

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF BAMBUTEROL FROM BULK AND DOSAGE FORM

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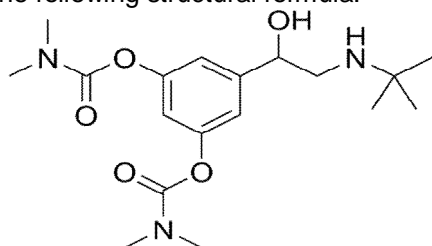
ABSTRACT

A Simple, fast and precise reverse phase high performance liquid chromatographic method developed for the determination of bambuterol in its tablets form. Phenomenex Luna C18 150mm X 4.6mm (l x d), 5 μ in isocratic mode with mobile phase Buffer and Acetonitrile in the ratio of 70 : 30 % v/v were used. The flow rate was 1ml/min. Linearity for bambuterol was 50mcg/ml to 150mcg/ml. The correlation coefficient (r^2) was found to be greater than (0.9992). Amount of bambuterol present in each tablet was found to be 9.99mg/tab. The % RSD values were less than 2% for method precession. The LOD for bambuterol was found to be 1.36 μ g/ml. The LOQ of bambuterol was found to be 5.02 μ g/ml. The percentage recoveries obtained for bambuterol was found to be 98.48. The Proposed method is accurate, precise, selective and rapid for the estimation of bambuterol in its tablets form.

Keywords: HPLC, Validation, bambuterol.

1. INTRODUCTION

Bambuterol is a long acting beta-adrenoceptor agonist (LABA) used in the treatment of asthma; it also is a prodrug of terbutaline. The pharmacologic effects of bambuterol are at least in part attributable to stimulation through beta-adrenergic receptors (beta 2 receptors) of intracellular adenylyl cyclase, the enzyme that catalyzes the conversion of adenosine triphosphate (ATP) to cyclic AMP. Increased cyclic AMP levels are associated with relaxation of bronchial smooth muscle and inhibition of release of mediators of immediate hypersensitivity from cells, especially from mast cells. Several methods such as GC/MS¹, HPLC^{2,3,4}, CE⁵ have been reported in the literature. The chemical name of bambuterol is (RS)-5-[2-(tert-butylamino)-1-hydroxyethyl] benzene-1,3-diyl bis(dimethylcarbamate). It has the following structural formula:



2. MATERIALS AND METHODS

Reference standard of Bambuterol, The bambec tablet contain label claim of bambuterol 10mg, Acetonitrile HPLC grade, water HPLC grade, methanol HPLC grade, Potassium Dihydrogen Orthophosphate AR grade, Stationary Phase C18, 5 μ , phenomenex Luna column 150mm X 4.6mm (l x d) was used.

2.1. Buffer preparation (0.01M)

1.36 gm of Potassium Dihydrogen Orthophosphate was dissolved in 1000 ml of HPLC grade water and PH adjusted to 5.6. Solution was sonicated and filtered through 0.45 μ Nylon filter.

2.2Preparation Of Mobile phase

Buffer and Acetonitrile were mixed in the ratio of 70 : 30 and sonicated to degas.

2.3. PREPARATION OF SOLUTION PREPARATION OF STANDARD STOCK SOLUTION (1000 μ g/ml)

Accurately weigh about 10 mg of Bambuterol and transferred into 10 ml volumetric flask and dissolved and volume were made up with diluent (methanol).

PREPARATION OF WORKING STANDARD SOLUTION (100µg/ml)

1 ml of above solution is diluted to 10 ml with diluent (methanol) to obtain the concentration of 100 µg/ml of Bambuterol.

PREPARATION OF SAMPLE STOCK SOLUTION

Five tablets was taken and crushed and transferred to 100 ml volumetric flask and little amount of diluent was added and shake well and soicated for 20min and volume were made up with diluent (methanol).

PREPARATION OF WORKING SAMPLE SOLUTION

To 2 ml of above solution is diluted to 10 ml with diluent(methanol).

3. RESULTS AND DISCUSSION

3.1. Assay:

10µl working standard solution and working sample solution(n=4) were injected in to an injector of liquid chromatograph. From the peak area of Bambuterol, the amount of drugs in sample (n=4) were computed. A typical HPLC chromatogram of Bambuterol as shown in fig 1. In replicate analysis n=4 of the drug by the proposed method the amount of bambuterol present in each tablet was found to be 9.99.6mg/tab. The result obtained by the proposed method was close to the label claim(10mg) of the drug indicating that the method is precise and accurate.

3.2. Linearity study

In to a serious of five standard measuring flask, varying amount of standard stock solution of bambuterol was taken and made up to various concentrations of 50,75,100,125,150 mcg/ml . The peak area response of the solutions were recorded at 217nm. The plot of peak area versus the concentrations of bambuterol was found to be linear in the range of 50-150 mcg/ml with coefficient of correlation (r=0.9999) as shown in fig.2.

3.3. Accuracy

Accuracy studies were performed at 50%,100%,150% spiked sample. Three replicate of each concentration were performed. The mean % recovery of bambuterol in the drug was 98.498. Since there is no significant difference between the theoretical and actual, the method is shown to be accurate and selective.

3.4. Robustness

Robustness of the method was evaluated by deliberately altering the method conditions from the original method parameters and verifying compliance of the system suitability requirements. Insignificant differences in peak areas and less variability in retention time were observed and results (system suitability results, assay values) were found to be satisfactory.

3.5. System suitability

System suitability tests were carried out on freshly prepared working standard solution of bambuterol and the parameters obtained with 10 µl injection volume and standard solution (n=6) are shown in Table 1.

3.6. Method Precision

The precision of the method was demonstrated by intraday variation studies. The assay on six test preparations were performed be injected into the chromatographic system as per the test method. The % assay of drug and percentage RSD were calculated. The % assay for the bambuterol was 99.99%. %RSD for the bambuterol was 0.34.

4. CONCLUSION

The proposed method is simple, precise and accurate for the determination of bambuterol in tablet dosage. In routine Quality control or in Test laboratories, when we have this formulation to be analysed, the method is best suited.

5. ACKNOWLEDGEMENT

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Table 1: System suitability parameters

Parameters	Bambuterol
Retention time	2.349
Tailing factor	1.29
Theoretical plate	3975
Peak area	1145892
RSD of peak area	0.3774

Fig. 1: Typical HPLC Chromatogram of Bambuterol

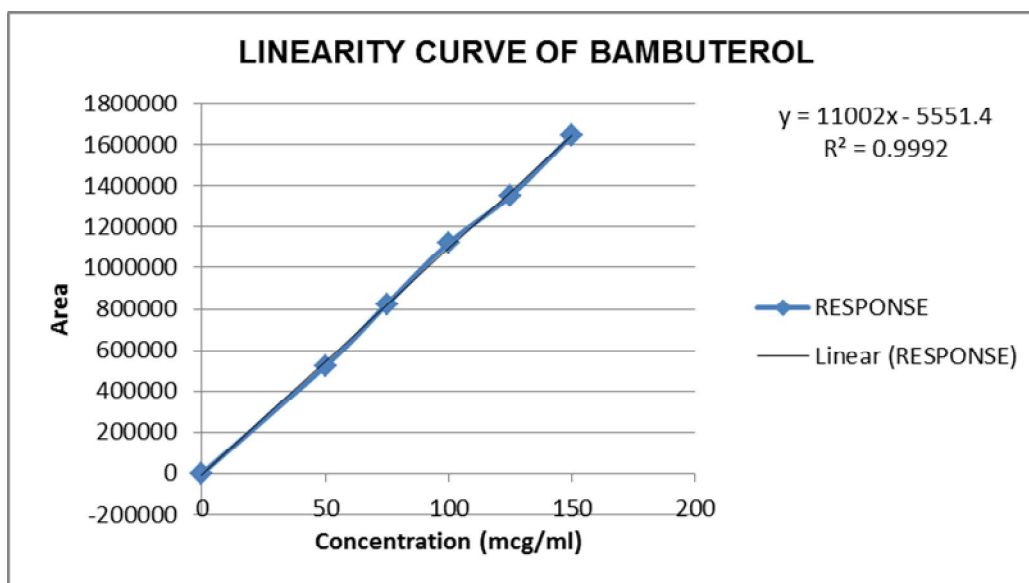
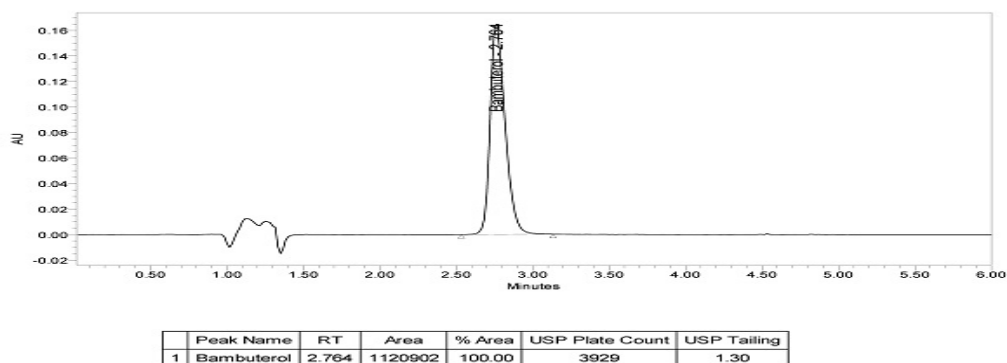


Fig. 2: Typical Linearity curve of Bambuterol

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