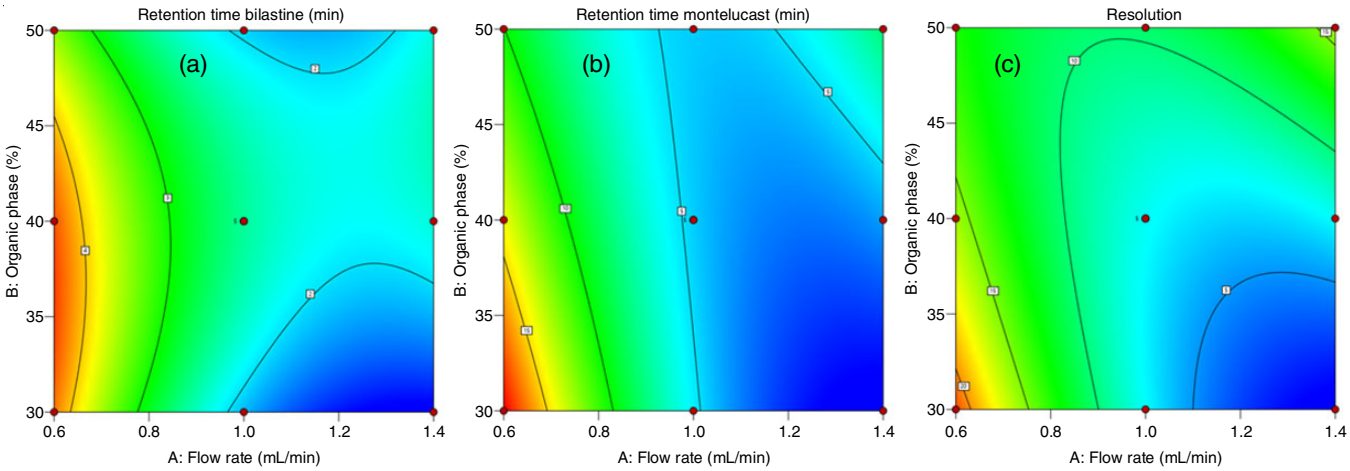

Fig. 3. Contour plots of (a) R_t BIL, (b) R_t MNT and (c) resolution(R_s), (d) 3D response

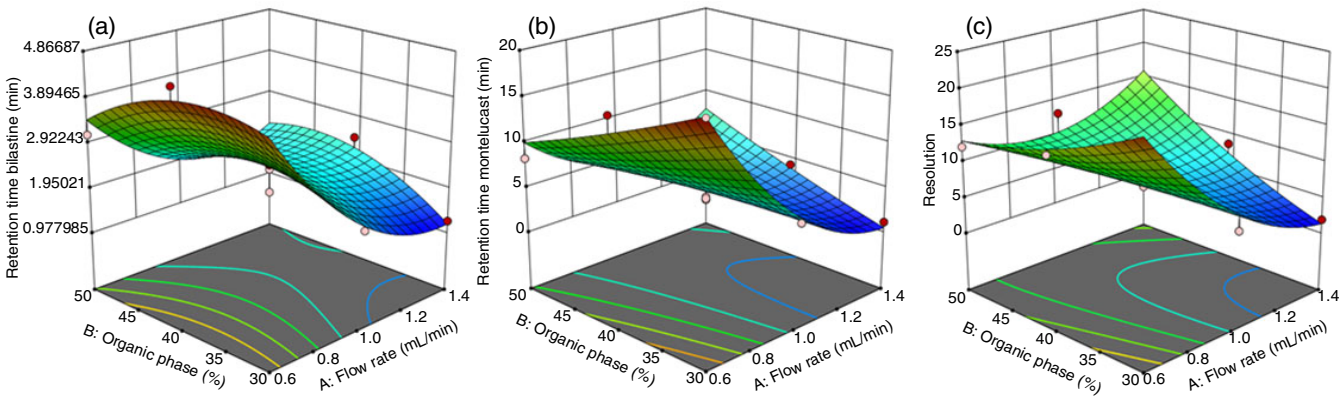

Fig. 4. Surface plots of (a) R_t BIL, (b) R_t MNT, (c) resolution

and concentration on X-axis and the results obtained are given on Table-4.

TABLE-4
LINEARITY, LOD AND LOQ

Parameters	BIL	MNT
Linearity level (%)	25-150	25-150
Linearity conc. (µg/mL)	5-30	2.5-15
Linearity coefficient (R^2 ± SD)	0.9997	0.9996
Linearity equation	y = 24768x + 3870	y = 25447x + 3401.1
LOD (µg/mL)	0.31	0.14
LOQ (µg/mL)	0.94	0.41

LOD and LOQ: The signal to noise technique was used to estimate the limit of detection (LOD) and limit of quantitation of BIL and MNT, as described by the ICH recommendations [45]. Each drug was injected into the chromatograph in increasingly dilute solutions and the signal-to-noise (S/N) ratio was computed at each concentration using equation a and b and the results are given in Table-1.

$$LOD = \frac{3.3\sigma}{S} \quad (a)$$

$$LOQ = \frac{10\sigma}{S} \quad (b)$$

where σ is the standard deviation of *y*-intercept of regression lines and S is the slope of the calibration curve.

Precision: 100% linearity level *i.e.* 20 µg/mL of BIL and 10 µg/mL of MNT solution was injected six times on the same day but different timing on the chromatogram to determine repeatability of the method and the results obtained were analyzed. Repeatability of the method was accomplished from RSD% values of the assays performed for intraday precision. The intermediate (inter-day) precision of the method was checked by performing same procedure on different days under the same experimental conditions. The results of precision experiment is summarized on Table-5.

Robustness: The robustness of method was determined by doing small intended small changes in chromatographic parameters like percent of organic solvent, flow rate, temperature. It was observed that the proposed HPLC method was unaffected by deliberate changes in experimental parameters and so the method shows robustness. Results of robustness of the proposed method is summarized on Table-6.

Specificity: The specificity of the method was found to be in accordance with ICH guidelines [46]. The injection of a placebo solution (extracted) demonstrated specificity (Fig. 5). In presence of excipients and degradants resolution of both peaks and RT are not affected (Fig. 6).

Accuracy: Accuracy of the proposed method was tested by applying it to a drug sample (BIL and MNT combination tablet) to which a known quantity of BIL and MNT standard

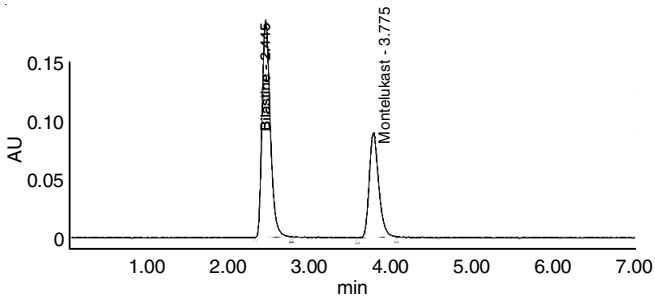

Fig. 5. Chromatogram of the formulation (BIL and MNT having R_t 2.445 min and 3.775 min respectively)

TABLE-5
PRECISION STUDIES

Drugs	Concentration (µg/mL)	Repeatability (n = 6)		Intermediate precision (n = 6)	
		% Assay found ± SD	RSD (%)	% Assay found ± SD	RSD (%)
BIL	20 ppm	100.36 ± 0.62	0.6	99.13 ± 1.25	1.26
MNT	10 ppm	100.97 ± 0.43	0.42	99.33 ± 0.85	0.86

TABLE-6
ROBUSTNESS OF THE METHOD

Factor	Level	Retention time		Asymmetry	
		BIL	MNT	BIL	MNT
% of ACN in the mobile phase					
39%	-1	2.46	3.80	1.27	1.17
40%	0	2.44	3.78	1.28	1.24
41%	+1	2.46	3.81	1.25	1.23
Mean ± SD (n = 3)		2.45 ± 0.011	3.79 ± 0.015	2.45 ± 0.011	1.26 ± 0.015
Flow rate (mL/min)					
0.9	-1	2.47	3.81	1.27	1.17
1.0	0	2.45	3.78	1.29	1.24
1.1	+1	2.43	3.77	1.26	1.20
Mean ± SD (n = 3)		2.45 ± 0.02	3.78 ± 0.02	1.27 ± 0.015	1.20 ± 0.035
Temperature					
29	-1	2.48	3.78	1.25	1.25
30	0	2.44	3.77	1.26	1.22
31	+1	2.47	3.76	1.23	1.18
Mean ± SD (n = 3)		2.46 ± 0.02	3.77 ± 0.01	1.25 ± 0.015	1.22 ± 0.035

TABLE-7
ACCURACY OF THE HPLC METHOD

Drugs	Label claim	Amount added (ppm	Standard (ppm)	Amount recovered ± SD	Percent recovery ± SD	% RSD
BIL	20 mg	10 (50%)	20	10.0 ± 0.115	99.79 ± 1.311	1.31
		20 (100%)	20	20 ± 0.2	100.00 ± 1.110	1.11
		25(150%)	20	25.03 ± 0.635	100.81 ± 1.242	1.23
MNT	10 mg	5 (50%)	10	4.97 ± 0.075	99.5 ± 1.52	1.53
		10 (100%)	10	9.88 ± 0.080	98.88 ± 0.79	0.80
		15 (150%)	10	15.08 ± 0.090	100.57 ± 0.55	0.55

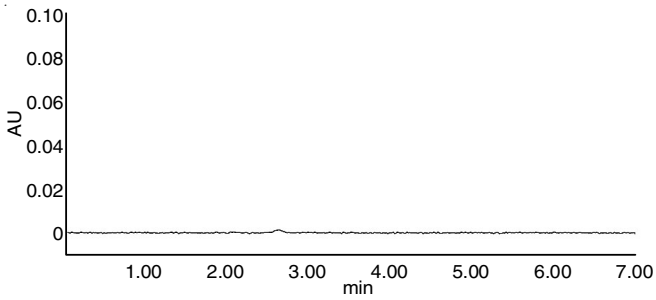

Fig. 6. Placebo chromatogram

drug corresponding to 50, 100 and 150% of label claim had been added (standard addition method). The method's accuracy was determined by calculating within the linearity range, the mean % recovery of triplicate determination of each drug at three distinct concentrations. The mean percent recovery of each drug was determined to be within compendial tolerance (98-102%) as shown in Table-7.

Application of assay method to marketed formulation: By applying the presented method, assay of BIL and MNT in their marketed formulation (tablet brand name Billargic M) were performed and the result (Table-8) was accounted to be in good accordance with the label claim (20 mg BIL and 10 mg MNT).

TABLE-8
ASSAY OF MARKETED TABLET FORMULATION

Drug	Label claim	Assay result (% ± SD) (n = 6)	% RSD
BIL	20 mg	100.36 ± 0.62	0.60
MNT	10 mg	100.97 ± 0.43	0.42

Forced degradation study: In forced degradation studies the drug sample was deliberately degraded to determine the method specificity. It also helps in determining the degradation pathway which in turn helps in wise formulation development in various dosage form of the drug.

Both bilastine and montelucast shown to be degraded under all degradation conditions; acidic (Fig. 7), alkaline (Fig. 8), neutral, oxidation (Fig. 9), thermal (Fig. 10), photolytic (Fig. 11). However prominent degradant peak of BIL and MNT was observed under oxidation condition. 4.33% and 4.44% degradation was observed for BIL and MNT, respectively. In acidic conditions, 6.19% and 6.27% degradation was observed for BIL and MNT, respectively. In alkaline condition, BIL undergo degradation of 5% and MNT undergo degradation of 4.96%. Though significant degradation under alkaline condition but

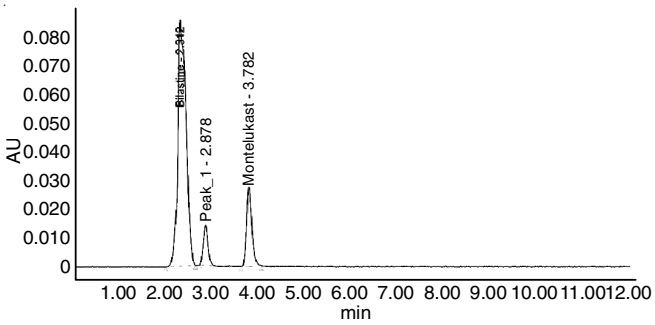

Fig. 7. Acidic treated chromatogram

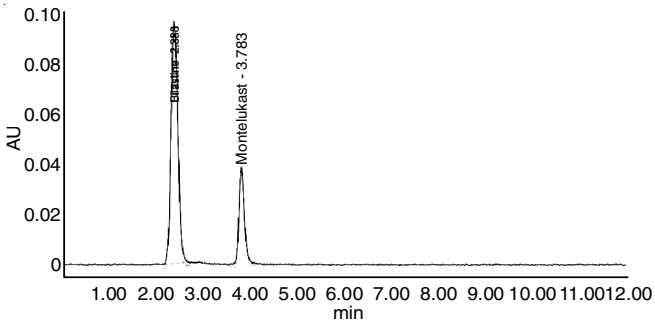

Fig. 8. Alkali treated chromatogram

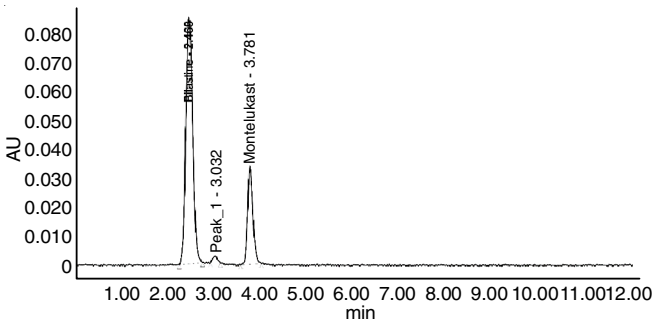

Fig. 9. Peroxide treated chromatogram

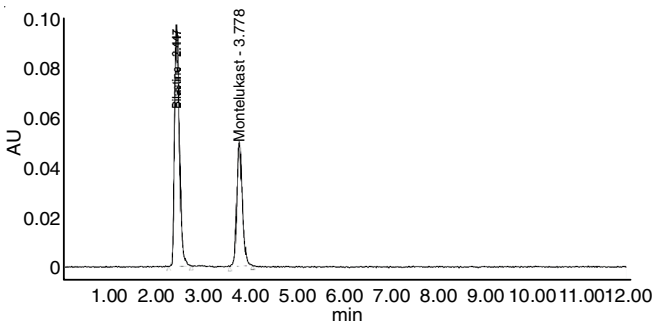

Fig. 10. Thermal treated chromatogram

TABLE-9
RESULTS OF FORCED DEGRADATION STUDIES OF BILASTINE (BIL) AND MONTELUCAST (MNT)

Degradation type	Degradation condition	Recovery (%)		Degradation (%)	
		BIL	Montelucast	BIL	Montelucast
Oxidative degradation	1 mL 20% H_2O_2, 30 min, 60 °C	95.67	95.56	4.33	4.44
Acid degradation	1 mL 2 N HCl, 30 min, 60 °C	93.81	93.73	6.19	6.27
Alkaline degradation	1 mL 2 N NaOH, 30 min, 60 °C	95.00	95.04	5.00	4.96
Dry heat degradation	105 °C, 6 h	96.96	97.08	3.04	2.92
Photodegradation	In UV chamber, 7 days	98.08	98.09	1.92	1.91
Neutral degradation	6 h, 60 °C	98.94	99.29	1.06	0.71

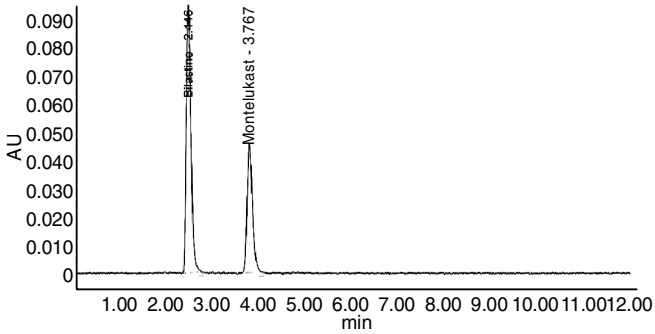

Fig. 11. UV radiation treated chromatogram

the degradant peak was not observed in the chromatogram. It can be attributed to inability of the degradant product to absorb UV light. Nearly 3% degradation for both the drugs occurred under thermal degradation. Similarly, under photo and neutral forced degradation 1% to 2% degradation occurred for both the drugs. The results of forced degradation is summarized in Table-9.

Conclusion

A stability indicating isocratic RP-HPLC method was developed, by applying quality by design (QbD) approach, The method resolves all the possible degradant peaks from the peaks of active constituents. The proposed method was optimized and validated according to ICH guidelines and suggested for the routine quality control analysis of bilastine and montelucast sodium in a fixed dose tablet formulation. The method shows chemical stability of bilastine and montelucast sodium. The proposed method is found to be economic, simple, precise, rapid, accurate and specific. The mobile phase is simple to prepare and shows good resolution of drugs in isocratic mode. The method shows broad linearity range and is specific and suitable for assay of bilastine and montelucast sodium in presence of their degradants and excipients used for their formulation in a tablet dosage form.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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