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 λmax 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 (R² = 0.9987) and excellent mean recovery (99-100%). 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 (C12H19Cl3N2O), 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]. Among the 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].

MATERIALS AND METHODS

Materials

Instrument used

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

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 up to the mark with distilled water to get concentrations 10, 20, 30, 40 and 50μg/ml respectively. The solutions were analysed in 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](image)

**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 | 0.95633333             | 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 0.359081556 respectively. 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 formulae:

\[
\text{LOD} = 3.3 \times \sigma/S
\]

\[
\text{LOQ} = 10 \times \sigma/S
\]
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                  |

### 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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