Research Article

Estimation of Bambuterol in Tablet dosage forms by RP-HPLC

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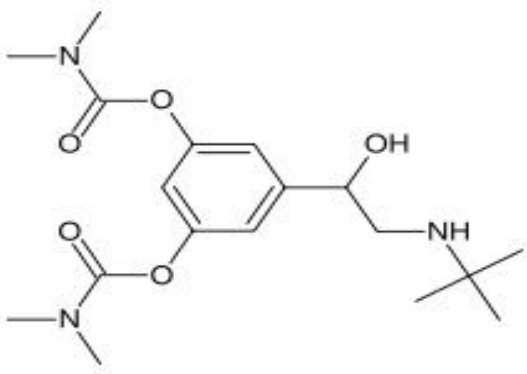
ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of 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 2.381min for bambuterol. 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. The limit of detection (LOD) is 0.175mcg and 0.35 mcg and the limit of quantification (LOQ) is 0.525mcg for Bambuterol. The percentage assay of Bambuterol was found to be 99.78%. 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 Bambuterol in bulk drug and in its pharmaceutical dosage forms.

Key words: Bambuterol, RP-HPLC, Estimation, and Tablets.

INTRODUCTION

Bambuterol[1] 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]. Literature survey reveals a few chromatographic methods [4-10] to deterimine the bambuterol in tablet dosage form and in biological fluids and only one spectrophotometric method[3] was repored for the estimation of bambuterol in tablet dosage form. So far, no chromatographic methods were reported for the estimation of Bambuterol in pharmaceutical dosage forms. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of Bambuterol in pharmaceutical formulations. The aim of the study was to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of Bambuterol in bulk drug samples and in pharmaceutical dosage form.



Bambuterol

EXPERIMENTAL:

Materials and Methods: Bambuterol was obtained as a gift samples from MSN Pharmachem Pvt. Ltd, Hyderabad. Acetonitrile and water used were of HPLC grade (Qualigens). Commercially available tablets (Bambudil, Cipla) were procured from local market.

Instrument: Quantitative HPLC was performed on liquid Chromatograph, Waters separation 2996, PDA detector 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 176.5 mg of Bambuterol hydrochloride (equivalent to 175mg of Bambuterol) 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 the standard stock solution of 1.75 mg/ml of 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 the concentration of 350µg/ml of Bambuterol.

***Preparation of Sample solution*:** Twenty tablets (Bambudil, Cipla) were weighed, and then powdered. A sample of the powdered tablets, equivalent to 176.5mg of the active ingredient, 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 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 Bambuterol.

Linearity: Aliquots of standard 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 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 Bambuterol **(Fig 2)**. The plot of peak areas of each sample against respective concentration of 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. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in **Table I.**

Assay: 20 µl of sample solution was injected into the injector of liquid chromatograph. The retention times were found to be 2.381min for Bambuterol. 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 II**.

Recovery Studies: Accuracy was determined by recovery studies of 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 II. The study was done at three different concentration levels.

Table I: Linear Regression Data for Calibration curves.

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

Table II: Assay & Recovery of Bambuterol in Tablet dosage form

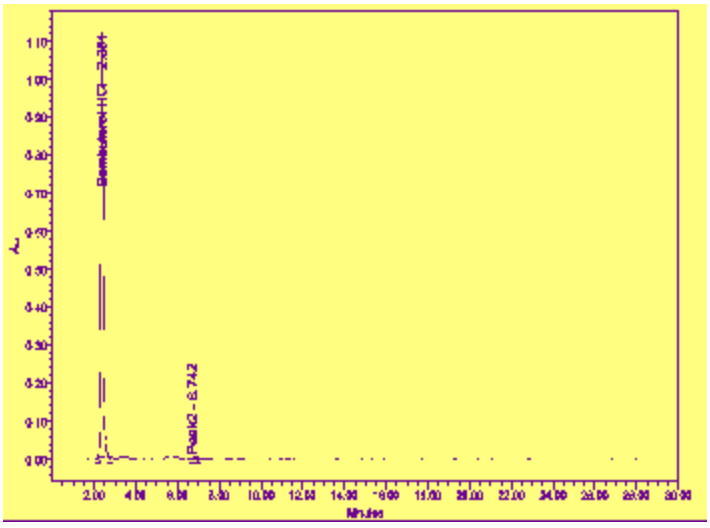
Sample	Amount claim (mg/tablet)	Amount Obtained (mg)* by proposed method	** % Recovery by the Proposed method
1.	10	9.87	103.3
2.	10	9.88	101.1
3.	10	9.86	99.2

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

Table III: Validation Summary

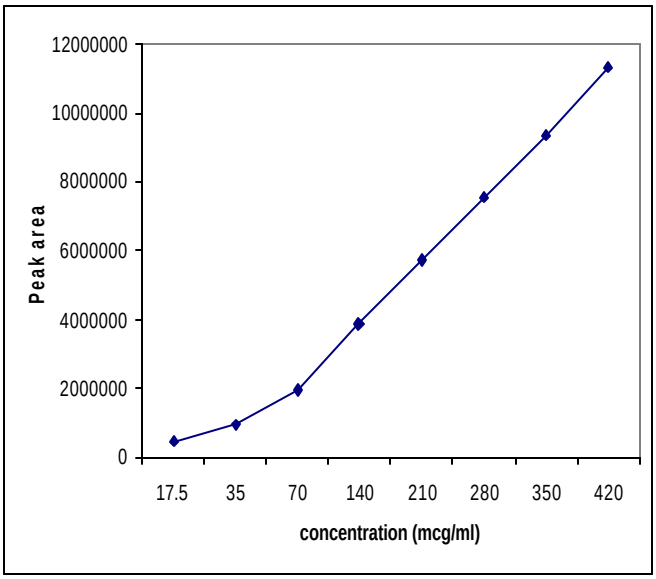
Parameter	Bambuterol
System Suitability	2007.45
Theoretical Plates (N)	1.15
Tailing factor	2.381
Retention time(min)	17.36
Resolution	99.82
% Peak Area	
LOD (µg/ml)	0.175
LOQ (µg/ml)	0.525

Fig 1: Typical Chromatogram of Bambuterol by RP-HPLC



Results and Discussion: The system suitability tests were carried out on freshly prepared standard stock solutions of Bambuterol. Parameters that were studied to evaluate the suitability of the system are given in **Table III.**

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



Limit of Detection (LOD) and Limit of Quantification (LOQ) : The limit of detection (LOD) is 0.175mcg and the limit of quantification (LOQ) is 0.525mcg for Bambuterol. From the typical chromatogram of Bambuterol as shown in **Fig 1**, it was found that the retention times were 2.381min. for bambuterol. 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 Bambuterol tablets was found to be 98.78% respectively. From the recovery studies, it was found that about 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 Bambuterol within a short analysis time.

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