Development and Validation of Ultraviolet Spectrophotometric Method for Estimation of Bambuterol Hydrochloride in Various Buffer and Solvent Systems

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Abstract

Introduction: Bambuterol is a long-acting β2-adrenoceptor agonist used in the treatment of asthma. It is a prodrug of terbutaline. Monograph of bambuterol hydrochloride describes non-aqueous titration for the estimation of bambuterol (B.P., 2001). Till date, simultaneous estimation of bambuterol hydrochloride and montelukast in distilled water, methanol, and chloroform by ultraviolet (UV) spectrophotometric method has been reported. Hence, the proposed method for the estimation of bambuterol hydrochloride in various buffers and solvent system was developed. **Materials and Methods:** Various solutions used during the study were freshly prepared. Distilled water, hydrochloric acid (Ramkem LR grade), potassium chloride (Qualigens AR grade), potassium dihyrogen phosphate (CDH LR), disodium hydrogen phosphate, sodium chloride (S.D. Fine Chem., extra pure), ethanol (Changshu Hongsheng Fine Chemical, AR), petroleum ether 60–80°C (Qualigen), and isopropyl alcohol (IPA) (Sisco Research Laboratories, extra pure) were used in the study. All the glasswares used were of borosilicate glass and were validated. Bambuterol hydrochloride was purchased from Yarrow Chem Products, Mumbai. UV-1800 Shimadzu spectrophotometer was used for the study. A simple, accurate, precise, and rapid UV spectrophotometric method was developed for the estimation of bambuterol hydrochloride in distilled water, pH 1.2 buffer, pH 6.8 buffer, pH 7.4 buffer, 90% v/v ethanol, IPA, and petroleum ether-ethanol (50%v/v) solution. **Results:** Bambuterol hydrochloride showed maximum absorbance at 264 nm in distilled water, pH 1.2 buffer, pH 6.8 buffer, pH 7.4 buffer, petroleum ether-ethanol (50%v/v) solution, and maximum absorbance at 265 nm in 90% ethanol and IPA. The proposed method is simple, rapid, and economical and R^2 value indicates good linearity. The method was validated statistically ($P < 0.05$). Further, the drug was found to be stable up to 24 h in various media studied. **Conclusion:** The method can be useful in the estimation of the content of bambuterol hydrochloride in the raw material and commercial tablet preparation. The method can be applied for the estimation of drug in dissolution studies at gastric and intestinal pH and estimation of drug content in oils and other water immiscible media.

Key words: UV spectrophotometric, method validation, bambuterol hydrochloride

INTRODUCTION[1-9]

Bambuterol hydrochloride is 5-[(1*RS*)-2-[(1,1-dimethylethyl) amino]-1-hydroxyethyl]-1,3-phenylene bis (dimethylcarbamate) hydrochloride.[1] Bambuterol is a long-acting β2-adrenoceptor agonist used in the treatment of asthma. It is a prodrug of terbutaline. Bambuterol causes smooth muscle relaxation, resulting in dilation of bronchial passages.[2-4] Monograph of bambuterol hydrochloride describes non-aqueous titration for the estimation of bambuterol.[1] Till date, simultaneous estimation

of bambuterol hydrochloride and montelukast sodium in distilled water,[5] chloroform,[6] and methanol[7] by ultraviolet spectrophotometric method has been reported. As far as

reported method in distilled water is concerned, calibration curve was developed for a range of 5–40 µg/ml, but in the, now, proposed method in distilled water, calibration curve was developed for a range of 10–1000 µg/ml.

The present study describes a simple, accurate, precise, and rapid method to determine the amount of bambuterol hydrochloride in distilled water, pH 1.2 buffer, pH 6.8 buffer, pH 7.4 buffer, 90% v/v ethanol, isopropyl alcohol (IPA), and petroleum ether-ethanol (50% v/v) solution. The calibration curve was developed for the concentration range of 10–1000 µg/ml for all the media except for IPA where the curve was developed for 10–500 µg/ml.

MATERIALS AND METHODS

Determination of λ_{max}

Unknown quantity of bambuterol hydrochloride was dissolved in freshly prepared distilled water, pH 1.2 buffer, pH 6.8 buffer, pH 7.4 buffer, 90% v/v ethanol, IPA, and petroleum ether-ethanol (50%v/v) solution. The resultant solution was filtered and scanned for the determination of λ_{max} against the respective blank. In distilled water, pH 1.2 buffer, pH 6.8 buffer, pH 7.4 buffer, petroleum ether-ethanol (50%v/v) solution, and bambuterol hydrochloride showed maximum absorbance at 264 nm, and in 90% v/v ethanol, IPA maximum absorbance was at 265 nm.

Preparation of stock solution and dilution

Stock solution of 1000 µg/ml of the drug was prepared by dissolving 50 mg of bambuterol hydrochloride in 50 ml freshly prepared distilled water, pH 1.2 buffer, pH 6.8 buffer, pH 7.4 buffer, 90% v/v ethanol, and petroleum ether-ethanol (50% v/v) solution, respectively, in a 50 ml volumetric flask. From the stock solution, dilutions of strength 10 µg/ml–1000 µg/ml were prepared in 10 ml volumetric flask. Similarly, stock solution of strength 500 µg/ml was prepared by dissolving 50 mg of bambuterol hydrochloride in 100 ml IPA in a volumetric flask. From the stock solution of IPA, dilutions of various strengths 10–500 µg/ml were prepared in 10 ml volumetric flask. The absorbance of the various dilutions prepared in distilled water, pH 1.2 buffer, pH 6.8 buffer, pH 7.4 buffer, and petroleum ether-ethanol (50% v/v) solution was measured at 264 nm. Similarly, absorbance of the various dilutions prepared in 90% v/v ethanol and IPA was measured at 265 nm. The absorbance for each dilution was measured in triplicate, and the mean value as well as standard deviation (SD) was calculated. The mean value was plotted against the concentration.

The method was further validated by the estimation of the content of bambuterol hydrochloride in the commercial preparation available in the market.

Estimation of drug content in commercially available product

The drug content of commercially available bambuterol hydrochloride tablets, strength 10 mg, was determined in IPA, ethanol (90% v/v), petroleum ether-ethanol (50% v/v) solution, distilled water, pH 1.2 buffer, pH 6.8 buffer, and pH 7.4 buffer. Twenty tablets were taken and weighed and finely powdered. The content equivalent to 10 mg of bambuterol hydrochloride was accurately weighed and transferred to a volumetric flask. The content was diluted with the given media to obtain a solution of strength 500 µg/ml. The resultant solution was sonicated for at least 5 min at 35°C. Thereafter, the solution was kept aside for 30 min to equilibrate and then filtered through Whatman filter no. 41. The absorbance of the filtrate was measured at respective λ_{max} [Table 1].

Limit of detection (LOD), limit of quantification (LOQ), precision, and stability[5,10-12]

LOD, which is the lowest amount of analyte in the sample, which can be detected but not necessarily quantitated under stated experimental conditions, was determined by the formula:

LOD=3.3*σ/S

Where σ = the SD of response and
S is the slope of the calibration plot.

Limit of quantification (LOQ), which is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy, was calculated from the formula:

LOD=10*σ/S

Precision

The intraday precision of the developed method was evaluated by analyzing sample ($n = 6$) of a particular concentration of bambuterol hydrochloride in different media on the same day. The interday precision was evaluated from the same concentration on next day.

Stability

The absorbance of the drug bambuterol hydrochloride for the highest dilution was also measured after a gap of 24 h to determine the stability of the drug in all the media.

Statistical analysis

Origin 9.0 software was used for statistical analysis. Where the studies have been done in triplicate, the data represent the mean ± SD. The statistical analysis was performed

using ANOVA. A difference below the probability level was considered as statistically significant ($P < 0.05$).

RESULTS

The λ_{max} in distilled water, pH 1.2 buffer, pH 6.8 buffer, pH 7.4 buffer, and petroleum ether-ethanol (50%v/v) solution was found to be 264 nm, whereas λ_{max} for 90% v/v ethanol and IPA was 265 nm [Table 2]. The statistical analysis of the data obtained from the estimation of bambuterol hydrochloride in all the media indicates the high level of precision of the proposed method as evident from low standard error, S,D and % real SD (RSD) values [Tables 2 and 3]. The spectra of bambuterol hydrochloride in distilled water, pH 7.4 Buffer, and 90% v/v ethanol are shown in Figures 1-3, respectively. The plot of mean absorbance against the concentration of pH 6.8 buffer, pH 7.4 buffer, and IPA is shown in Figures 4-6, respectively.

The value of R^2 ($P < 0.05$) indicates that a linear relationship exists between mean absorbance and concentration of bambuterol for all the buffer and solvent systems. The value of standard error for intercept and slope was found to be

within acceptable limit [Table 2]. The representative linear equation for each of the buffer and solvent system is provided in Table 4.

The value of LOD and LOQ indicates that low concentration of drug can be detected and quantified in all the buffer and solvent system studied in the proposed method [Table 2].

The interday and intraday precision data indicate that the proposed method is repeatable. The values of SD and % RSD is low and within acceptable limit <2% [Table 3].
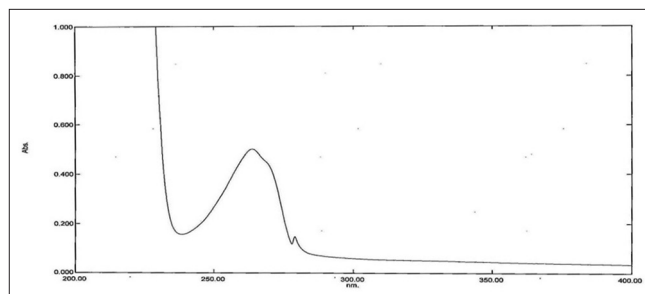

Figure 1: Spectrum of bambuterol in distilled water

Table 1: Assay results for the commercial pharmaceutical formulation in various buffer and solvent systems (*n*=3)

Media	Theoretical drug content (µg/ml)	Practical drug content (mean) (µg/ml)	Percentage drug content (mean) (%)	SD
IPA	500	517	103.4	0.494
Ethanol (90% v/v)	500	509	101.8	0.282
Petroleum ether-ethanol Solution (50%v/v)	500	516.5	103.3	0.544
Distilled Water	500	516	103	0.346
pH 6.8 buffer	500	514.8	102.96	0.636
pH 7.4 buffer	500	509	101.8	0.2828
pH 1.2 buffer	500	519	103.8	0.4242

SD: Standard deviation

Table 2: Result of analysis of data for the estimation of drug in various buffer and solvent systems (*n*=3)

Media	Wavelength of measurement	Intercept value	Standard error	Slope value	Standard error	R²	LOD µg/ml	LOQ µg/ml
Distilled water	264 nm	0.02573	0.00476	0.00117	1.2618E-5	0.9984	7.98	24.188
pH 1.2 buffer	264 nm	0.0273	0.00265	0.00109	3.19E-6	0.9999	14.98	45.41
pH 6.8 buffer	264 nm	−0.00109	0.00501	0.00108	1.1708E-5	0.9988	19.43	58.89
pH 7.4 buffer	264 nm	0.00751	9.005E-4	0.00111	5.48898E-6	0.99976	4.19	12.7
90% V/V ethanol	265 nm	0.00574	0.00199	0.00111	6.434E-6	0.9996	4.19	12.7
IPA	265 nm	0.01196	0.00471	0.00123	1.1298E-5	0.99933	3.78	11.46
Petroleum ether-ethanol solution (50%v/v)	264 nm	0.00461	0.00523	0.00111	1.3468E-5	0.99808	6.3	19.9

IPA: Isopropyl alcohol, LOD: Limit of detection, LOQ: Limit of quantification
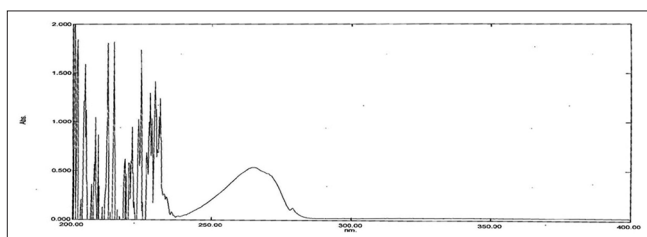
Table 3: Interday and intraday precision of the method in various buffer and solvent systems ($n=6$)

Media	Interday		Intraday	
	% mean assay±SD	%RSD	% mean assay±SD	% RSD
Distilled water	102.05±0.06364	0.062	101.2±0.9899	0.978
pH 1.2 buffer	103.57±0.060811	0.058	103.3±0.7707	0.746
pH 6.8 buffer	98.345±1.9445	1.97	98.27±1.775	1.806
pH 7.4 buffer	100.47±0.9121	0.90	100.32±0.968	0.96
90% v/v ethanol	96.730±0.9475	0.979	96.43±0.8061	0.835
IPA	101.57±1.3081	1.287	101.32±1.096	1.08

SD: Standard deviation, RSD: Real standard deviation, IPA: Isopropyl alcohol
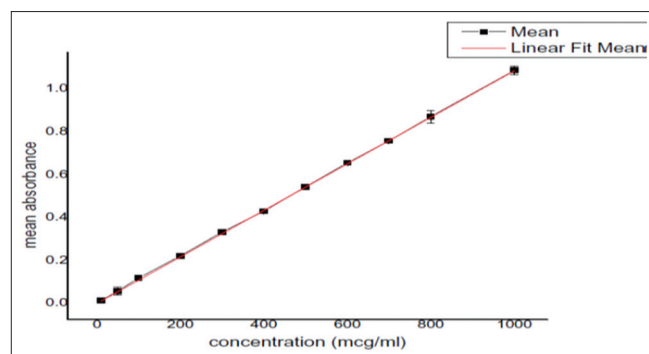
Table 4: Regression equation obtained by the proposed method for various buffer and solvent systems

Media	Wavelength of measurement	Regression equation	R^2
Distilled water	264 nm	Y = 0.00117x + 0.02573	0.9984
pH 1.2 buffer	264 nm	Y = 0.00109x + 0.0273	0.9999
pH 6.8 buffer	264 nm	Y = 0.00108x-0.00109	0.9988
pH 7.4 buffer	264 nm	Y = 0.00111x + 0.00751	0.99976
90% v/v ethanol	265 nm	Y = 0.00111x + 0.00574	0.9996
IPA	265 nm	Y = 0.00123x + 0.01196	0.99933
Petroleum ether-ethanol solution (50%v/v)	264 nm	Y = 0.00111x + 0.00461	0.99808
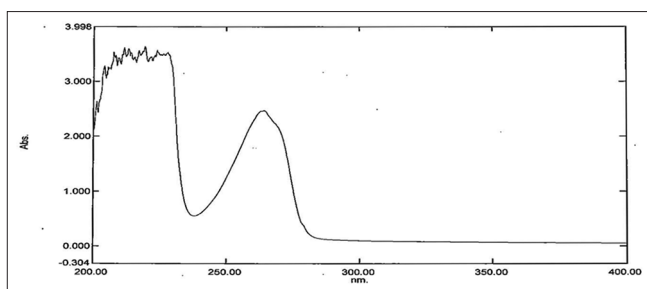
Figure 2: Spectrum of bambuterol in pH 7.4 buffer

Figure 4: Plot of mean absorbance against concentration for pH 6.8 buffer

Figure 3: Spectrum of bambuterol in 90% v/v ethanol

The method was found to be useful in the estimation of the content of bambuterol hydrochloride in the commercial tablet preparation (strength 10 mg) available in the market [Table 1].

The stability of the drug in each of the media was established by measuring the absorbance of the dilution samples after 24 h. It was found that there was no significant change in the readings of the absorbance for the given dilution even after 24 h in each of the medium.

DISCUSSION

From the studies, it can be concluded that the proposed method is simple, rapid, and economical and correlation coefficient indicates good linearity. Method validation has been demonstrated by a variety of tests for linearity, precision, and stability. The method can be applied for the estimation of drug in dissolution studies at stimulated gastric and intestinal pH and for the estimation of drug in dissolution diffusion studies at physiological pH.

The method can also be used for the estimation of the drug content in raw material and finish product. The studies conducted in non-aqueous solvent system can be useful in the estimation of drug content in oils and other water immiscible media.
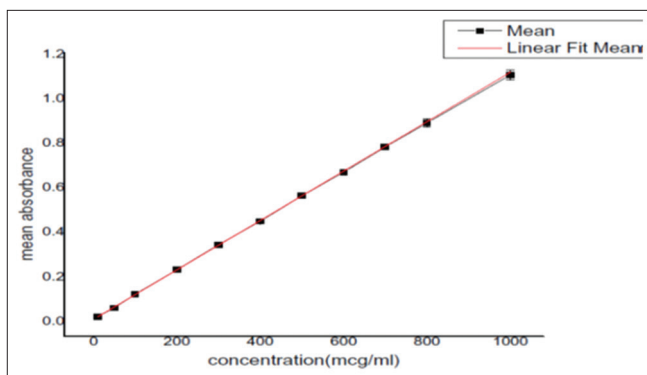
Figure 5: Plot of mean absorbance against concentration for pH 7.4 buffer
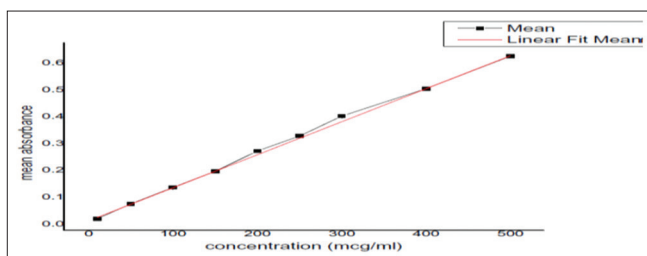
Figure 6: Plot of mean absorbance against concentration for isopropyl alcohol

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