International Journal of PharmTech Research
CODEN(USA):IJPRIF ISSN:0974-4304
Vol.2, No.3, pp 1767-1771, July-Sept 2010

Analytical Method Development and Validation of Montelukast Sodium and Bambuterol Hydrochloride in Combined Dosage Form by RP-HPLC

S.A.Patel , S. K. Patel, D. J. Patel*, N. J. Patel

Department of Pharmaceutical Chemistry, Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva, Mehsana - 382711, Gujarat, India.

Corres.author: sweetvpatel@gmail.com

Abstract: This research paper describes simple, specific, accurate and precise reverse phase high pressure liquid chromatographic method for the simultaneous determination of Montelukast sodium and Bambuterol hydrochloride in combined dosage form. The sample was analyzed by reverse phase C18 column (Phenomenex C18, 5µ, 250mm x 4.6mm) as stationary phase; methanol: acetonitrile : 1% trichloroacetic acid in the ratio of 80:10:10 v/v/v as a mobile phase at a flow rate of 1.0ml/min. Quantification was achieved with ultraviolet detection at 220 nm. The retention time for Montelukast sodium and Bambuterol hydrochloride was found to be 3.17 and 2.35 min, respectively. The linearity for both the drugs was in the range of 0.5-10 µg/ml with mean accuracies 100.06 ± 0.49 and 99.95 ± 0.63 for Montelukast sodium and Bambuterol hydrochloride, respectively. The method was successively applied to pharmaceutical formulation because no chromatographic interferences from the tablet excipients were found. The method retained its accuracy and precision when the standard addition technique was applied.
Keywords: Montelukast sodium, Bambuterol Hydrochloride, RP-HPLC.,Combined Dosage Form.

Introduction

Montelukast sodium (MTKT), 1-[({(R)-m-[(E)-2-(7-chloro-2-quinolyl) vinyl]-α-[o-(1-hydroxyl-1-methylethyl)phenethyl]benzyl}thio)methyl] cyclopropaneacetate sodium is a leukotriene receptor antagonist, used in the treatment of asthma[1],[2],[3]. It is not official in IP, BP and USP. Various analytical methods, such as liquid chromatography with fluorescence detection [4],[5],[6], stereoselective HPLC for MTKT and its S-enantiomer [7] , simultaneous HPLC and derivative spectroscopic method with loratadine [8], stability indicating HPLC method[9] for Montelukast sodium in tablets and human plasma have been reported. Bambuterol hydrochloride (BAM), (RS)- 5 -(2-tert-butylamino-1-hydroxyethyl) – m -phenylene bis (dimethylcarbamate) hydrochloride is a direct acting sympathomimetic with predominantly -adrenergic activity (β2 -agonist))[10]. It is an ester prodrug of b_2 adrenergic agonist terbutaline [2] . Bambuterol hydrochloride is official in BP [11]. Different HPLC methods have been reported for the estimation of BAM in pharmaceutical dosage form[12],[13],[14]. The drug has been also estimated by solid-state NMR [15] . The combined dosage forms of MTKT and BAM are available in the market for the prophylaxis and treatment of chronic asthma and chronic bronchitis in pediatrics.

At present one HPLC method [16] has been reported for simultaneous determination of MTKT and BAM in formulations. But it is more time consuming method because retention time for MTKT is 21.2 min and BAM is 5.8 min, so we are interested in development of less time consuming and more economic method for simultaneous analysis of both drugs in combination.

Hence, the aim was to develop validated HPLC method, which can simultaneously determine both components in marketed pharmaceutical dosage forms with better accuracy, precision and sensitivity.

Experimental

Instruments and Apparatus

The chromatography was performed on a Shimadzu (Columbia, MD) RP-HPLC instrument (LC-2010CHT) equipped with PDA detector and LC-solution software, Phenomenex (Torrance, CA) C_{18} column (250×4.6 mm id, 5 µm particle size) was used as stationary phase. Sartorius CP224S analytical balance (Gottingen, Germany) and ultrasonic cleaner (Frontline FS 4, Mumbai, India) were used during the research work.

Reagents and materials

Standard samples of MTKT and BAM were obtained from Sun Pharmaceutical Pvt Ltd, (Vadodara, Gujarat). Combination tablet formulation containing Montelukast sodium equivalent to Montelukast 10 mg and Bambuterol hydrochloride 10 mg was procured from local pharmacy. Triple distilled water, methanol, acetronitrile, trichloroacetic acid (S. D. Fine Chemicals) used were of HPLC grade.

Preparation of Standard Solution

Accurately weighed MTKT (10 mg) and BAM (10 mg) standards were transferred to a 50 ml volumetric flask, dissolved in and diluted up to the mark with methanol to obtain a standard stock solution (200 µg/ml) of MTKT and BAM, each. From the above stock solution, an aliquot (2.5 ml) of the solution was transferred to 50 ml volumetric flask, and diluted up to the mark with methanol to obtain a working standard solution (10 µg/ml) of MTKT and BAM, each.

Preparation of Calibration Curve

Aliquots (0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 , 4.5 5 ml) of mixed working standard solution (equivalent to 0.5, 1, 2, 3, 4 5, 6, 7, 8 ,9 and 10 µg/ml of MTKT and BAM, each) were transferred in a series of 10 ml volumetric flasks, and the volume was made up to the mark with methanol. An aliquot (10 µl) of each solution was injected under the operating chromatographic conditions as described above and responses were recorded. Calibration curves were constructed by plotting the peak areas versus the concentrations, and the regression equations were calculated. Each response was average of three determinations.

Preparation of Sample Solution

For determination of the content of MTKT and BAM in tablets; twenty tablets were weighed and average weight was determined. The accurately weighed powder equivalent to 10 mg MTKT and 10 mg of BAM was transferred in a 50 ml volumetric flask and methanol (30 ml) was added. The solution was sonicated for 15 min. The flask was allowed to stand for 5 min at room temperature and the volume was diluted up to the mark with methanol to obtain the sample stock solution of MTKT (200 µg/ml) and BAM (200 µg/ml). The solution was filtered through 0.45µm-47mm membrane filter. An aliquot (2.5 ml) was transferred to a 50 ml volumetric flask and diluted up to the mark with mobile phase used for HPLC, to obtain working sample solution of MTKT (10 µg/ml) and BAM (10 µg/ml). An aliquot (1 ml) of the working test solution was transferred to a 10 ml volumetric flask and diluted up to the mark with mobile phase to obtain the sample solution of MTKT (1 µg/ml) and BAM (1 µg/ml).

Method Validation

The methods were validated in compliance with ICH guidelines.

Accuracy

The accuracy of the methods was determined by calculating recoveries of MTKT and BAM by the standard addition method.

Intermediate Precision (Reproducibility)

The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of MTKT (1, 3 and 5 µg/ml) and BAM (1, 3 and 5 µg/ml).

Method Precision (Repeatability)

The repeatability was checked by repeatedly injecting (n = 6) solution of MTKT (4 µg/ml) and BAM (4µg/ml, each).

LOD and LOQ

The limit of detection (LOD) and the limit of quantification (LOQ) of the MTKT and BAM, were calculated using the standard deviation of responses (N) and slopes (S) of respective calibration curves using signal-to-noise ratio.

$LOD = 3.3 \times N/S$

$LOQ = 10 \times N/S$

Robustness

The robustness was studied by analyzing the same samples of MTKT and BAM by deliberate variation in the method parameters. The change in the responses of MTKT and BAM were noted. Robustness of the method was studied by changing the extraction time of MTKT and BAM from tablet dosage forms by ± 2 min, composition of mobile phase by ± 2 % of organic solvent, flow rate by ± 0.2 ml/min and column oven temperature by ± 2 ^{0}C. The parameters used in system suitability test were asymmetry of the chromatographic peak, peak resolution, theoretical plates and capacity factor, as RSD of peak area for replicate injections.

TABLE 1: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETER FOR THE PROPOSED METHOD

Parameters	RP-HPLC method	
	MTKT	BAM
Concentration range (µg/ml)	0.5-10	0.5-10
Slope	581164	526746
Intercept	17041	35052
Correlation coefficient	0.9973	0.9994
LOD(µg/mL)	0.04	0.05
LOQ(µg/mL)	0.5	0.5
% recovery (Accuracy, n = 6)	100.06 ± 0.49	99.95 ± 0.63
Repeatability (% RSD, n = 6)	0.24	0.31
Precision (%RSD)		
Interday (*n* = 6)	0.47-0.98	0.53-1.07
Intraday (*n* = 6)	0.34-0.82	0.65-1.19

[a] RSD is a Relative standard deviation, [b] n is number of determinations, MTKT is Montelukast Sodium, BAM is Bambuterol hydrochloride.

TABLE 2: SYSTEM SUITABILITY TEST PABAMETERS FOR MTKT & BAM FOR PROPOSED METHOD

Parameters	RP-HPLC method	
	MTKT ± % RSD[a]	BAM ± % RSD[a]
Retention time, min	3.17 ± 0.15	2.35 ± 0.17
Tailing factor	1.02 ± 0.67	1.29 ± 0.56
Asymmetry factor	1.22 ± 0.93	1.16 ± 0.39
Theoretical plates	6243.5 ± 1.13	4986.9 ± 1.33
Repeatability of measurement (n^b = 6)	0.67	0.48

[a] RSD is a Relative standard deviation, [b] n is number of determinations

TABLE 3: ASSAY RESULTS FOR TABLETS USING THE PROPOSED METHOD

Formulation	Amount of drug taken (mg)		Amount of drug found (mg)		% Amount found (n^a=3) ± SD[b]	
Tablets	MTKT	BAM	MTKT	BAM	MTKT	BAM
	10	10	10.13	9.99	101.30 ± 0.29	99.95 ± 0.45

[a]n is number of determinations, [b]SD is a Standard deviation

Results and Discussion

The responses of sample solutions were measured at 220 nm for quantitation of MTKT and BAM by the proposed methods. The amount of MTKT and BAM present in the sample solutions were determined by fitting the responses into the regression equations of the calibration curve for MTKT and BAM, respectively.

The mobile phase consisting of methanol: acetonitrile: (1%) trichloroacetic acid, at a flow rate of 1.0 ml/min was found to be satisfactory to obtain good peak symmetry, better reproducibility and repeatability for MTKT and BAM. Quantification was achieved with PDA detector at 220 nm based on peak area. The retention times were found to be 3.17 and 2.35 min for MTKT and BAM, respectively (Figure 1).

Linear correlation was obtained between peak area and concentration for MTKT and BAM, each, in the range of 0.5-10 µg/ml (Table 1). The method was found to be specific as no significant change in the responses of MTKT and BAM was observed after 24 h. The percent mean recoveries obtained for MTKT and BAM were

100.06 ± 0.49 % RSD and 99.95 ± 0.63 % RSD (Table 1), which suggest accuracy of the method. The values of % RSD for intraday and interday variations were found to be in range of 0.34-0.82 and 0.53-1.07 for MTKT, and 0.47-0.98 and 0.65-1.19 for BAM, respectively (Table 1). % RSD for repeatability was found to be 0.24 and 0.31 for MTKT and BAM, respectively. Low RSD values for precision suggest that the method is precise. The LOD and LOQ were found to be 0.04 and 0.5 µg/ml for MTKT, 0.05 and 0.5 µg/ml for BAM, respectively (Table 1), suggest sensitivity of the method. Results of system suitability testing are given in Table 2. The results obtained for MTKT and BAM were comparable with the corresponding labeled claim (Table 3).

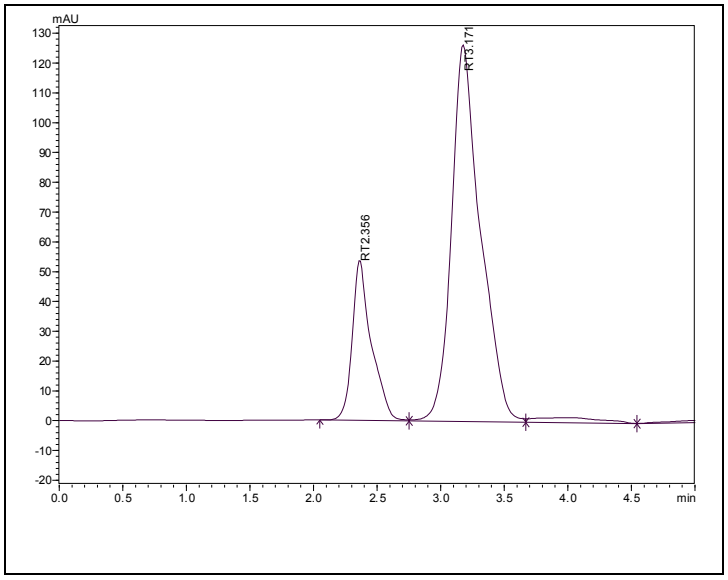

Figure1:High performance liquid chromatogram of MTKT (1 µg/ml) and BAM (1 µg/ml) from tablet dosage form, at 220 nm, with retention time of 3.17 and 2.35 min, respectively

Acknowledgements

The authors are thankful to managements of Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva, Mehsana for providing needed facilities for this work.

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