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Research Article

HPLC Method Development and Validation for Estimation of Chlorthalidone in Tablet Dosage Form

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ABSTRACT

The present work relates to development and validation of simple, precise and rapid high-performance liquid chromatographic (HPLC) method for the analysis of Chlorthalidone in tablet dosage form. Method was developed for qualitative and quantitative estimation of Chlorthalidone in tablet dosage form. The chromatographic separation was achieved by using mobile phase 20 mM potassium dihydrogen orthophosphate buffer pH 4.0: methanol (30:70 %v/v) on HiQ Sil C₈ (4.6 mm*250 mm* 5µm) column. The mobile phase was pumped at a flow rate of 1.0 ml/min and the eluent was monitored at 230 nm. Retention time was 3.334±0.042 min. Linearity was observed in the concentration range of 5-30 µg/ml with a correlation coefficient (R²) of 0.9915. All the parameters were validated as per ICH guidelines and found to be suitable for routine analysis of drug in pharmaceutical dosage form.

Keywords: Chlorthalidone, Quantitative and Qualitative estimation, HPLC.

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INTRODUCTION

Chlorthalidone is a long acting thiazide-like diuretic of the sulfamoylbenzamide class that is devoid of the benzothiadiazine structure. Chlorthalidone directly inhibits sodium and chloride reabsorption on the luminal membrane of the early segment in the distal convoluted tubule (DCT) in the kidney. This leads to an increase in sodium, chloride, bicarbonate, and potassium secretion resulting in the excretion of water. In addition, this agent, like other thiazide diuretics, decreases calcium and uric acid secretion. In addition, this agent inhibits many carbonic anhydrase (CA) isoenzymes. Chlorthalidone is considered first-line therapy for management of uncomplicated hypertension as there is strong evidence from meta-analyses that thiazide diuretics such as Chlorthalidone reduce the risk of stroke, myocardial infarction, heart failure, and cardiovascular all-cause mortality in patients with hypertension. Chlorthalidone is used in the treatment of high blood pressure, edema and congestive heart failure [1-2]. Chlorthalidone is chemically described as 2-chloro-5-(2, 3-dihydro-1-hydroxy-3-oxo-1H-isindol-1-yl) benzenesulfonamide [3].

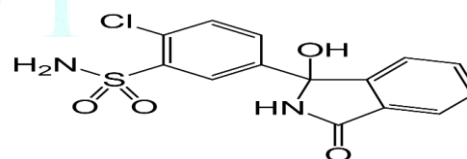


Figure 1: Chemical structure of Chlorthalidone

Literature review indicated that few UV spectrophotometric [4-6], HPLC [7-10] methods was reported for estimation of drug as individual or in combination with other drugs.

literature survey reveals that the data of reported HPLC methods is incomplete hence based on these observations we have developed HPLC method and validated as per International Conference on Harmonization Guidelines [12] for estimation of Chlorthalidone.

MATERIALS AND METHODS

Reagents and chemicals

Chlorthalidone tablets I.P labeled to contain Chlorthalidone 6.50 mg were purchased from local market. Methanol (AR grade), Methanol (HPLC grade), potassium dihydrogen phosphate, ortho-phosphoric acid, HPLC Grade water was used in study.

Preparation of standard stock solution:

Standard stock solution of drug was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml. From this solution 5 ml was taken in 50 ml volumetric flask and volume was made up with methanol to get concentration of solution 100 µg/ml. Further 1 ml of this solution was diluted to 10 ml with mobile phase to get concentration of solution 10 µg/ml.

Selection of detection wavelength:

From the standard stock solution (1000 µg/ml) further dilutions was made using methanol and scanned over the range of 200-400 nm and the spectra was obtained. It was observed that the drug showed linear, stable and considerable absorbance at 230 nm. Representative UV spectrum was obtained shown in Figure II.

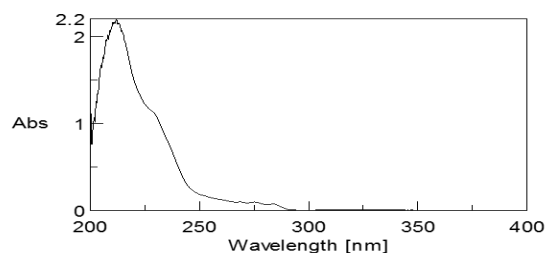


Figure II: UV Spectrum of Chlorthalidone (10 µg/ml)

Selection of mobile phase:

The standard solution of Chlorthalidone (10 µg/ml) was injected into the HPLC system. Solvents 20 mM potassium dihydrogen orthophosphate buffer (pH 4) and methanol (30:70v/v) was chosen as the mobile phase, which gave acceptable peak parameters. Representative HPLC chromatogram is given in Figure III

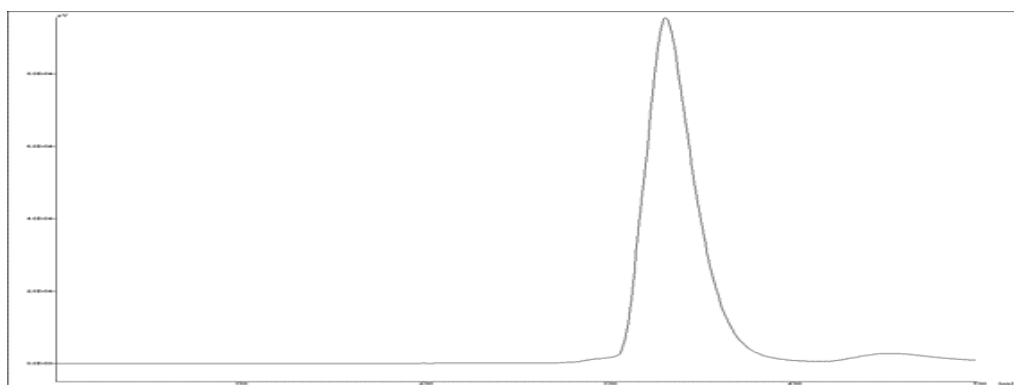


Figure III: Chromatogram of Chlorthalidone (10 µg/ml)

Preparation of 20 mM potassium dihydrogen phosphate buffer (pH 4) and mobile phase:

20 mM potassium dihydrogen phosphate buffer (pH 4) was prepared by dissolving 272 mg of potassium dihydrogen phosphate in 200 ml of HPLC grade water and pH of the solution was checked. pH was adjusted to 4 by orthophosphoric acid. Mobile phase was prepared by mixing potassium dihydrogen phosphate buffer (pH 4) and methanol in the ratio of 30:70 v/v. It was then filtered through 0.45µm nylon 6, membrane filter and sonicated for 15 min

Preparation of sample solution:

A content of 20 tablets was weighed and triturated to powder. A quantity of powder equivalent to 10 mg of Chlorthalidone was transferred to a 10 ml volumetric flask containing 10 ml of methanol. The mixture was ultrasonicated for 15 min and the resulting sample stock solution was filtered with Whatman filter paper 41 and the volume was made up with the methanol to get concentration of 1000 µg/ml. Further dilution were made to get concentration 10 µg/ml.

RESULTS AND DISCUSSION

Validation of analytical method [11]:

Linearity:

From the standard stock solution (1000 µg/ml) of Chlorthalidone, solution was prepared containing 100 µg/ml with methanol. This solution was further used to prepare range of solution containing six different concentrations by using mobile phase. The linearity (relationship between peak area and concentration) was determined by analyzing six solutions over the concentration range of 5-30 µg/ml. The equation of calibration curve was found to be $y = 172250x - 102173$. The peak area of drug was plotted against the corresponding concentrations to obtain the calibration curve as shown in Figure IV. The results obtained are shown in Table I

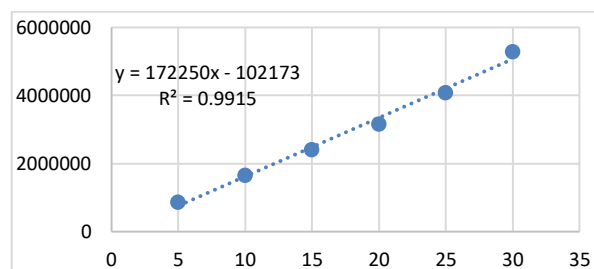


Figure IV: Calibration Curve of Chlorthalidone

Table I: Linearity study data for Chlorthalidone

| Replicates | Concentrations of Chlorthalidone | | | | | |
|------------|----------------------------------|----------|----------|----------|----------|----------|
| | 5 µg/ml | 10 µg/ml | 15 µg/ml | 20 µg/ml | 25 µg/ml | 30 µg/ml |
| | Peak Area | | | | | |
| 1 | 865843.2 | 1639680 | 2480303 | 3124996 | 4221263 | 5352837 |
| 2 | 862836 | 1679193 | 2409482 | 3176863 | 4077366 | 5267557 |
| 3 | 844065.7 | 1632744 | 2409482 | 3144434 | 4025417 | 5360332 |
| 4 | 860010.8 | 1639680 | 2373585 | 3226756 | 4024086 | 5318589 |
| 5 | 865843.2 | 1639680 | 2399873 | 3124996 | 4121263 | 5122837 |
| 6 | 892836 | 1660599 | 2380303 | 3176863 | 4077366 | 5287557 |
| Mean | 865239.2 | 1660599 | 2408838 | 3162485 | 4091127 | 5284951 |
| Std. Dev. | 15771.75 | 26072.96 | 38066.79 | 39194.09 | 73531.66 | 87192.51 |
| %RSD | 1.82 | 1.57 | 1.58 | 1.23 | 1.79 | 1.64 |

Precision:

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the Intra-day studies, 3 replicates of 3 different concentrations was analyzed in a

day and percentage RSD was calculated. For the inter-day variation studies, 3 different concentrations was analyzed on 3 consecutive days and percentage RSD was calculated. The results obtained are shown in Table II.

Table II: Intra-day and Inter-day variation studies data for Chlorthalidone.

| Conc. (µg/ml) | Intra-day precision * | | Inter-day precision * | |
|---------------|-----------------------|-------|-----------------------|-------|
| | Peak area | % RSD | Peak area | % RSD |
| 10 | 1823101.316 | 0.60 | 1852005.012 | 1.38 |
| | 1819680.109 | | 1805020.147 | |
| | 1839193.102 | | 1835120.596 | |
| 15 | 2709482 | 1.33 | 2719622.251 | 1.47 |
| | 2649951 | | 2647745.153 | |
| | 2709523 | | 2705902.457 | |
| 25 | 4521263.115 | 1.37 | 4392567.115 | 1.15 |
| | 4421356.005 | | 4457635.005 | |
| | 4528747.103 | | 4490727.103 | |

Limit of detection (LOD) and limit of quantitation (LOQ):

From the linearity data the LOD and LOQ was calculated, using the formula $LOD = 3.3 \sigma/S$ and $LOQ = 10 \sigma/S$, where

σ = standard deviation of the response at lowest concentration or standard deviation y intercept
S = average of slope of the calibration curve.

Table III: LOD and LOQ of Chlorthalidone

| Method | Avg slope | SD | LOD (µg/ml) | LOQ (µg/ml) |
|--|-----------|----------|-------------|-------------|
| 1. Using S.D of the response at lowest concentration | 145829.16 | 15771.74 | 0.357 | 1.082 |
| 2. Using S.D of y-intercept | 145829.16 | 35107.64 | 0.794 | 2.407 |

Specificity:

The specificity of the method was ascertained by peak purity profile studies. The peak purity values were found to be more than 0.996, indicating the no interference of any other peak of degradation product, impurity or matrix.

Accuracy:

To check accuracy of the method, recovery studies were carried out at three different levels around 50, 100 and 150 %. Basic concentration of sample solution chosen was 10 µg/ml. % recovery was determined from linearity equation. The results obtained are shown in Table IV

Table IV: Accuracy of Chlorthalidone.

| Level% | Sample (µg/ml) | Standard (µg/ml) | Area | %Recovery * |
|--------|----------------|------------------|-------------|----------------|
| 50 | 10 | 05 | 2799837.603 | 98.68-101.85% |
| | | | 2707995.207 | |
| | | | 2699289.618 | |
| 100 | 10 | 10 | 3533289.618 | 99.60-101.94% |
| | | | 3614057.529 | |
| | | | 3555662.151 | |
| 150 | 10 | 15 | 4473624.401 | 100.72-101.83% |
| | | | 4487091.839 | |
| | | | 4439326.564 | |

Assay:

Twenty tablets were weighed and crushed in mortar by pestle. Powder equivalent to 10 mg of Chlorthalidone was accurately weighed and transferred into a 10 ml volumetric flask and volume was made up with methanol as mentioned under section preparation of stock solution (1000 µg/ml). The volumetric flask was sonicated for 15 min to enable complete dissolution of Chlorthalidone and the solution was

filtered through 0.45 µm whatmann filter paper. From the filtrate further dilution was made with methanol to get 100 µg/ml solution. Finally this solution was further diluted with mobile phase to yield a concentration of 10 µg/ml. Sample solution was injected and area was recorded. The procedure was repeated for six times. Concentration and % recovery was determined from linear equation. The results obtained are shown in Table V.

Table V: Assay of marketed formulation

| Sr. No. | Peak Area | Amount Recovered (µg/ml) | %Recovery | Mean ± % RSD |
|---------|-----------|--------------------------|-----------|--------------|
| 1 | 1812734 | 9.93 | 100.8 | 100.26 |
| 2 | 1819993 | 9.97 | 98.64 | |
| 3 | 1826278 | 10 | 99.12 | |
| 4 | 1810798 | 9.92 | 101.6 | |
| 5 | 1855520 | 10.12 | 99.36 | |
| 6 | 1850574 | 10.15 | 101.4 | |

Robustness:

Robustness of the method was checked by carrying out the analysis under conditions during which flow rate ($\pm 0.5\%$ Composition), detection wavelength (± 1 nm) was altered and the effect on the area was noted. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters indicating that the method is robust.

Table VI: Robustness study.

| % RSD Found for Robustness Study (peak area) | | | | | |
|--|------|------|------------------------------------|-------|-------|
| Flow Rate | | | Detection Wavelength (± 1 nm) | | |
| 0.95 | 1 | 1.05 | 229 | 230 | 231 |
| 1.324 | 1.61 | 1.2 | 1.518 | 1.618 | 1.326 |

CONCLUSION

All the parameters were validated as per ICH guidelines for the method validation and found to be suitable for routine analysis of drug in pharmaceutical dosage forms. The result of linearity, accuracy, precision proved to be within limits with lower limits of detection and quantification. Robustness of method was confirmed as no significant difference was observed on analysis by subjecting the method to slight change in the method condition. Assay results obtained by proposed method are in good agreement.

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