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**ORIGINAL ARTICLE** **OPEN ACCESS**

# Development and Validation of the UV-Spectrophotometric and TLC Method for Determination of Chlorthalidone in Bulk and Dosage Form

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**ABSTRACT**
*A simple, rapid, precise, and accurate UV Spectrophotometric method and thin layer chromatography-densitometry (TLC-Densitometry) had been developed for the determination of Chlorthalidone in bulk and tablet dosage forms. For the determination of Chlorthalidone in bulk and (200-400nm) in 1cm quartz cell in a double beam UV Spectrophotometer. The spectrophotometric detection was carried out at an absorption maximum of 227 nm using Methanol as solvent. The detector response for the Chlorthalidone was linear over the selected concentration range 5-25µg /ml with a correlation coefficient of 0.988 and equation for the regression curve was found to bey=0.0359x + 0.1184. The LOD and LOQ was 0.6179 and 1.8727µg/ml respectively. Normal phase thin layer chromatography plate (silica gel 60 F254) was used as stationary phase and acetone: cyclohexane (8:2) as mobile phase. This system gave a good resolution for Chlorthalidone (Rf value of 0.8). Determination was done by densitometry in the absorbance mode at 254 nm. The method was validated for linearity, LOD and LOQ. The linear regression data for the calibration plot showed a good relationship with r = 0.9994for chlorthalidone. According to the results, both methods were in accordance with good validation requirements.*
***Keywords:*** *Chlorthalidone, Validation, TLC, Densitometer*

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## INTRODUCTION

Chlorthalidone is a thiazide-like sulfonamide-derived diuretic that has been FDA approved since 1960 for the management of hypertension and oedema associated with congestive heart failure, it was first introduced in Switzerland in 1959. It has a longer duration of action but a similar diuretic effect at maximal therapeutic doses [1].

Chlorthalidone analysis as anti-hypertensive drug is of great interest, since hypertension is very common disorder, particularly in the past middle age. Accordingly, the development and validation of new analytical methods for estimation of antihypertensive drug is required. Literature survey reveals that there are various analytical methods for estimation of Chlorthalidone [2].

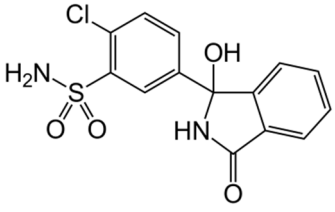


**Fig 1. Molecular Structure of Chlorthalidone**

## MATERIAL AND METHODS

### Instruments

For weighing, a calibrated weighing balance (Mettler Toledo, Model No. ME 203) of 1mg sensitivity was used. A PharmaspecUV-1700 UV-visible double beam spectrophotometer was used. All the glass wares and were made of borosilicate and were calibrated.

**Chemicals**

API- Chlorthalidone (CHL) pure drug was gifted by Emcure Pharmaceuticals. Chlorthalidone 12.5 mg strength was purchased from the local pharmacy in Nanded under commercially available brand name Thaloric (12.5mg) (Zuventus Healthcare), Methanol LR, Acetone, Cyclohexane was used in this study.

**UV SPECTROSCOPIC METHOD VALIDATION**

**Solvent Selection**

Chlorthalidone is freely soluble in Methanol so, it was used as the solvent.

**Preparation of Standard Stock Solution**

The standard stock solution Chlorthalidone was prepared by transferring accurately weighed 10 mg of Chlorthalidone into 10ml volumetric flask containing Methanol dissolved properly. Then volume was made up to the mark by using Methanol to give a concentration of 1000µg/ml. From this, 1ml of the solution was transferred to a 10ml volumetric flask and make up the volume with Methanol to give a concentration of 100µg/ml which is a standard stock solution and it is further diluted with Methanol to get concentration range of 2-10µg/ml [3].

**Determination of Absorption Maxima**

The standard stock solution of 10µg/ml was scanned in the range of 200-400 nm to determine the wavelength of Maximum Absorption. The drug showed Absorption maxima at 227 nm [4].

**Preparation of calibration curve**

Preparation of Calibration Curve For the preparation of calibration curve, the concentration of 2-10µg/ml was prepared by pipetting out 0.2, 0.4, 0.6, 0.8 and 1 ml of the 100µg/ml solution into 10 ml volumetric flasks and made up the volume with Methanol. The absorbance of each solution was measured at 227 nm against Methanol as blank. Calibration curve of the Chlorthalidone was plotted by taking the absorbance obtained on the y-axis and concentration of the solution on the x-axis. The curve showed linearity in the range of 5-25µg/ml with correlation coefficient 0.998 [5].

**Method Validation**

The developed method was validated as per ICH guidelines for the following parameters:

**Linearity**

0.5, 1, 1.5, 2.0, 2.5 ml of standard CHL solution was transferred into a series of 10 ml volumetric flasks. The volume was made up to the mark with Methanol to obtain the concentration of 5, 10, 15, 20,25 µg/ml. Then absorption of these solutions was recorded and the graph was plotted of absorption against concentration. The correlation coefficient(r2) of least square linear regression of CHL was calculated.

**Range**

The Range of the analytical method was decided from the interval between upper and lower level of calibration curve by plotting curve.

**Precision**

The precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same sample under the prescribed conditions. The precision of the method was determined in terms of repeatability and intraday and inter-day precisions. Intra-day and inter-day precision (Intermediate Precision) Intraday precision was determined by analyzing the drugs at concentrations (15µg/ml) and each concentration for three times, on the same day. Inter-day precision was determined similarly, but the analysis being carried out daily, for two consecutive days.

**Limit of Detection**

Detection limit was determined based on the standard deviation of absorbance of same concentration that is a standard solution of CHL (15µg/ml) and LOD calculated by LOD = 3.3(SD/S) Where, SD- standard deviation; S= slope of the curve.

**Limit of Quantification**

Quantification limit was determined based on the standard deviation of peak area of same concentration that is standard solution CHL (15µg/ml) prepared six times and LOQ calculated by LOD = 10(SD/S) Where, SD= standard deviation; S= slope of Curve.

**THIN LAYER CHROMATOGRAPHY METHOD VALIDATION [6-8]**

**Instrumentation and chromatographic conditions**

The TLC system consisted of a twin trough chamber (20 x 20 cm). Pre-coated silica gel 60 F254 TLC plates (20 x 20 cm, Merck, Darmstadt, Germany) were used as stationary phase. TLC plates were activated at 105$^{o}$C for 10 min prior to sample application. The standard and formulation samples of5 µL chlorthalidone was spotted manually on pre-coated TLC plates. The mobile phase consists of Acetone: Cyclohexane (8:2). Linear ascending development was carried out in twin trough chamber. The optimized chamber saturation time for mobile phase was 20 min, at room temperature; the length of chromatogram run was 6 cm. Densitometric scanning was performed on MAC MSW-508 in Absorbance

mode. The spots were analyzed at wavelength 254 nm. Evaluation was performed using linear regression analysis of peak areas.

**Analysis of Chlorthalidone marketed tablet formulation**

To determine the content of chlorthalidone in conventional tablets (label claim 12.5 mg chlorthalidone); twenty tablets were accurately weighed, average weight determined and ground to fine powder. A quantity of powder equivalent to 100 mg chlorthalidone was transferred into 100 mL volumetric flask containing 100 mL acetone, sonicated for 10 min and diluted to mark with same solvent to obtained 1000 µg/mL chlorthalidone. The resulting solution was centrifuged 3000 rpm for 5 min and was filtered using filter paper. From each solution then was diluted into 10 mL volumetric flasks with acetone and was obtained100 µg/chlorthalidone, 1 µL of chlorthalidone sample solution was applied on TLC plate followed by development and scanning at 254. The analysis was repeated for three times

**Validation of the TLC Method**

The proposed method was validated by linearity, detection limit, quantitative limit according to the ICH guidelines.

**Linearity**

The linearity of the TLC method was evaluated by analysis of nine standard solutions of chlorthalidone of concentrations 10, 20, 30, 40µg/ml. The solutions (5 µL) were applied to the same plate. The plates were developed using above-mentioned mobile phases (in thin layer chromatography section) and scanned.

**Detection Limit and Quantitative Limit Based on the Calibration Curve**

A specific calibration curve was studied using samples containing acetaminophen in the range of the detection limit, namely, 0.12, 0.25, and 0.35 µg spot.

The detection limit (DL) was calculated as the quantitative limit (QL) was calculated as where is the standard deviation of the response and is the slope of the calibration curve.

## RESULTS AND DISCUSSION

**Validation of U.V Method**

Determination of wavelength of maximum absorption the wavelength of maximum absorption was found to be 227 nm.

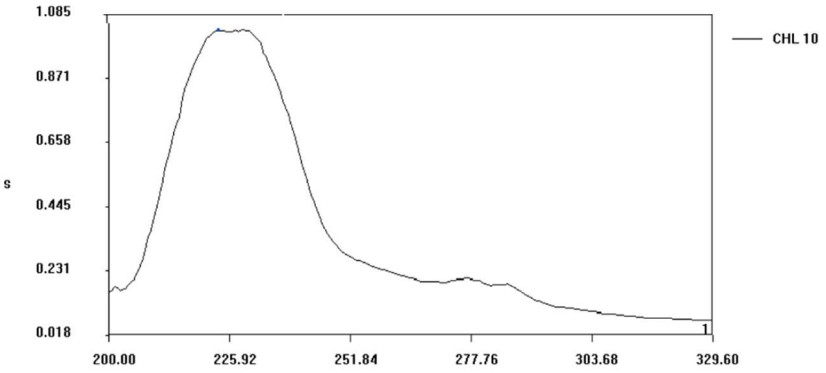


**Fig 2. Absorption Maxima of Chlorthalidone**

**Linearity and range**

The linearity of this method was determined at ranges from 5-25µg/ml for Chlorthalidone. The regression equation was found to be *Y=0.0824x + 0.0609*, $R^2$=0.9989.

| Sr.No | Conc.(µg/ml) | Absorbance |
|---|---|---|
| 1. | 5 | 0.4912 |
| 2. | 10 | 0.8848 |
| 3. | 15 | 1.2697 |
| 4. | 20 | 1.6952 |
| 5. | 25 | 2.1471 |

**Table No 1. Linearity Table**

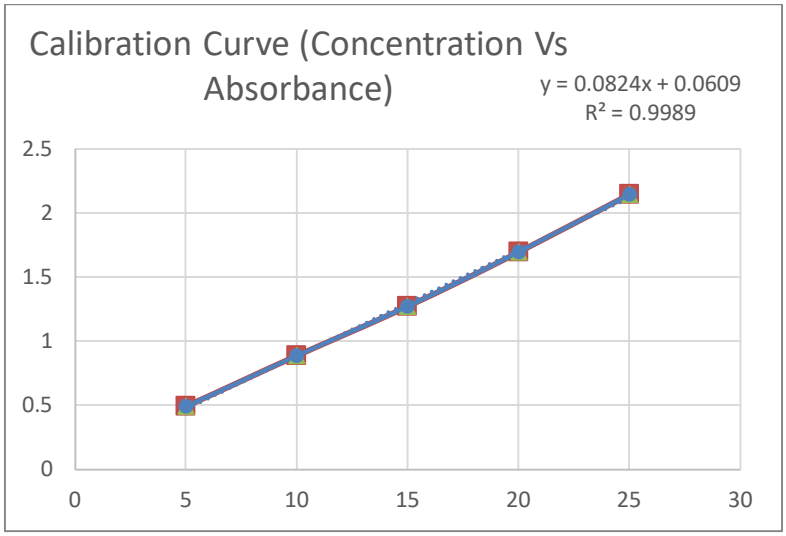


**Fig 3. Calibration curve of Chlorthalidone**

**Precision**

The precision (measurement of intra-day, inter-day, repeatability) results showed good reproducibility with the relative standard deviation (% RSD) below 0.05%. This indicated that method was highly precise.

**Intra-day Precision**

| S.No | Concentration (µg/ml) | Absorbance | SD | %RSD |
|---|---|---|---|---|
| 1 | 15 | 1.269 | | |
| 2 | 15 | 1.268 | 0.000748 | 0.05 |
| 3 | 15 | 1.269 | | |
| 4 | 15 | 1.270 | | |
| 5 | 15 | 1.268 | | |

**Table No 2. Intra-Day Precision**

**Inter-day Precision**

| S.No | Concentration (µg/ml) | Absorbance | SD | %RSD |
|---|---|---|---|---|
| 1 | 15 | 1.269 | | |
| 2 | 15 | 1.269 | | |
| 3 | 15 | 1.270 | 0.0007 | 0.05 |
| 4 | 15 | 1.270 | | |
| 5 | 15 | 1.268 | | |

**Table No 3. Inter-day Precision**

**Limit of Detection and Limit of Quantification**

| Drug | LOD(µg/ml) | LOQ(µg/ml) |
|---|---|---|
| Chlorthalidone | 0.6179 | 1.8727 |

**Table No 4. LOD and LOQ**

**Validation of TLC Method**

During the stage of method development different mobile phases were tried and the mobile phase comprising of acetone and cyclohexane (8: 2, v: v) was confirmed. This method showed that Rf value 0.81 was the best mobile phase for TLC of chlorthalidone analysis by using silica gel 60 F254 plate. A good linear relationship was obtained over the concentration range 10-50 µg/mL with linear regression Y = 66.85x - 9.7and coefficient correlation of 0.9994 (Fig.4). The LOD was found to be 0.025 µg/mL. The LOQ was found to be 0.080 µg/mL.

**Fig 4 Visualization in UV Chamber**

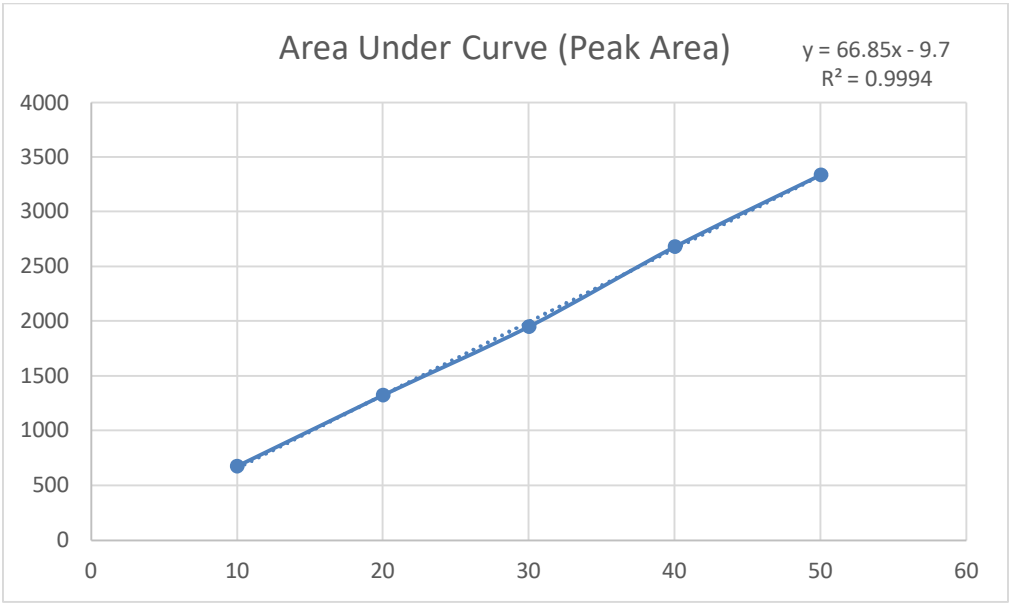


**Fig 5. Calibration curve (TLC) of Chlorthalidone**

**Detection Limit (DL) and Quantitative Limit (QL) Based on the Calibration Curve**

The limits of detection and the limit of quantification were 0.025 mg per spot and 0.080 mg/spot respectively.

| Sr. No | Parameters | Values |
|---|---|---|
| 1. | $R_f$ | 0.80 |
| 2. | Linearity range | 10-40 µg/spot |
| 3. | Straight line equation | 66.85x - 9.7 |
| 4. | Correlation coefficient (r) | 0.9994 |
| 5. | LOD (µg/ml) | 0.025 |
| 6. | LOQ (µg/ml) | 0.080 |

**Table No 6. Summary**

**CONCLUSION**

The UV-Spectrophotometric and TLC method was developed and it is found to be simple, accurate, precise, highly sensitive, reproducible and inexpensive. The proposed method was found suitable for determination of Chlorthalidone in bulk and its dosage form without any interference from the excipients. This method can be successfully applied for the routine analysis of Chlorthalidone in bulk and for dosage forms. Its advantages are the low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

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