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Simultaneous HPTLC determination of bambuterol HCl in pharmaceutical preparations

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

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

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

E-mail : preetibarde1974@gmail.com

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ABSTRACT

A simple, fast, precise and accurate high performance thin layer chromatographic method has been developed for the simultaneous determination of Bambuterol HCl using Mebeverine HCl as an internal standard. Chromatographic separation of these two drugs was performed using Silica gel 60F 254 as stationary phase with a mobile phase comprising of Toluene : Ethyl acetate: Methanol and Glacial acetic acid (70:20:10:2 v/v). Under these conditions the Rf for Bambuterol peak was 0.10. Quantitation was achieved by densitometric scanning at 254nm. The Rf values for Bambuterol HCl and Mebeverine HCl were 0.10 and 0.30 respectively. The proposed method was validated for linearity, accuracy, precision. The response to Bambuterol HCl was a linear function of concentration over the range 125-1000 μg mL^{-1} i.e. 25% to 200% of the working assay concentration. The method permits reliable quantification of Bambuterol HCl and good resolution and separation of Bambuterol HCl from internal standard of Mebeverine HCl. It can be conveniently adopted for routine quality control analysis.

KEYWORDS

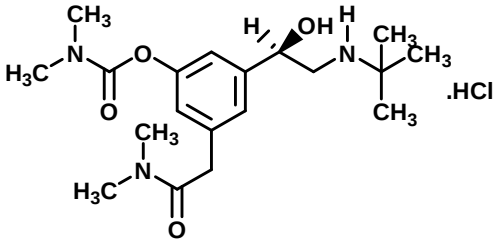
ICH Guidelines;
Validation;
HPTLC;
Pharmaceutical preparations;
Bambuterol HCl.

INTRODUCTION

Bambuterol HCl 5-[(CRS)-2-[(1,1-dimethylethyl) amino]-1-hydroxyethyl]-1,3-phenylene bis (dimethylcarbamate)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 Fig I. 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 HPTLC. Therefore an HPTLC 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.

Chemicals and reagents

Standards were supplied from Accutest lab., Mumbai, India. Bambudil-20 tablets manufactured by Cipla, India was procured from the market. Methanol, Ethyl alcohol, Toluene, Ethyl acetate and Glacial acetic acid used was from Rankem. All dilutions were performed in standard volumetric flasks.



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

Figure 1 : Structures of Bambuterol HCl

EXPERIMENTAL

Method development and optimization of high performance thin layer chromatographic conditions:

To develop a suitable HPTLC 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 using a CAMAG TLC system comprising of a Linomat-5 applicator and CAMG TLC scanner. Chromatography was performed on HPTLC silicagel 60F as stationary phase . The mobile phase comprising of Toluene: Ethyl acetate : Methanol: GAA (70: 20 : 10:2 *v/v)*., 5 µL of sample was spotted in the chromatographic system and detection wavelength was set at 254nm for simultaneous determination of Bambuterol HCl. The plate was developed to a distance of 70 mm using Toluene: Ethyl acetate : Methanol: GAA (70: 20 : 10:2 *v/v)*, as a mobile phase with Camag twin trough chamber. The developed plates were dried with the help of a drier. Evaluation of the plates was performed at =254nm using tungsten lamp with the help of Camag TLC scanner. The wavelength used for evaluation was selected after acquiring spectra of the standard and the sample. Typical chromatograms obtained from the standard and the sample are shown in figure 2.

Preparation of standard stock solutions

The stock solution of Bambuterol HCl (1000 µg mL^{-1}) was prepared by dissolving 25.04 mg of Bambuterol HCl (99.9 %) in ethyl alcohol in a standard 25 mL volumetric flask (solution A). Internal standard (Mebeverine HCl) stock solution (1000 µg mL^{-1}) was prepared by dissolving 25.14 mg of Mebeverine HCl in ethyl alcohol in a 25mL standard volumetric flask (solution B).

Working standard solution

Transferred 5.0 mL of each stock solutions A & B to a 10 mL volumetric flask, and mix.

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 100 mg of Bambuterol HCl was transferred in a 100mL volumetric flask, dissolved in ethyl alcohol, and filtered through Whatman no. 41 filter paper. The filtrate (5mL) was quantitatively transferred to a 10mL volumetric flask, 5mL of internal standard solution (solution B) was added to it, and mix.

RESULTS AND DISCUSSION

Linearity

Standard stock solution preparation

The stock solution of Bambuterol HCl (1000 µg mL^{-1}) was prepared by dissolving 100.24 mg of Bambuterol HCl (99.9 %) in ethyl alcohol in a standard 100 mL volumetric flask (solution A).

The linearity of response was determined by preparing 5 different concentrations of standard solution of assay ranging from 25% to 200% of the working assay concentration (500 ppm)[3,4].

The test was carried out by injecting 5-µL standard solutions of Bambuterol HCl of strengths 500µg mL^{-1} using Mebeverine HCl as an internal standard. Each of these solutions (5µl) was applied to a plate, the plate was developed and detector response to the different concentrations was measured. A graph of peak area against concentration was plotted. The plot was linear in the range 125 to 1000 µg mL^{-1} . The experiment was performed twice and mean was used for calculations. The data is summarized in TABLE 1.

The result shows that with-in the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration as evident from Graph 1. The correlation coefficient was found to be 0.99994.

Precision

Repeatability was studied by carrying out system precision. System precision was determined from results for

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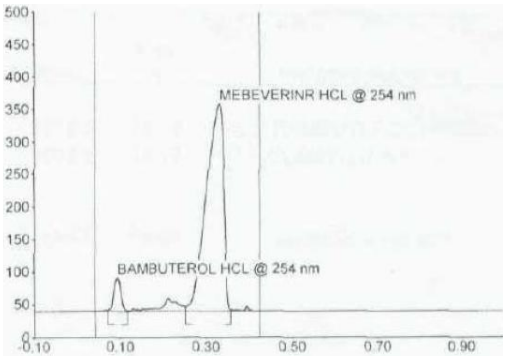
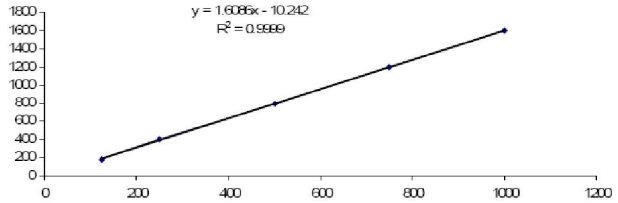


Figure 2 : Chromatogram of Bambuterol HCl with Mebeverine HCl (internal standard)



Graph 1 : Graph of Linearity/Range

six replicate injections of the mixed standard solutions [3-4]. The relative standard deviations were 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 0.34. Refer TABLE 2.

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% and 120% 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 III 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 Benzene, Toluene, Methanol, Ammonia 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.

TABLE 1 : Result of Linearity/ Range

Sr. No.	% Level	Conc. in ppm	Mean peak Area
1	25	125	182.48
2	50	250	399.10
3	100	500	797.7
4	150	750	1196.61
5	200	1000	1595.5
	Slope		1.6086
	Intercept		-10.242
	Correlation coeff. (r)		0.99994
	r^2		0.9999

TABLE 2 : Results of Assay experiment

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

TABLE 3 : Accuracy of the method

Analyte	Initial conc. (mg)	Conc. added (mg)	Total conc. (mg)	Conc. found (mg)	RSD (%) n= 3	Recovery (%)
Bambuterol HCl	500	0	500	499.92	0.21	99.98
	500	50	550	548.37	0.14	99.70
	500	100	600	599.01	0.05	99.84

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