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RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF RP- HPLC SIMULTANEOUS ESTIMATION METHOD OF MONTELUKAST AND BAMBUTEROL FOR STABILITY INDICATING ASSAY

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ABSTRACT

The objective of the current study was to develop and validate a simple, accurate, precise and selective stability-indicating gradient reverse phase high performance liquid chromatographic method for simultaneous estimation of Montelukast (MTK) and Bambuterol (BBL) in pharmaceutical formulation in presence of degradation products. The chromatographic separation of MTK and BBL was achieved on Shimadzu LC-20AT series HPLC having C_{18}-ODS bonded column (250 ×4.6 mm, 40 °C, 10 µL) using UV/Visible detector at 231 nm. The optimized mobile phase was consisted of a acetonitrile (100%) at a flow rate of 1.5 mL/min. The retention times were 4.65 and 2.69 min for bambuterol and montelukast respectively. The proposed method provided linear responses within the concentration ranges 0-50µg/mL for bambuterol and montelukast. The limit of detection (LOD) and limit of quantification (LOQ) values were found to be 0.0259, 0.078 µg/mL and 0.0206, 0.062 µg/mL for bambuterol and montelukast, respectively. The developed method was validated as per ICH guidelines with respect to specificity, linearity, accuracy, precision, robustness and ruggedness. The studies data revealed that developed method was convenient, fairly reliable, sensitive, less expensive and reproducible.

Keywords: RP-HPLC, Validation, Stability-indicating assay, Montelukast, Bambuterol, Tablet.

INTRODUCTION:

Montelukast is chemically known as 2-[1-[[(1*R*)-1-[3-[2-(7-chloroquinolin-2-yl)e phenyl]-3-[2-(2-hydroxypropane-2-yl)phenyl] propyl] sulfanylmethyl] cyclopropyl acetic acid belongs to category of leukotriene antagonist, used in the treatment of asthma[1–4]. Montelukast sodium is an orally active compound that binds with high affinity and selectivity to the CysLT1 receptor. Montelukast inhibits physiologic actions of LTD 4 at the CysLT1 receptor without any agonist activity. Various analytical methods, like liquid chromatography with fluorescence detection[5-7], simultaneous HPLC and derivative spectroscopic method with loratadine[8], stability indicating HPLC method for MTK in tablets and human plasma[9], stereoselective HPLC for MTK and its S-enantiomer [10] have been already reported.

Bambuterol is chemically known as 5-(2-tert-butylamino-1-hydroxyethyl)-m-phenylene bis (dimethylcarbamate) hydrochloride is a direct acting sympathomimetic with predominantly active precursor of the Selective beta-2 adrenergic agonist[2]. Bambuterol is an active precursor of the selective beta2-adrenergic agonist, terbutaline. It is an ester prodrug of terbutaline which is an β_2 adrenergic agonist[3]. It is the first once daily oral beta 2 agonist with 24-hour duration for the treatment of asthma. Following slow absorption from the GI-tract, the drug is metabolized via hydrolysis (plasma cholinesterase) and gets converted into its active metabolite terbutaline. Bambuterol is the bis-dimethylcarbamate of terbutaline.

The objective of the present investigation was to developed a simple, specific, precise and rapid RP-HPLC method for simultaneous estimation of Montelukast and Bambuterol in same dosage form. The developed method is further validated in terms of specificity, accuracy, precision and reproducibility.

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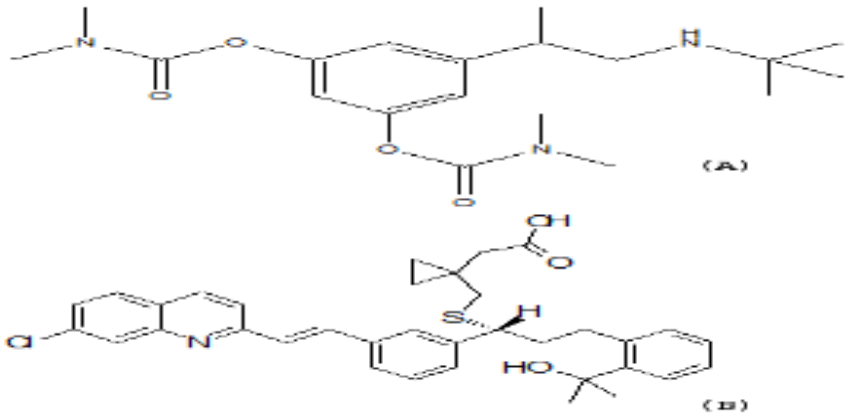

Figure 1: Chemical structure of (A) Bambuterol (B) Montelukast.

MATERIAL:

Montelukast and Bambuterol was generously supplied as a gift samples by Cipla Pharmaceuticals Ltd., Mumbaiand was confirmed by the official methods. Acetonitrile, water and all other chemicals were purchased from Himedia Labs, Mumbai. All other chemicals of HPLC and analytical grades were used without any further purification.

METHOD:

Instrumentation:

The HPLC system consisted of a model LC-20AT (Prominence Shimadzu, Japan) was used having UV (SPD-20A) Prominence UV/Visible detector. Column Phenomenex Gemini C18-ODS bonded column of length 250 mm and an inter diameter 4.6 mm was selected for analysis. The particle size of the stationary phase was 5µm. The Mobile phase was a degassed and filtered (0.45 µm, Millipore, Watford, UK) Acetonitrile 100% Flow rate 1.5 mL/min. Frontline Ultra Sonic Cleaner Fs-10 Sonicator was used for sonication The column temperature was maintained at 40°C using a model 7716 HPLC column block heater CTO-20AC.

Standard solution preparation:

Acetonitrile was used as solvent. The stock solutions of montelukast (1000 µg/mL) and bambuterol (1000 µg/mL) were prepared by dissolving in acetonitrile separately. Further aliquots were prepared from standard stock solution separately. Mixed standard solution with concentration of 100 µg/mL of montelukast and bambuterol were prepared in acetonitrile by proper dilution of pre analysed stock solution of both drug. The solution was found to be stable for at least 3 days.

Sample solution preparation:

Tablet powder equivalent to 100 mg of montelukast and bambuterol was transferred into 100 mL volumetric flask, added 35 mL of diluent and ultrasonicated for 20 min by using UV/Visible detector. Furthermore dilutions make for to give a solution 10 µg/mL of montelukast and bambuterol with mobile phase and then ultrasonicated for 5 min. The solution was filtered through Whatman filter paper No. 41 (Himedia, India). The 10 µL sample solution was injected at flow rate of 1.5 ml/min in C18-ODS column (250 ×4.6 mm) at 40 °C. The chromatogram was obtained and the peak areas were recorded is shown in Table 1.

Table 1: Analysis of commercial formulation.

Drug	Label claim (mg/tablet)	Label claim found (mg)	% Label claim estimated	%RSD (n=6)
Bambuterol	10	9.9731	100.269	0.173
Montelukast	10	9.9775	100.225	0.312

ACCELERATED STABILITY STUDIES:

Accelarated decomposition studies were performed at 20 µg/mL concentration of montelukast and bambuterol on tablets to provide an indication of the stability-indicating assay and specificity of the proposed method. Peak purity test was carried out for the montelukast and bambuterol peaks by using UV/Visible detector on stress samples. All the solutions used in accelerated stability studies were prepared by dissolving the tablet powder in small volume of stressing agents. After decomposition, these solutions were diluted with acetonitrile to yield stated montelukast and bambuterol of about 20 µg/mL.

Conditions employed for performing the stress studies were as follows [11-13].

Base Degradation:

Tablet powder equivalent to 20 mg of montelukast and bambuterol was accurately weighed and dissolved in 20 mL of diluent, added 5 mL 0.05 N NaOH and the mixture was kept at 70°C for 1 hr. The solution was brought to ambient temperature, neutralized by addition of 5 mL 0.1 N HCl and diluted to 100 mL with diluent. 5 mL of this solution was diluted to 50 mL with diluent.

Acid Degradation:

Tablet powder equivalent to 20 mg of montelukast and bambuterol was accurately weighed and dissolved in 20 mL of diluent, added 5 mL 0.1 N HCl and the mixture was kept at 70°C for 1 hr. The solution was brought to ambient temperature, neutralized by addition of 5 mL 0.05 N NaOH and diluted to 100 mL with diluent. 5 mL of this solution was diluted to 50 mL with diluent.

Oxidation Degradation:

Tablet powder equivalent to 20 mg of montelukast and bambuterol was accurately weighed and dissolved in 20 mL of diluent, added 5 mL of 1% hydrogen peroxide. The mixture was kept at room temperature for 1 hr and diluted to 100 mL with diluent. 5 mL of this solution was diluted to 50 mL with diluent.

Hydrolytic Degradation:

Tablet powder equivalent to 20 mg of montelukast and bambuterol was accurately weighed and dissolved in 15 mL of diluent, added 15 mL of water and the mixture was kept at 70°C for 24 hr. The solution was brought to ambient temperature and diluted to 100 mL with diluent. 5 mL of this solution was diluted to 50 mL with diluent.

Thermal Degradation:

Tablet powder equivalent to 20 mg of montelukast and bambuterol was stored at 100°C for 12 hr, dissolved and diluted to 100 mL with diluent. 5 mL of this solution was diluted to 50 mL with diluent.

RESULT AND DISCUSSION:

Method Development and Optimization:

The two component formulation have gained a lot of advantages as there is greater patient acceptability, increased potency and decreased side effect. A calibration curve was prepared for the MTK and BBL in the range of 5-30 and 2-12 µg/mL to allow an assessment of the assay and the plot equation was used for quantization. On the optimization of gradient program, montelukast and bambuterol peaks were well resolved form degradation products. Based on these experiments, the final optimized conditions are described below.

The objective of the chromatographic method was to separate and quantitate MTK and BBL in presence of degradation products. And isocratic method was employed using acetonitrile as mobile phase. Column Phenomenex Gemini C18 –ODS bonded column (250 x 4.6 mm) the particle size of the stationary phase was 5 µ with flow rate of 1.5 mL/min on UV (SPD-20A) Prominence UV/Visible detector. The column temperature was maintained at 40°C and detection wavelength was selected by overlain spectra at 231 nm (Figure **2**). The typical retention time of MTK and BBL was found to be 4.658 and 2.692 min, respectively (Figure **3**). The regression and intercept values, the determination coefficient (R^2) and the %RSD were also calculated.

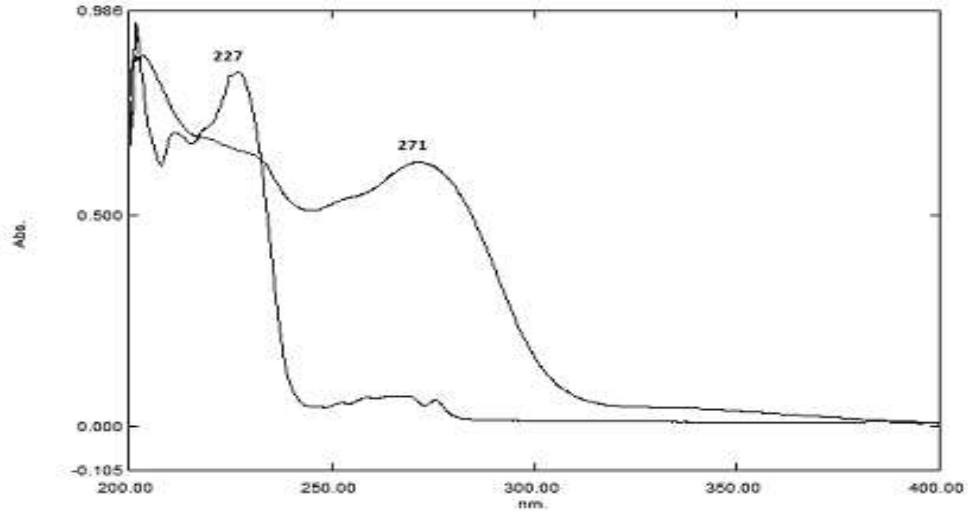

Figure 2: Overlain spectra of MTK and BBL.

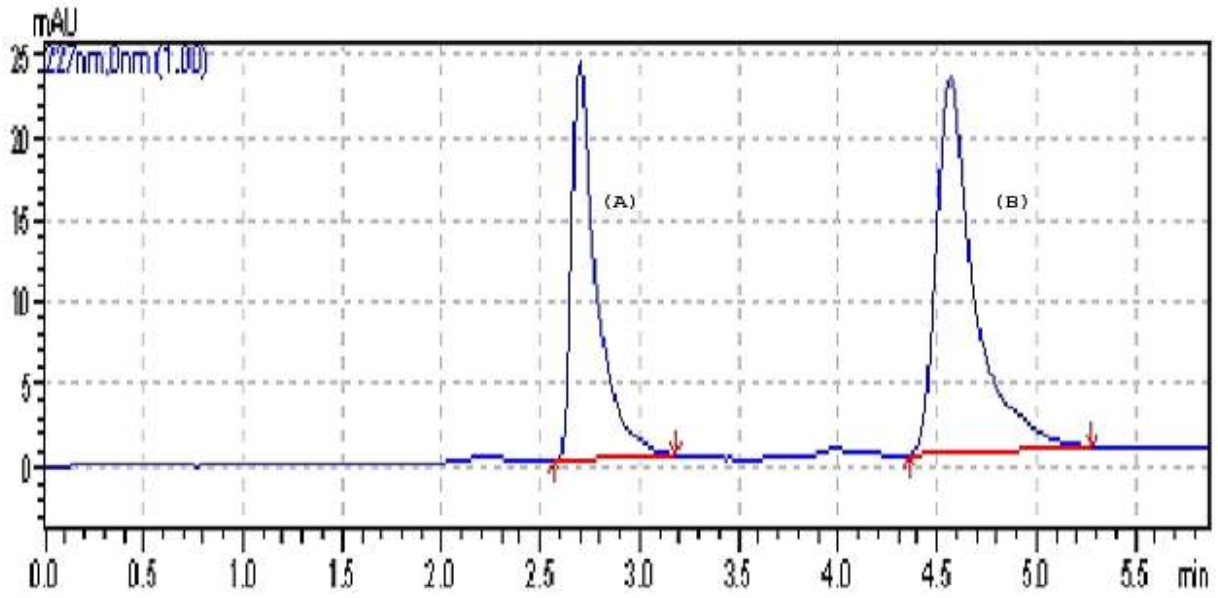

Figure 3: Preliminary chromatograms of BBL and MTK solution prepared in acetonitrile, as the mobile phase (1.5 ml/min, C18 –ODS column (250 ×4.6 mm, 40 °C, 10 µL).

STABILITY STUDY:

The purpose of the accelerated stability studies was to ensure the peak purity of the MTK and BBL in the presence of degradation products and established the stability indicating ability of the method under basic, acidic, peroxide and thermal degradation conditions. Bambuterol was found stable but montalukast degrades significantly. MTK and BBL were found stable under thermal degradation and degraded slightly under hydrolytic degradation. Peak purity test was carried out for the MTK and BBL peak by using UV/Visible detector in stress samples analysis. The purity threshold was within the limit in all of the stressed samples demonstrating the homogeneity of the peaks. The purity of both drug was unaffected by the presence of degradation products and thus confirms the stability-indicating power of the developed method. A summary data of accelerated stability studies is shown in Table 2.

Table 2: Summary of Forced Degradation Results

Stress condition	Montelukast		Bambuterol	
	% Degradation	Purity threshold	% Degradation	Purity threshold
Base hydrolysis 0.05 N NaOH at 70°C, 1 h	11.4	0.268	2.4	0.099
Acid hydrolysis 0.1 N HCl at 70°C, 1 h	19.6	0.213	1.4	0.091
Oxidation 1% H2O2 at RT, 1 h	20.9	0.211	1.4	0.064
Hydrolytic Water at 70°C , 1 h	1.8	0.214	4.9	0.090
Thermal 100°C, 12 h	0.6	0.210	0.9	0.031

VALIDATION OF THE METHOD:

System Suitability:

System suitability was determined before sample analysis from six replicate injections of the standard solution containing 10 µg/mL of MTK and BBL. The chromatograph to determine peak areas, retention times (*tR*) and chromatographic relative standard deviation (%RSD). The retention factor (*k*), theoretical plates (*N*), tailing (*Tf*) and peak asymmetry (*As*) chromatographic parameters were also be evaluated (Table 3).

Table 3: System suitability parameters.

Parameters	Bambuterol	Montelukast
Calibration range(µg/mL)	5-30	2-12
Retention time (min)	4.658	2.692
K	4.09	1.32
T_f	1.57	1.83
A_s	1.18	1.27
Resolution	4.85	2.73

Specificity:

The specificity is performed to check ability of method to measure the analyte accurately and specifically in presence of other components or drugs. The other compound should not interfere with drug and should be estimated separately.

Linearity:

Linearity test solutions were prepared by diluting the stock solutions to the required concentrations. The solutions were prepared in mobile phase at six concentration levels from 5-30 µg/mL and 2-12 µg/mL of BBL and MTK respectively. Calibration curves were plotted between the responses of peak versus drug concentrations. The coefficient correlation, slope, y-intercept of the calibration curve at 100% response are reported (Table 4) and result shows that an excellent correlation existed between peak area and concentration of bambuterol and montelukast.

Table 4: Evaluation of Linearity data, LOD and LOQ.

Parameters	Bambuterol	Montelukast
Detection wavelength (nm)	231	231
Linearity range (µg/mL)	5-30	2-12
Correlation coefficient	0.994	0.996
Intercept	4739	19020
Slope	12892	50414
Detection limit (µg/mL)	0.0259	0.0206
Quantitation limit (µg/mL)	0.078	0.062

Accuracy:

Accuracy is performed to check similarity of results obtained by analytical value to the true value. To check the accuracy of the proposed methods, recovery studies were carried out triplicate at 60, 80, 100, 120 and 140% of the standard concentration as per ICH guidelines. The percentage recovery for both components was calculated. The percentage mean recovery of bambuterol and montelukast from the formulation varied from 99.07 to 100.4 % indicating that the developed method was accurate for the determination of bambuterol and montelukast in pharmaceutical formulation (Table 5).

Table 5: Recovery Data Study for Accuracy of montelukast and Bombuterol.

Amount Spiked[a]	Sample concentration (µg/mL)	% Recovery[b]	
		Bambuterol	Montelukast
60%	16	99.9±0.1	100.3±0.3
80%	18	99.5±0.5	99.15±0.5
100%	20	99.6±0.3	99.4±0.7
120%	22	100.4±0.8	99.07±0.6
140%	24	99.4±0.7	99.6±0.4

a Amount of both analytes spiked with respect to target concentration.
b Mean + %RSD, *n=3*.

Precision:

The precision of method was verified by repeatability and intermediate precision. Repeatability was checked by injecting six individual preparations of sample containing bambuterol and montelukast at 60%, 80%, 100%, 120% and 140% level of test concentration (16, 18, 20, 22, 24 µg/mL for bambuterol and montelukast). The intra-day and inter-day precision was determined by assay of the sample solution on the same day at different time intervals and on different days respectively. The relative standard deviation of the percentage assay of each analyte was calculated and found to be less than 0.87% in repeatability and less than 0.84% in intermediate precision study, which confirms the good precision of the method. The % RSD values are presented in (Table 6).

Table 6: Precision results determined during method validation.

Parameter	Spiked level	Sample concentration (µg/mL)	% RSD (n=6)	
			Bambuterol	Montelukast
Repeatability	60	16	0.24	0.21
	80	18	0.45	0.73
	100	20	0.81	0.54
	120	22	0.63	0.87
	140	24	0.35	0.44
Intermediate Precision	60	16	0.55	0.36
	80	18	0.72	0.56
	100	20	0.61	0.73
	120	22	0.84	0.49
	140	24	0.32	0.38

Limit of Detection (LOD) & Limit of Quantitation (LOQ):

For LOD and LOQ, 1µg/mL of solution of drugs was prepared from standard stock solution of 100 µg/mL by diluting appropriate volume with mobile phase. Five standard solutions for bambuterol 0.02, 0.04, 0.06, 0.08, 0.10 µg/mL and for montelukast 0.05, 0.06, 0.07, 0.08, 0.09, 0.10 µg/mL were prepared in mobile phase from 1 µg/mL of solution. The LOD and LOQ of bambuterol and montelukast by proposed methods were determined using calibration standards. The Data for LOD and LOQ for different drugs was shown in Table **4**. The limit LOD and LOQ values were found to be 0.0259, 0.078 µg/mL and 0.0206, 0.062 µg/mL for bambuterol and montelukast, respectively.

Robustness and Ruggedness:

As per ICH guidelines, small but deliberate variations in concentration of the mobile phase were made to check the accuracy of the method. These variations did not cause any significant difference in the resolution of HPLC method, shown in (Table 7 & 8).

Table 7: Robustness result of HPLC method.

Variation in Chromatographic Condition	**Observed System Suitability Parameters**							
	Bambuterol				**Montelukast**			
	tR [a]	***A*** [b]	***T*** [c]	***Rs*** [d]	***tR*** [a]	***A*** [b]	***T*** [c]	***Rs*** [d]
70:30	4.498	15382	1.63	3.98	2.876	51973	1.98	2.38
80:20	4.575	14587	1.58	3.76	2.339	52430	1.77	2.54
90:10	4.698	14672	1.56	3.52	2.573	52787	1.78	2.65
Flow rate 1.5ml/min	4.658	145315	1.57	4.85	2.692	529703	1.83	2.73
Flow rate 1.6ml/min	4.567	13267	1.53	4.67	2.542	51878	1.82	2.63
Flow rate 1.7ml/min	4.332	12334	1.52	4.66	2.267	50456	1.79	2.54

a Retention time (min) of the analyte peak.
b % RSD of the analyte peak areas from 5 injections.
c Tailing factor of the analyte peak., **d** Plate count of the analyte peak.

Table 8: Ruggedness result of HPLC method.

Parameters	Bambuterol		Montelukast	
	% Content	% RSD	% Content	%RSD
Analyst-1	99.82	1.28	100.30	1.39
Analyst-2	99.74	1.09	100.93	1.30
Analyst-3	99.69	1.37	100.87	1.28

Average of 5 determinations

CONCLUSION:

A simple and efficient reverse-phase HPLC method was developed and validated for quantitative determination of bambuterol and montelukast in pharmaceutical dosage forms. The method found to be precise, accurate, linear, robust and rugged during validation. Satisfactory results were obtained from the validation of the method. The method is stability indicating and can be used for routine analysis of production samples and to check the stability of the montelukast and bambuterol tablets.

CONFLICT OF INTEREST:

The authors confirm that this article content has no conflicts of interest.

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