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Simultaneous estimation of Montelukast and Bambuterol in tablet dosage forms by RP-HPLC

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ABSTRACT

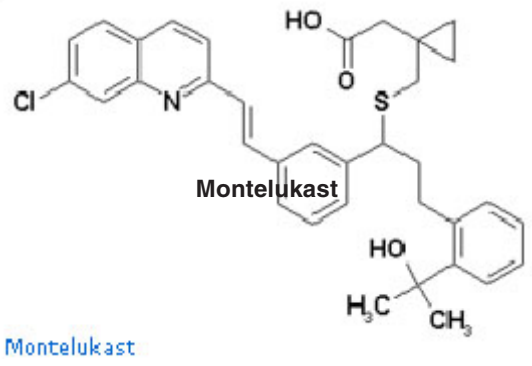
A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of Montelukast & Bambuterol in tablet dosage forms. An Inertsil ODS-3CV C18, 250x4.6 mm, coloumn with 5 µm particle size and the mobile phase consisting of 0.1% Trifluoro Acetic acid: Methanol in the ratio of 10:90 v/v was used. The flow rate was 0.8 ml/min and the effluents were monitored at 220 nm. The retention times were 2.373min for bambuterol and 6.899 min for Montelukast. The detector response was linear in the concentration of 17.5-420 mcg/ml. The respective linear regression equation being Y= 26705.167x+67644.9694 for Bambueterol and Y=75702.306x+114141.09 for Montelukast. The limit of detection (LOD) is 0.175mcg and 0.35 mcg for Bambuterol and Montelukast respectively. The limit of quantification (LOQ) is 0.525mcg for Bambuterol and 1.05mcg for Montelukast. The percentage assay of Montelukast & Bambuterol was 99.48 % and 99.78% respectively. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Montelukast & Bambuterol in bulk drug and in its pharmaceutical dosage forms.

Key words: Montelukast & Bambuterol, RP-HPLC, estimation, and tablets.

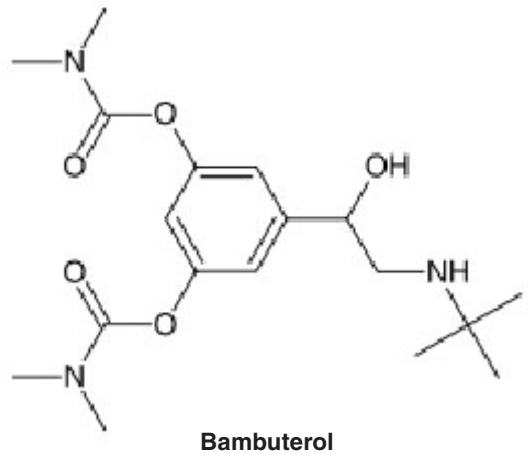
INTRODUCTION

Montelukast [1] is a selective leukotriene receptor antagonist used in the management of chronic asthma. Chemically it is 1-[[[(1R)-1-[3-[1E)-2-(7-chloro-2-quinolinyl) ethenyl]phenyl]thio]methyl] cyclopropane acetic acid[1]. Its molecular weight is 586.18 and molecular formula is $C_{35}H_{36}NSClO_3$. Montelukast is a selective cysteinyl leukotrien type I receptor antagonsit[4]. Bambuterol[3] is inactive prodrug of terbutaline, a direct acting sympathomimetic with predominantly beta-adrenergic activity and a selective action on $beta_2$ireceptors. It is used as bronchodilator for persistent asthma. Chemically it is Dimethyl carbamic acid 5-[2-[(1,1-dimethyl ethyl)amino]-1-hydroxy ethyl]-1,3,-phenylene ester[2] with molecular weight 367.4 and molecular formula $C_{18}H_{29}N_3O_5$. Literature survey reveals a few chromatographic methods [6-12] to deterimine the montelukast in tablet dosage form and in biological fluids and only one spectrophotometric method[5] was reported for the estimation of bambuterol in tablet dosage form. So far, no chromatographic methods were reported for the simultaneous estimation of Montelukast & Bambuterol in pharmaceutical dosage forms. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the

determination of Montelukast & Bambuterol in pharmaceutical formulations. The aim of the study was to develop a simple, precise and accurate reverse-phase HPLC method for the estimation of Montelukast & Bambuterol in bulk drug samples and in pharmaceutical dosage form.



Montelukast



Bambuterol

EXPERIMENTAL

Materials and Methods

Montelukast & Bambuterol were obtained as a gift samples from MSN Pharmachem Pvt.Ltd, Hyderabad. Acetonitrile and water used were of HPLC grade (Qualigens). Commercially available tablets (Montair Plus, Cipla) were procured from local market.

Instrument

Quantitative HPLC was performed on liquid Chromatograph, Waters separation 2996, PDA detector module equipped with automatic injector with injection volume 20 µl, and 2693 pump. An Inertsil-ODS-3V C18 column (250 x 4.6 mm i.d; particle size 5 µm) was used. The HPLC system was equipped with Empower Software.

HPLC Conditions

The contents of the mobile phase were 0.1% Trifluoro Acetic acid: Methanol in the ratio of

Table 1: Linear Regression Data for Calibration curves

Parameter	Montelukast	Bambuterol
Conc.range (µg/ml)	17.5-420	17.5-420
Slope (m)	75702.306	26705.167
Intercept (b)	114141.0925	67644.9694
Correlation coeff.	0.9999	0.9999
% RSD	0.93	0.35
Standard error of estimate	118206.6442	48619.7117

Table 2: Assay & Recovery of Montelukast & Bambuterol in Tablet dosage form

Tablet formulation	Amount claim (mg/tablet)	Amount claim (mg/tablet)	Amount Obtained (mg)* by proposed method		** % Recovery by the by Proposed method	
	Monteleukast	Bambuterol	Montelukast	Bambuterol	Montelukast	Bambuterol
1	10	10	9.84	9.87	99.5	100.3
2	10	10	9.88	9.88	99.0	101.1
3	10	10	9.80	9.86	99.1	99.2

*Average of three determinations.** After spiking the sample.

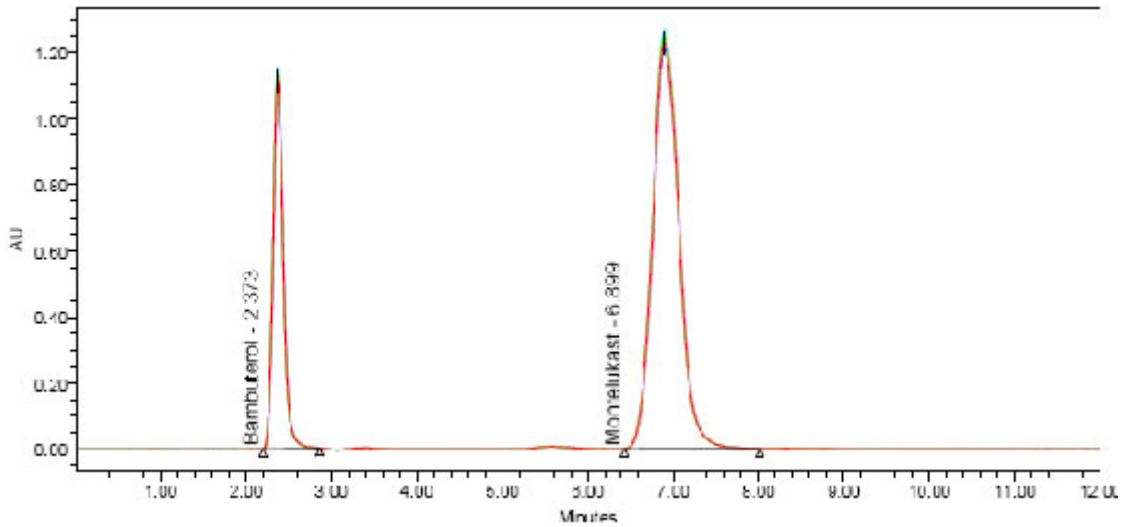
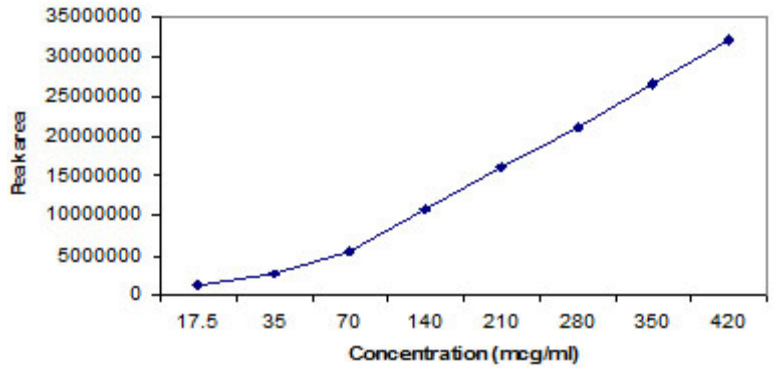
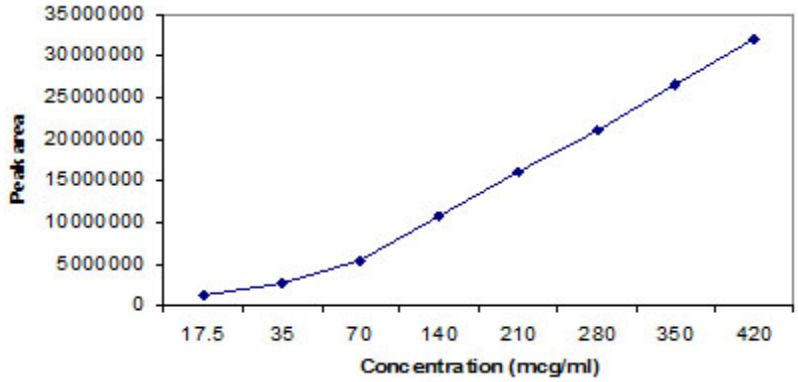


Fig 1: Typical chromatogram of montelukast & bambuterol by RP-HPLC



Montelukast



Bambuterol

Fig. 2: Caliberation curves of the Montelukast & Bambuterol by RP-HPLC

10:90 v/v. They were filtered before use through a 0.45 µm membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 0.8 ml/min. The run time was set at 12.0 min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 220 nm.

Preparation of standard stock solution

A standard stock solution of the drug was prepared by dissolving 193.5 mg of Bambuterol hydrochloride (equivalent to 175mg of Bambuterol) and 182 mg of Montelukast sodium (equivalent to 175 mg of montelukast) in 100 ml volumetric flask containing 30 ml of methanol as diluent, sonicated for about 15 min and then made up to 100 ml with methanol to get standard stock solution of 1.75 mg/ml each of montelukast and Bambuterol.

Working standard solution

10ml of the above stock solution was taken in 50 ml volumetric flask and made up to 50 ml with methanol as diluent to get a concentration of each 350µg/ml of Montelukast and Bambuterol.

Preparation of sample solution

Twenty tablets (Montair Plus, Cipla) were weighed, and then powdered. A sample of the powdered tablets, equivalent to mixture containing 175 mg of montelukast and 175 mg of bambuterol active ingredients, was mixed with 30 ml of methanol as diluent in 50 ml volumetric flask. The mixture was allowed to stand for 1 hr with intermittent sonication to ensure complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by adding methanol up to100 ml to obtain a stock solution each of 1.75mg/ml of Montelukast and Bambuterol. 10ml of the above sample stock solution was taken in 50 ml volumetric flask and made up to 50 ml with methanol as diluent to get a concentration of each 350 µg/ml of Montelukast and Bambuterol.

Table 3: Validation summary

Parameter	Montelukast	Bambuterol
System Suitability		
TheoreticalPlates(N)	2400.38	2007.45
Tailing factor	1.14	1.15
Retention time(min)	6.899	2.373
Resolution	17.52	17.36
% Peak Area	99.98	99.82
LOD (µg/ml)	0.35	0.175
LOQ (µg/ml)	1.05	0.525

Linearity

Aliquots of standard Montelukast & Bambuterol stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of Montelukast & Bambuterol are in the range of 17.5-420 µg/ml. Each of these drug solutions (20 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 220 nm and a Calibration graphs were obtained by plotting peak area versus concentration of Montelukast & Bambuterol (Fig 2). The plot of peak areas of each sample against respective concentration of Montelukast & Bambuterol was found to be linear in the range of 17.5-420 µg/ml.with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in table I. The respective linear regression equation being Y= 26705.167x+67644.9694 for Bambueterol and Y=75702.306x+114141.09 for Montelukast. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in Table 1.

Assay

20 µl of sample solution was injected into the injector of liquid chromatograph. The retention times were found to be 2.373min for Bambuterol and 6.899 for Montelukast.. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Table 2.

Recovery studies

Accuracy was determined by recovery studies of Montelukast & Bambuterol; known amount of standard was added to the preanalysed

sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table 2. The study was done at three different concentration levels.

RESULTS AND DISCUSSION

The system suitability tests were carried out on freshly prepared standard stock solutions of Montelukast & Bambuterol. Parameters that were studied to evaluate the suitability of the system are given in Table 3.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) is 0.175mcg and 0.35 mcg for Bambuterol and Montelukast respectively. The limit of quantification (LOQ) is 0.525mcg for Bambuterol and 1.05mcg for Montelukast.

From the typical chromatogram of Montelukast & Bambuterol as shown in Fig 1, it was found that the retention times were 2.373min. for bambuterol and 6.899 min. for Montelukast. A mixture of 0.1% Trifluoro Acetic acid: Methanol in the ratio of 10:90 v/v was found to be the most suitable as mobile phase to obtain the peaks well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extractions were involved. A good linear relationship (r=0.9999) was observed between the concentration range of 17.5-420 µg/ml. Low values of standard deviation are indicative of the high precision of the method. The assay of Montelukast & Bambuterol tablets was found to be 98.48% and 98.78% respectively. From the recovery studies it was found that about 99.14% of Montelukast & 100.2% of Bambuterol was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage forms of Montelukast & Bambuterol within a short analysis time.

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REFERENCES

1. The Merck Index (monograph#953), **14**: 1080 (2006).
2. The Merck Index (monograph#6258), **14**: 161 (2006).
3. Martindale-The Complete Drug Reference, **34**: 781 (2005).
4. Martindale-The Complete Drug Reference, **34**: 788 (2005).
5. S. Appala Raju, Shobha Manjunath, *Asian J. Chem.* **15**: 1117-1120 (2003).
6. Ibrahim A. Alsarra, *Saudi Pharmaceutical Journal*, **12**(4): 136-143 (2004).
7. Chauhan B, Rani Shubha ; Nivasarkar M. PadhH. ; *Indian journal of pharmaceutical sciences*, **68**(4): 517-520 (2006).
8. Alsarra I, Khalil NY, Sultan M, Al-Ashban R, *Pharmazie,* **60**(11): 823-6 (2005).
9. Smith GA, Rawls CM, Kunka RL. *Pharm Res,* **21**(9): 1539-44 (2004).
10. Radhakrishna T, Narasaraju A, Ramakrishna M, Satyanarayana A. *J Pharm Biomed Anal*. **31**(2): 359-68 (2003).

11. Al-Rawithi S, Al-Gazlan S, Al-Ahmadi W, Alshowaier IA, Yusuf A, Raines DA. *J Chromatogr B Biomed Sci Appl*, **754**(2): 527-31 (2001).

12. Ochiai H, Uchiyama N, Takano T, Hara K, Kamei T. *J Chromatogr B Biomed Sci Appl.* **713**(2): 409-14 (1998).