WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 14, 975-987. Research Article ISSN 2277– 7105

METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF CHLORTHALIDONE IN BULK AND TABLET DOSAGE FORM BY UV SPECTROPHOTOMETRY

Greeshma S.*, Anagha Sathian, Jithendra P., Sahla, Shaheena Sharaf and Vishnu Sekhar

Department of Pharmaceutical Analysis, Prime College of Pharmacy, Prime Nagar, Near Govt. Polytechnic, Erattayal, Palakkad, Kerala, India.

Article Received on
25 Sept. 2021,

Revised on 15 October 2021,
Accepted on 05 Nov. 2021

DOI: 10.20959/wjpr202114-22297

***Corresponding Author**
Greeshma S.
Department of Pharmaceutical Analysis, Prime College of Pharmacy, Prime Nagar Near Govt. Polytechnic, Erattayal, Palakkad, Kerala, India.

ABSTRACT

The present research work discussed the development of a simple, sensitive, rapid, accurate, precise and economical UV Spectrophotometric method for the quantitative estimation of Chlorthalidone in bulk and pharmaceutical dosage form. Double beam UV visible spectrophotometer, Shimadzu, Model UV1800 with 1cm matched quartz cells and 0.1N sodium hydroxide as solvent was used, an absorption maximum was obtained at 229 nm. Developed method obeyed the Beer's law in the concentration range of 4 to 9µg/ml having line equation y = 0.059x – 0.012 with correlation coefficient of 0.996. Method was validated statistically as per ICH guidelines. Percentage recovery of the drug for the proposed method ranged from (99.52%) indicating no interference of the excipients. The developed method was validated with respect to linearity, precision, accuracy (recovery), limit of detection (LOD) and limit of quantitation (LOQ).

KEYWORDS: Chlorthalidone, UV Spectroscopy, Validation Parameters, Sodium Hydroxide.

INTRODUCTION

Pharmaceutical analysis is a branch of chemistry, which involves the series of process for the identification, determination, quantitation, and purification. This is mainly used for the separation of the components from the mixture and for the determination of the structure of the compounds.

Based upon the determination type, there are mainly two types of analytical methods. They are as follows:

Qualitative analysis

This method is used for the identification of the chemical compounds.

Quantitative analysis

This method is used for the determination of the amount of the sample.

Spectroscopy

Spectroscopy is the branch of science, which deals with the study of interaction of the electromagnetic radiation with sample substances. The interaction is mainly based upon the absorption or emission of the radiation by the sample. The absorbed or emitted radiation is in the form of quantum energy.[1,3]

Applications of UV spectroscopy

1. **Detection of impurities**

UV absorption spectroscopy is one of the best methods for determination of impurities in organic molecules. Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material

2. **Structural elucidation of organic compounds**

UV spectroscopy is useful in the structural elucidation of organic molecules, the presence or absence of unsaturation, the presence of hetero atoms.

3. **Quantitative analysis**

UV absorption spectroscopy can be used for the quantitative determination of compounds that absorb UV radiation. This determination is based on Beer's law [2]

4. **Qualitative analysis**

UV absorption spectroscopy can characterize those types of compounds which absorbs UV radiations. Identification is done by comparing the absorption spectrum with the spectra of known compounds. UV absorption spectroscopy is generally used for characterizing aromatic compounds and aromatic olefins.

It has also wide range of applications in the determination of dissociation constants of acids and bases, in chemical kinetics, In molecular weight determinations and it is also used as a hplc detector.[2,4,5]

Analytical parameters for validation

Validation may be defined as a process involving confirmation or establishing by laboratory

studies that a method/ system/analyst gives accurate and reproducible result for intended analytical application in a proven and established range.

Validation parameters

The parameters for method validation as defined by the ICH guidelines and other organisation are summarized below.

Selectively (Specificity)

It is the ability to asses unequivocally the analyte in the presence of components which may be expected to be present. For selectivity, analysis of blank samples of appropriate biological matrix should be obtained from at least six sources.

Linearity

It is the ability of the method within a given range to obtain test results in direct proportion to the concentration of analyte in the sample calibration curve of the analyte.

Range

It is the interval between the upper and lower of analyte, which is studied, the range is normally expressed in the same units as the test results obtained by the analytical method. The ICH guidelines specify a minimum of five concentration levels.

Precision

It is a measure of degree of repeatability of an analytical method under normal operation and it is normally expressed as % of relative standard deviation (%RSD). This involves

- Repeatability
- Reproducibility
- Intermediate precision

$$\% \text{ RSD} = 100\ S/X$$

Where, S= Standard deviation X= Mean

It is determined at three levels.

Repeatability

Precision of the method when repeated by the same analysis, same test method and under

same set of laboratory condition (reagent, equipments), within a short interval of time, the only difference being the sample.

Reproducibility

When the subjected method is carried out by different analysis in different laboratories using different equipments, reagents and laboratory settings and on Different days of variability of analytical results as function of analyst, day to day, laboratory to laboratory, equipment to equipment etc., using the sample from same homogenous batch**.**

Intermediate precision

It is determined by comparing the results of a method within the same laboratory but different days, analysis, equipments and reagents.

Accuracy

It is defined as the closeness of agreement between the actual (true) value and mean analytical value obtained by applying the test method for number of time. Accuracy is acceptable if the difference between the true value and mean measured value does not exceed the RSD values obtained for repeatability of the method.

Limit of Detection

LOD is defined as the lowest concentration of an analyte in a sample that can be detected but not quantified. LOD is expressed as a concentration at a specified signal to noise ratio.

S/N=2/1 or 3/1

Limit of Quantification

It is defined as lowest concentration of analyte in a sample that can be determine with acceptable precision and accuracy and reliability by a given method under stated experimental conditions.

S/N=10/1

Ruggedness

Degree of reproducibility of test results obtained by analyzing the same sample under variety

of normal test conditions such as different analysts, instrument, days, reagents etc.

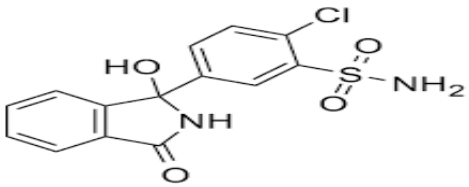
Robustness

It is the measure of the capacity of the analytical method to remain unaffected by small but deliberate variation in procedure. It provides an indication about variability of the method during normal laboratory conditions.

The aim of the present work is to develop and validate simple, specific, sensitive, accurate spectrophotometric method for estimation and evaluation of chlorthalidone in bulk and tablet dosage form.[8,9]

Chlorthalidone

Chlorthalidone is a thiazide-like diuretic used for the treatment of hypertension and for management of edema caused by conditions such as heart failure or renal impairment. Chlorthalidone improves blood pressure and swelling by preventing water absorption from the kidneys through inhibition of the Na+/Cl− symporter in the distal convoluted tubule cells in the kidney. The exact mechanism of chlorthalidone's anti-hypertensive effect is under debate, however, it is thought that increased diuresis results in decreased plasma and extracellular fluid volume, decreased cardiac output and therefore overall reduction in blood pressure. [10]



MATERIALS AND METHODS

Instruments

UV Spectrophotometer (SHIMADZU UV 1800)

Digital balance (electronic balance type AY-120)

Chemicals

1. NaOH- Spectro reagents and chemicals Pvt. ltd Edayar, Cochin
2. Chlorthalidone 6.25 mg Tablet
3. Chlorthalidone (Pure drug) Dr.Milton’s labs pvt.

Methods

Selection of solvent

Solubility of Chlorthalidone was checked in various solvents like methanol, ethanol, water and alkali hydroxides, the one which showed good spectrum and good solubility was selected as solvent of choice for determination of Chlorthalidone.

Preparation of standard stock solution

Standard Chlorthalidone 10 mg was weighed accurately and transferred in to volumetric flask it was dissolved properly in 0.1 N NaOH and made up to the mark to get a concentration of 100 microgram per ml.

Preparation of working standard solution

Working standard solution was prepared by series of dilutions of 0.4 - 0.9 ml of standard stock solution to 10 ml with 0.1N NaOH separately to get a concentration of 4 - 9 µg/ml of Chlorthalidone.

Selection of wavelength

After selection of appropriate solvent for Chlorthalidone and preparation of stock solution a solution containing 5 µg/ml of Chlorthalidone was prepared and scanned in the range of 200 to 400 nm against 0.1 N NaOH as blank.

Calibration curve of chlorthalidone

Working standard solution was prepared by series of dilutions of 0.4 - 0.9 ml of standard stock solution to 10 ml with 0.1 N NaOH to get concentration of 4 - 9 µg of Chlorthalidone. These solutions were scanned in the wavelength range of 200 - 400 nm and absorbance was measured at 229 nm against reagent blank. Calibration curve was prepared by plotting concentration Vs absorbance.

Analysis of tablet formulation

10 tablets were accurately weighed and triturate thoroughly to get fine powder. The powder equivalent to 10mg of Chlorthalidone was weighed and transferred into 100 ml volumetric flask. The contents of flask were dissolved in 50 ml of 0.1 N NaOH with aid of ultrasonication for 10 minutes. The solution was filtered through Whattmann filter paper no.41 and volume was made up to 100 ml with 0.1 N NaOH.

From the resultant solution, further dilutions were prepared with 0.1 N NaOH separately to

get final concentration of Chlorthalidone. The absorbance was measured at selected wavelengths and concentration of each analyte was determined with the equation obtained from calibration curve.[6]

RESULTS AND DISCUSSION

Selection of solvent

Solubility of the drug was checked in solvents like water, methanol, ethanol and alkali hydroxides. UV spectra of the drug in those solutions were recorded. Absorbance of the drug was higher and exhibited distinct λmax in 0.1 N NaOH and hence 0.1 N NaOH was selected. as the solvent for the further studies. There was no method using 0.1 N NaOH as the solvent.[9]

Selection of wavelength

From the stock solution of 100 µg/ml of chlorthalidone, a solution containing 5 µg/ml of chlorthalidone was prepared for scanning in the UV range of 200-400 nm against 0.1 N NaOH as blank. Chlorthalidone showed good absorption spectrum at 229 nm, where linear response was very good with maximum acceptable absorbance.[7]

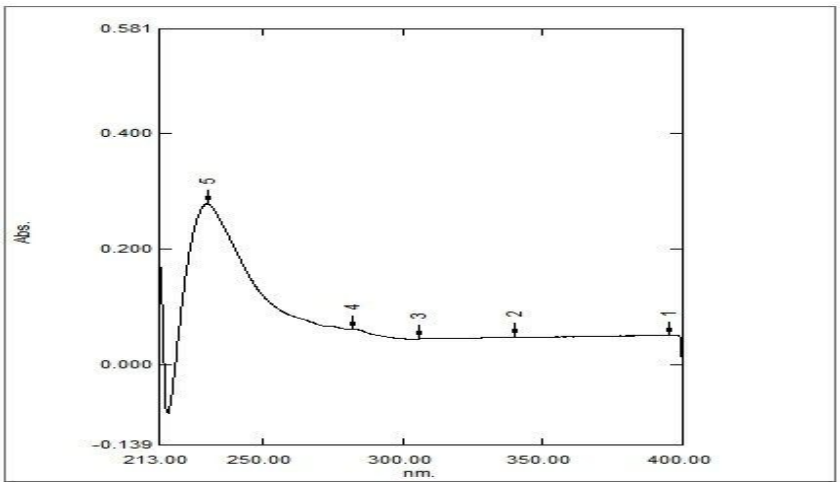


Figure 1: Calibration curve of chlorthalidone.

Working standard solution was prepared by series of dilutions of 0.4 - 0.9ml of standard stock solution to 10ml with 0.1 N NaOH to get concentrations of 4 - 9 µg/ml for Chlorthalidone. These solutions were scanned in the wavelength range of 200 - 400 nm and absorbance was measured at 229 nm against reagent blank. Calibration curve was prepared by plotting concentration versus absorbance.[12]

Table 1

Sl. no.	Concentration of Chlorthalidone (µg/ml)	Absorbance at 229nm (0.1 N NaOH)
1	4	0.214
2	5	0.278
3	6	0.332
4	7	0.346
5	8	0.474
6	9	0.530

Analysis of tablet formulation

10 tablets were accurately weighed and triturate thoroughly to get a fine powder. The powder equivalent to 10 mg of Chlorthalidone was weighed and transferred into 100 ml volumetric flask. The contents of the flask were dissolved in 50 ml of 0.1N NaOH with aid of ultrasonication for 10 minutes. The solution was filtered through whatmann filter paper no.41 and volume was made up to 100 ml with 0.1 N NaOH. From the resultant solution, further dilutions were prepared with 0.1 N NaOH separately to get final concentration of Chlorthalidone. The absorbance was measured at selected wavelengths and concentration of each analyte was determined with the equation obtained from the calibration curve[22] (figure 3) table 1.

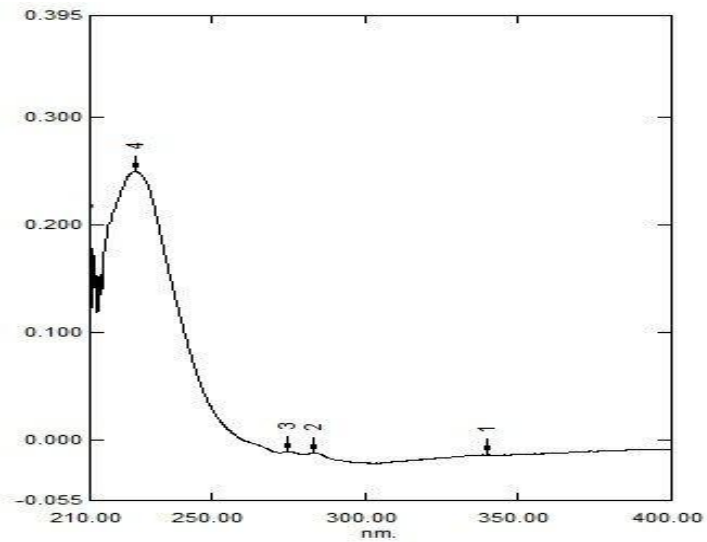


Fig. 2

Table 2

Formulation	Label claim	Amount estimated	% Amount estimated	%RSD
Chlorthalidone	6.25 mg	6.203 mg	99.2 %	1.8032

Validation of the method

Developed method was validated in terms of parameters like linearity and range, precision, LOD & LOQ, ruggedness, robustness & recovery studies.[10]

Linearity and Range

Linear regression data showed good correlation coefficient over a concentration range of 4 - 9 µg/ml. The absorbance of these solutions was noted at selected wave length 229 nm.

Calibration curve was plotted using the concentration and absorbance .The slope, intercept and co-relation co-efficient value were noted.[12]

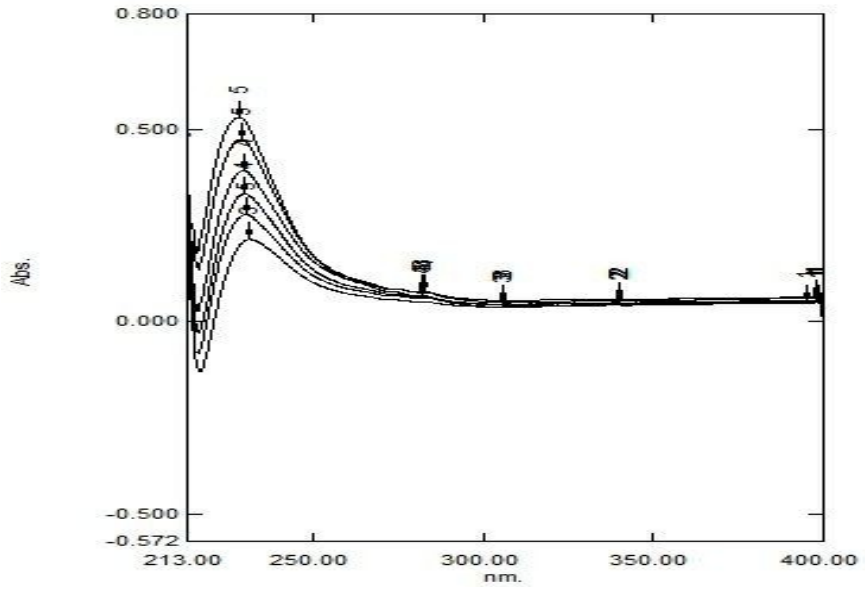


Fig. 3

Table 3

Sl. no.	Concentration of Chlorthalidone (µg/ml)	Absorbance at 229nm (0.1 N NaOH)
1	4	0.214
2	5	0.278
3	6	0.332
4	7	0.346
5	8	0.474
6	9	0.530

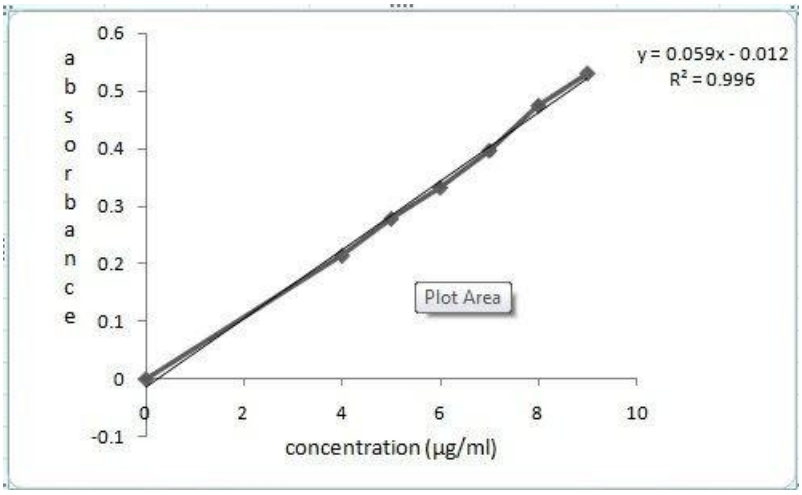


Figure 4

Table 4

Parameters	Values
λmax	229 nm
Linearity range	4 - 9 µg/ml
Regression equation	Y = 0.059 x - 0.012
Slope	0.059
Intercept	0.012
Correlation coefficient	0.996
LOD	1.495 µg/ml
LOQ	4.537 µg/ml
Molar absorptivity (mean)	553 L/mol/cm

LOD and LOQ

In this study LOD and LOQ were based on the standard deviation of response (σ) and the slope of the corresponding curve using the following equation

$LOD = 3.3\sigma/S$

$LOQ = 10\sigma/S$

Accuracy

The accuracy of the proposed method was determined by calculating the recoveries of Chlorthalidone by the standard addition method. It was determined by preparing solutions of different concentrations at 80%, 100% and 120% in which the amount of marketed formulation was kept constant and the amount of pure drug was varied. The amount of Chlorthalidone was estimated by applying obtained values to the regression line equation and the percentage RSD was also calculated.[15]

Table 5

Standard drug added (%)	Amount of pure drug added(µg/ml)	Concentration of sample present (µg/ml)	% Recovery	%RSD
80	4	5	99.52	1.033
100	5	5	99.92	1.028
120	6	5	100.83	0.547

Precision

Precision of the method was determined by performing interday variation, intraday variation and expressed in the forms of %RSD. In interday variation, the absorbance of working standard solutions of chlorthalidone was measured on three consecutive days. In intraday variation the absorbance were measured three times a day.[13,14]

Table 6

Particulars	Fortified amount (µg/ml)	Amount found(µg/ml)	%RSD
Repeatability	5	5.007	0.376
Reproducibility	5	4.06	1.5

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. In this method it was performed by changing temperature (i.e., room temperature and at 21˚C). The percentage purity of the drug was determined.[15]

Table 7

Component	Label claim	At 21° C		At room temperature	
		% amount estimated	% RSD	% amount present	% RSD
Chlorthalidone	6.25 mg	99.65	0.682	99.465	0.487

Ruggedness

Ruggedness is a measure of reproducibility of test result under normal expected operational conditions from laboratory to laboratory and from analyst to analyst. In the present study the determination of chlorthalidone was carried out by different analysts. The percentage purity of the drug was determined and %RSD was calculated.[16]

Table 8

Component	Label claim	Analyst 1		Analyst 2	
		%amount estimated	% RSD	%amount present	% RSD
Chlorthalidone	6.25 mg	98.52	0.887	99.15	0.62

CONCLUSION

A simple, rapid, precise and economical UV-spectrophotometric method has been developed for the quantitative estimation of Chlorthalidone in bulk and pharmaceutical formulation. The present work complied with our initial research objectives and successfully demonstrated the applicability of simple UV-spectrophotometric method for analysis of Chlorthalidone.

The method was developed and validated as per ICH guidelines and shown a high degree of sensitivity, selectivity, reproducibility and good recovery when compared with previously reported methods. Hence this method can be successfully and suitably acquired for routine quality control analysis of Chlorthalidone in bulk and pharmaceutical dosage form.

The promising result from the present research reveals the need of further extensive study of the drugs using the tremendous potential of various analytical instruments. The development of innovative methodologies will unquestionably expand future research capabilities in terms of shorter run times, high rugged and reproducible methods with less precision and high accuracy.

REFERENCE

1. Glenn L.jenkins; Adelbert M. Knevel; Frank. E. Digangi. Quantitative pharmaceutical chemistry sixth edition, 327-360.
2. Skoog, D. A, principles of instrumental analysis, CBS publishing and sons, 1988; 40-58.
3. Anees A Siddiqui; pharmaceutical analysis second edition, 2: 52 – 98.
4. R P. Khare. Analysis instrumentation an introduction, 59-82.
5. Rajesh Kumar Nema; S N Meyyanatham; Chandra Shekhar Sharma; Mahesh Varma; C S Bhan. A practical approach to pharmaceutical analysis instrumental and manual, 1-64.
6. Gurdeep R Chatwal, Sham K Anand. Instrumental method of chemical analysis, 2. 107-2.184.
7. Dr. A V Kasture, Dr. S G Wadodar, Dr. K R Mahadik, Dr H N More pharmaceutical analysis volume-2 instrumental methods, 169-181.
8. G.P.Carr, J.C.Wahlich, A practical approach to method validation in pharmaceutical

analysis, J. Pharm. Biomed. Anal, 1990; 86: 613–618.

9. ICH, Q2 (R1) Validation of Analytical Procedure: Text and Methodology, International Conference on Harmonization, 2005.
10. www.ncbi.nlm.nih.gov/books/NBK553174/
11. Narmeen S.AbdullahMedea A.HassanRebwar O.Hassan; Spectrophotometric determination of chlorthalidone in pharmaceutical formulations using different order derivative methods, Arabian Journal of Chemistry, 2017; 10(2): S3426-S3433.
12. Ranjit Singh and Rehman Z. current trends in forced degradation study for pharmaceutical product development. Journal of pharmaceutical and educational research, 2012; 2: 54-63.
13. Jitendra Kumar: ICH guidelines, Q1A (R2): Stability Testing of New Drug Substances and products (revision2), International Conference on Harmonization, 2013.
14. Mariko M, Toure H P: International Journal of Recent Scientific Research, 2016; 7(9): 13299-13301.
15. G.C Gomes, H.R.N. Salgado. Validation of UV spectrophotometric method for determination of Lomefloxacin in pharmaceutical dosage form. Lat. Am. J. Pharm, 2005; 24(3): 406-408.
16. Anindya Bagchi, P. Mukherjee: Development and validation of UV spectrophotometric method for estimation of Deflazacort in bulk drug and Pharmaceutical formulation; International Journal of Drug Development & Research; July-September, 2012; 4(3): 369-373.