SPECTROPHOTOMETRIC ESTIMATION OF CHLORTHALIDONE IN PHARMACEUTICAL FORMULATION USING MBTH & FC REAGENT


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ABSTRACT
Two visible spectrophotometric methods have been developed for the determination of Chlorthalidone either in pure form or in their pharmaceutical formulations. The developed methods are based on reaction of Chlorthalidone with MBTH, FC Reagent. They are quantified spectrophotometrically at their absorption maximum at 540nm (Method A), 680nm (Method B). Beer’s law was obeyed in the concentration ranges of 10-50 µg/ml, 10-50 µg/ml and 10-50 µg/ml for the two methods respectively. The colors were found to be stable for more than 4 hours. The proposed methods were successfully applied for determination of the Chlorthalidone in their pharmaceutical formulations and the results compared favorably to that of reference methods, hence are recommended for quality control and routine analysis.

Key Words: Spectrophotometer, Pharmaceutical dosage forms, Chlorthalidone, MBTH, FC Reagent.

INTRODUCTION[1-5]
Chlorthalidone is chemically 2-chloro 5-(1-hydroxy 3-oxo 2,3-dihydro-1H-isoindol-1-yl)benzene 1-sulfonamide is widely used in anti hypertensive pharmaceutical preparations, reduces active sodium reabsorption and peripheral vascular resistance. Chlorthalidone is a diuretic drug used to treat hypertension. It is described as a thiazide diuretic. Compared with other medications of the thiazide class, chlorthalidone has the longest duration of action. It is often used in the management of hypertension and edema. Literature survey revealed that few
sophisticated analytical methods[6-8] have been reported for the estimation of chlorthalidone. There is no single method existed for simple colorimetric estimation of chlorthalidone. So the purpose of the described method is to develop and estimate simple, precise, accurate and reproducible spectrophotometric method for the estimation of chlorthalidone in pharmaceutical dosage form. The present work reports on simple, precise, colorimetric methods for the determination of chlorthalidone in pure and pharmaceutical formulations which has not been reported till date. The structure of chlorthalidone is given in Fig.1.

Literature survey reveals few HPLC9-11, GLC12, UV13 methods reported for the estimation of chlorthalidone. However there is no simple and accurate method reported for the determination of chlorthalidone in pharmaceutical formulation by Colorimetry.

![Graph showing absorbance vs concentration](image)

**Figure1: Structure of Chlorthalidone**

**MATERIALS AND METHODS:**

Instruments used: LAB India UV-3092 UV/Visible Spectrophotometer with 1 cm matched quartz cell was used for recording spectra and absorbance measurements.

Reagents: All reagents used were of analytical grade and were obtained from s.d. fine chemicals, Mumbai. chlorthalidone was kindly supplied by PRONTER PHARMA Pvt. Ltd. (India). chlorthalidone tablets were purchased from a local market.
EXPERIMENTAL

Preparation of standard chlorthalidone solution
Standard chlorthalidone 10mg was dissolved in 10ml of methanol and further dilutions were prepared in distilled water.
Working standard solution of 100µg/ml was prepared by further dilution of the above standard stock solution.

Method A: Using MBTH
Diluted standard solution of chlorthalidone 50µg/ml was scanned over the range of 400-800nm to select the λmax wavelength of 540nm was selected for analysis of chlorthalidone drug. To the drug solution add 1.0ml of 1% FeCl3 Reagent and 1ml of 0.5 % of MBTH. Keep aside for two minutes and makeup the volume to 10ml with distilled water and measure the absorbance of resulting yellow coloured solution against the reagent blank prepared in the same manner without drug solution.
A calibration graph was constructed by plotting the absorbance against the concentration of the drug as shown in Fig. 1.

Method B: FC Reagent
Diluted standard solution of chlorthalidone 50µg/ml was scanned over the range of 400-800nm to select the λmax wavelength of 680 nm was selected for analysis of chlorthalidone drug. To the drug solution add 1N FC Reagent and 2ml of 10% Na2CO3 keep aside for two minutes and makeup the volume to 10ml with distilled water and measure the absorbance of the resulting blue coloured solutions against reagent blank.
A calibration graph was constructed by plotting the absorbance against the concentration of the drug as shown in Fig. 2.
RESULTS AND DISCUSSION

Determination of absorption maximum

Chlorthalidone was treated with, MBTH and FC Reagent. To determine the absorption maxima, 100μg/ml of drug concentration was prepared from that dilution was made to 50μg/ml of drug solution. To this add 1ml of 1% FeCl₃ and 1ml of 1% MBTH and the volume was made up to the mark with distilled water. The λmax was seen at 540nm. Similarly 50μg /ml of drug solution were mixed with 1ml of1N FC reagent and 1ml of 10%-sodium carbonate. The volume was made up to the mark with distilled water. The λmax was seen at 680nm.

Fig-4: Absorption spectrum of chlorthalidone with MBTH Reagent

Fig-5: Absorption spectrum of chlorthalidone with FC Reagent

Optimization of reaction conditions

Optimum reaction conditions for quantitative determination of drug were achieved through a number of preliminary experiments. This was done by measuring the
absorbance’s of a series of solutions at 540 & 680nm (Method A & B ) by varying one and fixing the other parameters. These conditions were incorporated in the procedure.

Table1. Analytical parameters of spectrophotometric methods.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>METHOD A</th>
<th>METHOD B</th>
</tr>
</thead>
<tbody>
<tr>
<td>λmax</td>
<td>540nm</td>
<td>680nm</td>
</tr>
<tr>
<td>Beers law limit(μg/ml)</td>
<td>10-50 μg/ml</td>
<td>10-50 μg/ml</td>
</tr>
<tr>
<td>Sandell's sensitivity(μg/ml)</td>
<td>0.0029</td>
<td>0.0033</td>
</tr>
<tr>
<td>Regression equation*</td>
<td>Y=0.018x</td>
<td>Y=0.016x</td>
</tr>
<tr>
<td>Slope</td>
<td>0.018</td>
<td>0.016</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.995</td>
<td>0.9981</td>
</tr>
<tr>
<td>Stability of coloured products</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>% range of error</td>
<td>0.143</td>
<td>0.258</td>
</tr>
<tr>
<td>Limit of detection (μ/ml)</td>
<td>0.0946</td>
<td>0.783</td>
</tr>
<tr>
<td>Limit of quantification(μ/ml)</td>
<td>0.28</td>
<td>0.5218</td>
</tr>
</tbody>
</table>

**Y=bX where Y is the absorbance and X is the concentration of the drug in μg/ml * average of six determinations.

Stability
The resultant colored products of the proposed methods were found to be stable for more than four hours, which was sufficient time to make proper determination of chlorthalidone drug.

Table 2. Determination of chlorthalidone in formulations by the proposed and Reference Method

<table>
<thead>
<tr>
<th>Tablet Brand name</th>
<th>Labeled Amount</th>
<th>% Recovery</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>chlorthalidone</td>
<td>5mg</td>
<td>100.6%</td>
<td>99.23%</td>
<td>99.33%</td>
</tr>
</tbody>
</table>
Optical characteristics and validation of the method

Optical characteristics such as Beer’s law limits and Sandell’s sensitivity, for chlorthalidone, is given in Table 1.

Data of the regression analysis using the least squares method made for the calibration curves are also given in the same table. The accuracy and precision of the proposed methods were checked by analyzing six replicate samples within the Beer’s law range containing the same amount of each drug. The lower values of RSD indicate the good precision and reproducibility of the methods. The validity of the proposed procedure for the determination of drug in their pure state was checked by analyzing this drug using the proposed methods. The results obtained for pure drug were reproducible with low relative standard deviations (RSD). The LOD and LOQ for chlorthalidone by the proposed method were determined by using calibration standards.

Applicability of the method

The applicability of the proposed spectrophotometric methods for the assay of chlorthalidone was tested by analyzing various available commercial formulations. The results given in Table 2 of the analysis showed that the data are consistent with the label claim of the formulations. The calibration curves showed a linear response over the concentration ranges used in the general procedures. The RSD values for the reproducibility and recovery studies show that the methods are precise and accurate. In addition it is observed that there is no interference (Table 3) from the excipients used in the formulations. Hence, these methods can be adopted for the routine quality control of chlorthalidone in bulk and in formulations.

CONCLUSIONS

To summarize, our studies showed a possibility to use MBTH, FC reagents for the spectrophotometric determination of chlorthalidone. The determination procedures are characterized by low detection limits, simplicity, cheap and good reproducibility. The statistical analyses showed that the data from the proposed methods are in good agreement with those of the reported methods. The color reaction does not require stringent conditions nor any specific reagent or buffer. The colored species was stable for more than four hours, which is sufficient time for the analyst to perform the analysis. Moreover they do not require any pretreatment
of the drug and tedious extraction procedure. Hence, the data presented in the thesis by spectrophotometric methods for the determination of chlorthalidone in its pure and dosage form demonstrate that the proposed methods are accurate, precise and linear. Thus it can be extended for routine analysis of chlorthalidone in bulk and pharmaceutical dosage.

ACKNOWLEDGEMENTS
Authors are thankful PRONTER PHARMA drugs Pvt. Ltd. (India) for providing the gift samples of drug and also thankful to Chairman, Department of pharmaceutical Analysis, GNANA JYOTHI COLLEGE OF PHARMACY, Hyderabad for providing laboratory facilities to carry out this research work.

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