Original Article

DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF CLENBUTEROL HYDROCHLORIDE IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

Objective: The objective of the present work is to develop a simple, rapid, economic UV spectrophotometric method for quantification of clenbuterol hydrochloride in bulk and pharmaceutical formulation as per ICH guidelines.

Methods: A UV spectrophotometric method has been developed using water as solvent to determine the Clenbuterol hydrochloride in bulk and pharmaceutical dosage formulation. The λ_{max} of Clenbuterol hydrochloride in water was found to be 242 nm.

Results: The drug was proved linear in the range of 10-50μg/ml and exhibited good correlation coefficient (R^2 = 0.9987) and excellent mean recovery (98-100%). The %RSD for intra-day and inter-day precision was found to be 0.053997676 and 0.359081556 respectively. The LOD and LOQ of clenbuterol hydrochloride was found to be 3.704448 and 11.2256 respectively. This method was successfully applied to clenbuterol content in marketed brands and the results were in good agreement with the label claims.
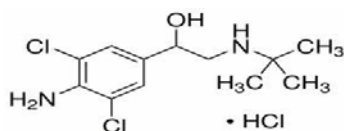
Conclusion: The method was validated for linearity, precision, repeatability and reproducibility. The obtained results proved that the method can be employed for the routine analysis of clenbuterol in bulks as well as in commercial formulations.

Keywords: Clenbuterol hydrochloride, UV, Water, Method development, Validation

INTRODUCTION

Clenbuterol hydrochloride ($C_{12}H_{19}Cl_3N_2O$), 1-(4-amino-3,5-dichlorophenyl)-2-(tert-butylamino)ethan-1-ol hydrochloride, is a hydrochloride that is the mono-hydrochloride salt of clenbuterol. It has a role as a bronchodilator agent, a beta-adrenergic agonist and a sympathomimetic agent [1]. Clenbuterol is a sympathomimetic amine used by sufferers of breathing disorders as a decongestant and bronchodilator. It is most commonly available as the hydrochloride salt, clenbuterol hydrochloride [2]. Amongthe various method available for the determination of drugs, spectrophotometry continues to be very popular, because of their simplicity, specificity and low cost. This study presents a new spectrophotometric method for the determination of clenbuterol hydrochloride in bulk and pharmaceutical formulations. It is effective in improving peak expiratory flow rate (PEFR) in asthma patients [3]. Clenbuterol is also a performance-enhancing drug as it stimulates the central nervous system and improves oxygen transport. Clenbuterol elicits cardio protective functionality in patients with dilated cardiomyopathy [4].

Fig. 1: Structure of clenbuterol hydrochloride

MATERIALS AND METHODS

Materials

Instrument used

A double beam UV-visible spectrophotometer (Shimatzu, model 1800) was used for recording of spectra and measuring absorbance.

An electronic analytical weighing balance (Shimadzu, AY 220) were used in this study.

Chemicals used

Clenbuterol hydrochloride pure drug was a gift sample from Vamsi Labs Ltd., Solapur. Distilled water is used as solvent.

Methods

Preparation of standard stock solution

Accurately weighed 10 mg of clenbuterol hydrochloride was transferred to a 100 ml volumetric flask, dissolved in 20 ml of distilled water by shaking manually. The volume was adjusted with the same up to the mark to give the final strength (100μg/ml).
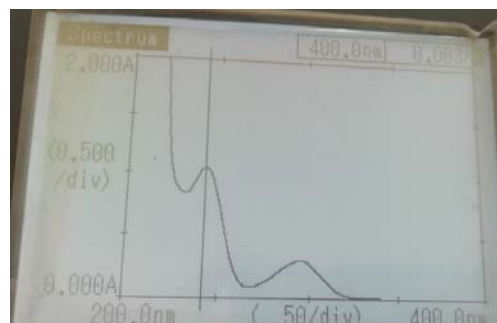
Fig. 2: UV spectrum of clenbuterol hydrochloride at 242 nm

Selection of wavelength for analysis of clenbuterol hydrochloride

Appropriate volume 5 ml of standard stock solution of clenbuterol hydrochloride was transferred into a 10 ml of volumetric flask,

diluted to a mark with distilled water to give concentration of 50µg/ml. The resulting solution was scanned in the UV range (200-400 nm), clenbuterol hydrochloride showed absorbance maximum at 242 nm (fig. 2).

Validation of the method

The method was validated as per ICH guidelines in terms of linearity, accuracy, precision, sensitivity, ruggedness and robustness.

Linearity

Different aliquots of clenbuterol hydrochloride in the range 1-5 ml were transferred into series of 10 ml volumetric flasks, and the volume was made upto the mark with distilled water to get concentrations 10, 20, 30, 40 and 50µg/ml, respectively. The solutions were analysedin the UV range on spectrophotometer. The spectrum was recorded at 242 nm. The calibration plot was plotted as absorbance vs. concentration.

Table 1: Linearity of clenbuterol hydrochloride

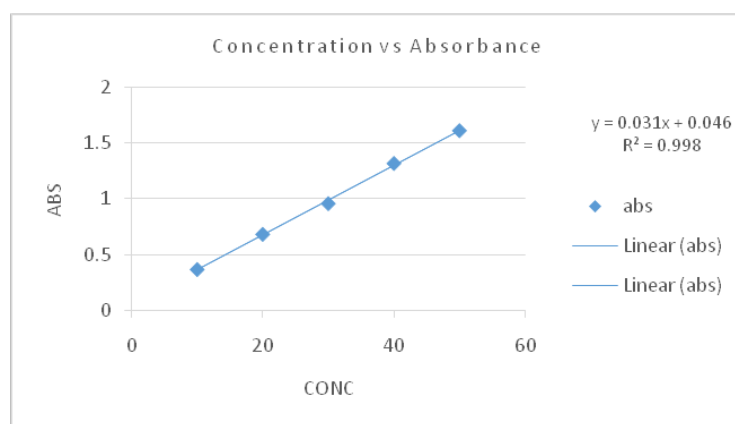| S. No.	Concentration (µg/ml)	Absorbance
1	10	0.364
2	20	0.68
3	30	0.956
4	40	1.317
5	50	1.611

Fig. 3: Calibration curve of clenbuterol hydrochloride

Table 2: Recovery studies

Concentration (µg/ml)	% of standard spiked to sample	Total including spiked sample (µg/ml)	Spiked sample determined (µg/ml)	% of drug recovered
100	80	16	15.81	98.81
100	100	20	19.74	98.70
100	120	24	23.9	99.58

Table 3: Precision studies

S. No.	Concentration (µg/ml)	Intra-day precision	Inter-day precision
1	30	0.956	0.931
2	30	0.957	0.933
3	30	0.957	0.926
4	30	0.956	0.935
5	30	0.956	0.932
6	30	0.956	0.935
	Average	0.956333333	0.932
	Standard deviation	0.000516398	0.00334664
	% RSD	0.053997676	0.359081556

Accuracy

To the preanalyzed sample solutions, a known amount of standard stock solution was added at different levels, i.e. 80%, 100% and 120%. The solutions were reanalysed by the proposed method.

Precision

Precision of the method is studied as intra-day precision and inter-day precision. Intra-day precision was determined by analysing the solution of known concentration i.e. 30 µg/ml six times in the day. Inter-day precision was determined by analysing the same solution for 3 d over period of week. The %RSD was found to be 0.053997676 and 0.359081556respectively. The %RSD values found to be less than 2.

Sensitivity

The sensitivity of clenbuterol hydrochloride by proposed method was estimated by limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were calculated using following formula-

LOD $= 3.3 \times \sigma/S$

LOQ $= 10 \times \sigma/S$

Where 'σ' is standard deviation of the response and 'S' is the slope of the corresponding calibration curve of analyte.

The linearity equation was found to be y = 0.0313x+0.0463. The LOD and LOQ of clenbuterol hydrochloride was found to be 3.704448 and 11.2256 respectively.

Robustness

Robustness of the proposed method is determined for 10µg/ml concentration of clenbuterol hydrochloride by analysis of aliquots from a homogenous slot for two different wavelengths, at two different temperature using same environmental conditions.

Table 4: Robustness studies

S. No.	Concentration (µg/ml)	Wavelength	Absorbance
1	10	241	0.284
2	10	243	0.290

Table 5: Ruggedness studies

S. No.	Concentration (µg/ml)	Absorbance (analyst 1)	Absorbance (Analyst 2)
1	10	0.294	0.293
2	10	0.290	0.295
3	10	0.297	0.291
4	10	0.289	0.290
5	10	0.292	0.295
6	10	0.295	0.287

Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from a homogenous slot by two analysts for 10µg/ml concentration of clenbuterol hydrochloride using same operational and environmental conditions.

RESULTS AND DISCUSSION

A validated simple, rapid, sensitive and accurate UV spectrophotometric method was developed for determination of clenbuterol hydrochloride in bulk and pharmaceutical formulation. Clenbuterol hydrochloride shows maximum absorbance at 242 nm in water. The clenbuterol hydrochloride follows Beer's Lambert's law in the linearity range of 10-50µg/ml. The linear regression of coefficient was found to be 0.9987 with equation y = 0.0313x+0.0463. Accuracy of drug was performed by recovery studies and it is near 100% i.e. 98-100%. The % recovery studies indicated that there is no interferents from the excipients present in the formulation. The LOD and LOQ was found to be 3.704448 and 11.2256 respectively.

CONCLUSION

The developed UV-spectrophotometric technique for quantification of clenbuterol hydrochloride in pharmaceutical formulation is quite simple, accurate, precise, reproducible and sensitive. The validation confirms that this is an appropriate method for their quantification in the different dosage form. It is also used in the routine quality control of the formulations containing this compound.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declare none

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