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Simultaneous RP HPLC determination of bambuterol HCl in pharmaceutical preparations

V.V.Vaidya[1]*, A.Y.Desai[2], P.S.Barde[2], Shikha M.N.Roy[2], K.V.Mangaokar[1]

[1]Department of Chemistry, S.P.Mandali's Ramnarain Ruia College, Matunga, Mumbai-400019, (INDIA)

[2]Department of Chemistry. G.N.Khalsa College, Sion, Mumbai-400019, (INDIA)

Tel.: 09322404966

E-mail: mpc26@rediffmail.com

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ABSTRACT

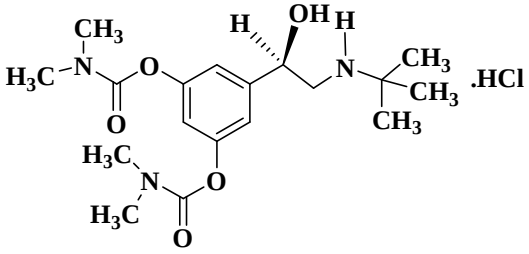
A simple, fast and precise reversed phase high performance liquid chromatographic method is developed for the simultaneous determination of bambuterol HCl using mebeverine HCl as an internal standard. Chromatographic separation of these two drugs was performed on a inertsil C_{18} column (250mm×4.6 mm, 5µm) as stationary phase with a mobile phase comprising of 0.02 M potassium dihydrogen orthophosphate: acetonitrile (50:50 v/v), at a flow rate of 0.7ml min^{-1} and UV detection at 215nm. The Retention time of Bambuterol HCl and Mebeverine HCl were 3.17 min and 4.88 min respectively. The proposed method was validated for linearity, accuracy, precision, LOD, LOQ. Linearity, accuracy and precision were found to be acceptable over the ranges of 100-300 -µg ml^{-1} for bambuterol HCl. It can be conveniently adopted for routine quality control analysis.

KEYWORDS

ICH Guidelines;
Validation;
Column liquid chromatography;
Pharmaceutical Preparations;
Bambuterol HCl.

INTRODUCTION

Bambuterol HCl 5-[(CRS)-2-[(1,1-dimethylethyl) amino]-1-hydroxyethyl]-1,3-phenylene bis (dimethyl carbamate)hydrochloride. is a drug used for the treatment of asthma, breathing difficulties due to a narrowing of the airways (bronchospasm), and for chronic obstructive pulmonary disease. The structure of the drug is shown in figure 1. One such combination contains 20mg of bambuterol HCl. The literature revealed no method was available for simultaneous determination of this drug in such pharmaceutical preparation by HPLC. Therefore an HPLC method was developed for determination of bambuterol HCl from their combined dosage form[1,2,5]. The method described is simple, fast, precise and accurate for simultaneous determination of bambuterol HCl from pharmaceutical preparation.



Bambuterol HCl ($C_{18}H_{29}N_3O_5$.HCl)

Figure 1: Structures of bambuterol HCl

Chemicals and reagents

Standards were supplied from Accutest lab., Mumbai, India. Bambudil-20 tablets manufactured by Cipla, India was procured from the market. Acetonitrile and potassium dihydrogen orthophosphate were from Qualigens. Double distilled water was employed

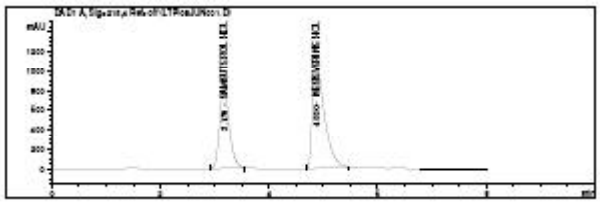


Figure 2: Chromatogram of bambuterol HCl with mebeverine HCl(internal standard) in standard preparation

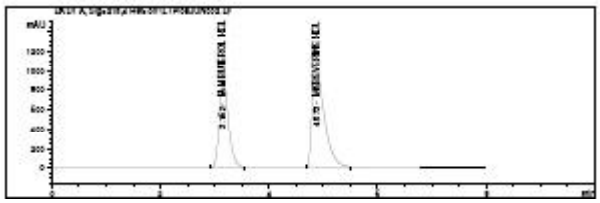


Figure 3: Chromatogram of bambuterol HCl with mebeverine HCl (internal standard) in sample preparation

throughout the work. All dilutions were performed in standard volumetric flasks.

EXPERIMENTAL

Method development and optimization of chromatographic conditions

To develop a suitable LC method for the analysis of Bambuterol HCl in their combined dosage form, different mobile phases were tried. The criteria employed for selecting the mobile phase for the analyses of the drugs were cost involve, time required for the analysis, better separation of drugs. Chromatographic separation was preformed with Agilent 1100 series High performance liquid chromatography having HPLC isocratic pump, equipped with auto sampler and a photo-diode array detector. The uv spectrum of bambuterol HCl was scanned on photo diode array detector for selecting the working wavelength. Peak purity of bambuterol HCl was checked using photo diode array detector. Chromatograms and data were recorded by means of chemstation software. An Inertsil C_{18} column (250mm×4.6 mm, 5µm particle) was used for the analysis. The mobile phase comprising of 0.02 M potassium dihydrogen orthophosphate: acetonitrile (50:50 υ/υ). The system was run at a flow rate of 0.7ml min^{-1}, 20µl of sample was injected in the chromatographic system and detection wavelength was set at 215nm for simultaneous determination of bambuterol HCl. A typical HPLC chromatogram for simultaneous determination of bambuterol HCl from pharmaceutical formulation is shown in figure 2 and figure 3.

Preparation of standard stock solutions

The stock solution of bambuterol HCl (2000µg ml^{-1}) was prepared by dissolving 100.2 mg of bambuterol HCl (99.9%) in water:acetonitrile (1:1) in a standard 50ml volumetric flask (solution A). Internal standard (mebeverine HCl) stock solution (2000µg ml^{-1}) was prepared by dissolving 99.8 mg of mebeverine HCl in water:acetonitrile (1:1) in a 50ml standard volumetric flask (solution B).

Working Standard Solution:

Transferred 10.0 ml of each stock solutions A and B to a 100 ml volumetric flask and diluted up to the mark with water:acetonitrile (1:1).

Sample preparation

Twenty tablets were weighed and their average weight was calculated. The tablets were crushed into a homogeneous powder and a quantity equivalent to ten tablet was transferred in a 100ml volumetric flask, dissolved in water:acetonitrile (1:1), and filtered through Whatman no. 41 filter paper. The filtrate (10ml) was quantitatively transferred to a 100ml volumetric flask, 10ml of internal standard solution was added to it, and solution was diluted up to the mark with water: acetonitrile (1:1).

RESULTS AND DISCUSSION

System suitability

System suitability tests are used to verify that the reproducibility of the equipment is adequate for the analysis to be carried out[3,4]. System suitability tests were performed as per the USP 31 to confirm the suitability and reproducibility of the system. The test was carried out by injecting 20-µl standard solutions of bambuterol HCl of strengths 200µg ml^{-1} using mebeverine HCl as an internal standard. This was repeated five times. The RSD values of bambuterol HCl was 0.28. The RSD values was found to be satisfactory and meeting the requirements of USP 31 (RSD less than 2.0 %). Theoretical plates, resolution, tailing factor were determined and are presented in TABLE 1.

Linearity

Linearity was evaluated by analysis of working stan

TABLE 1: Result of system suitability

Parameters	Bambuterol HCl	Mebeverine HCl (IS)
Resolution	-	4.84
Tailing factor	1.26	1.96
Theoretical plates	1770	2325

TABLE 2: Results of linearity

Analyte	Slope	Intercept	Correlation coefficient (r^2) (n =7)
Bambuterol HCl	105.3	-47.5	0.9998

TABLE 3: Results of assay experiment

	Bambuterol HCl
Drug found in mg/tablet (mean)	19.96
Mean %	99.93
RSD	0.34

TABLE 4: Accuracy of the method

Analyte	Initial conc. (mg)	Conc. added (mg)	Total conc. (mg)	Conc. found (mg)	RSD (%) n=3	Recovery (%)	% Bias
Bambuterol HCl	20	0	20	19.92	0.21	99.96	+0.04
	20	2	22	21.99	0.09	99.91	+0.09
	20	4	24	24.05	0.14	100.25	-0.25
	20	6	26	26.01	0.05	98.86	+1.14

dard solutions of bambuterol HCl of seven different concentrations[3,4]. The range of linearity was from 100-300 μg ml^{-1} for bambuterol HCl. The peak area ratio and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. The regression data obtained for the bambuterol HCl is represented in TABLE 2. The result shows that with-in the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration.

Limit of detection and limits of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively[3,4]. The LOD and LOQ of bambuterol HCl was experimentally determined by six injections of each drug. The LOD of bambuterol HCl was found to be 0.1μg ml^{-1}. The LOQ of bambuterol HCl was found to be 0.3μg ml^{-1}.

Precision

Repeatability was studied by carrying out system precision. System precision was determined from results for six replicate injections of the mixed standard solutions[3-4]. The relative standard deviations was less than 2%. Method precision was determined from results from ten independent determinations at 100% of the test concentrations of bambuterol HCl in the product. The RSD was found to be 0.49. Refer TABLE 3.

Accuracy

To study accuracy of the method, recovery experiment was carried out by applying the standard addition method. A known quantity of drug substance corresponding to 100%, 110%, 120% and 130% of the label claim of drug was added, to determine if there are positive or negative interferences from excipients present in the formulation[4]. Each set of addition was repeated three times .The accuracy was expressed as the percentage of analytes recovered by the assay. TABLE 4 lists the recoveries of the drug from a series of spiked concentrations. The results indicate the method is highly accurate for simultaneous determination of bambuterol HCl.

DISCUSSION AND CONCLUSION

Several mobile phases such as water-methanol, water-acetonitrile in different ratios were tried but good peak shape and good resolution between Bambuterol HCl and Mebeverine HCl was observed using the mobile phase mentioned in chromatographic conditions. The method after being completely validated showed satisfactory data for all the method validation parameters. The method was found to be specific. The low values of %RSD for Method precision suggested that the method is precise. Linearity evaluated for the analyte peak showed a good linear response over a wide range of concentration. The linearity, precision, accuracy of the method proves that the method is specific, accurate,easily reproducible and can be used for simultaneous determination of bambuterol HCl from pharmaceutical preparations.

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