QUANTIFICATION OF CHLORTHALIDONE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY UV, INFRARED SPECTROPHOTOMETRY AND SPECTROFLUORIMETRY

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In partial fulfilment of the requirements
for the award of the degree
MASTER OF PHARMACY
IN
PHARMACEUTICAL CHEMISTRY

Submitted by
Reg.No.261215709

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APRIL 2014

CERTIFICATE

This is to certify that the dissertation entitled "**QUANTIFICATION OF CHLORTHALIDONE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY UV, INFRARED SPECTROPHOTOMETRY AND SPECTROFLUORIMETRY**" submitted by ***Reg No*: 261215709** in partial fulfillment for the award of the degree of **MASTER OF PHARMACY** in **PHARMACEUTICAL CHEMISTRY** by the Tamil Nadu Dr.M.G.R.Medical University is a work done by him during the academic year 2013-2014 at the Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai 600 003.

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LIST OF ABBREVIATIONS

S.NO	ABBREVIATIONS	EXPANSION
1	Avg	Average
2	KBr	Potassium bromide
3	LOD	Limit of Detection
4	LOQ	Limit of Quantification
5	KSCN	Potassium Thiocyanate
6	Mm	Millimole
7	µg	Microgram
8	µL	Microlitre
9	mg	Milligram
10	ml	Milli litre
11	R.S.D	Relative standard deviation
12	R_f	Retention factor
13	R_t	Retention time
14	Std	Standard
15	S.D	Standard deviation
16	SE	Standard error
17	UV	Ultra-Violet spectroscopy
18	Wt	Weight
19	IR	Infra red
20	Abs	Absorbance

Dedicated to the Almighty & My Parents

CONTENTS

INTRODUCTION

Analytical chemistry is the science of making quantitative measurements. It is the study of chemical composition of natural and artificial materials. Properties studied in this include geometric features such as molecular morphologies and distributions of species, as well as features such as composition and species identity. Modern analytical chemistry is dominated by instrumental analysis and it employs a range of techniques that vary from simple quantitative chemical test to the use of more sophisticated and expensive computer controlled instruments.

Traditional analytical techniques

Although modern analytical chemistry is dominated by sophisticated instrumentation, the roots of analytical chemistry and some of the principles used in modern instruments are from traditional techniques which include

Titration

Titration involves the addition of a reactant to a solution being analysed until some equivalence point is reached. Often the amount of material in the solution being analysed maybe determined. The titrations employ different types of indicators to detect equivalence point.

Gravimetry

Gravimetric analysis involves determining the amount of material present by weighing the sample before and / or after some chemical transformation/reaction.

Inorganic qualitative analysis

Inorganic qualitative analysis generally refers to a systematic scheme to confirm the presence of certain, usually aqueous, ions or elements by performing a series of reactions that eliminate ranges of possibilities and then confirms suspected ions with test. With modern instrumentation these tests are rarely used but can be useful for educational purposes and in field work or other situations where access to state of the art instruments is not available.

Instrumental methods

- Spectroscopy
- Mass spectrometry
- Thermal Analysis
- Electrochemical Analysis
- Chromatographic techniques
- Hyphenated techniques

Scope of Pharmaceutical analysis

Pharmaceutical companies rely upon both qualitative and quantitative chemical analysis to ensure that the raw material used meet all the desired specifications, and also to check the quality of the final product. The examination of raw material is carried out to ensure that there is no unusual substance present which might deteriorate the manufacturing process or appear as a harmful impurity in the final product. The quantity of required ingredient in raw material is determined by a procedure known as Assay.

The final manufactured product is subjected to quality control to ensure that desired components are present within a range and impurities do not exceed certain specified limits.

Some specific use of analysis is under mentioned:

- Quantitative analysis of air, water and soil samples is carried out to determine the level of pollution.
- Chemical analysis is widely used to assist in the diagnosis of illness and in monitoring the condition of patients.
- In farming, nature of soil and level of fertilizer application is analyzed
- In geology, composition of the rock and soil is carried out.
- In general analysis is divided into two major part:
    - Qualitative analysis (what substances are present in the given sample)
    - Quantitative analysis (to determine the quantity of each component in the given sample)

Quality assurance

It is a wide ranging concept covering all matters that individually or collectively influence the quality of a product .It is the totality of the arrangements made with the object of ensuring that pharmaceutical products are of the quality required for their intended use. Quality assurance therefore incorporates GMP.

Quality control

It is primarily designed to detect and correct defects or checking to demonstrate whether the anticipated results are compiled with. It is designed to ensure that the results of laboratory analysis are consistent, comparable and accurate and within specified limits of precision.

Instrumental methods

Spectrophotometric methods are based on measurement of the interaction between electromagnetic radiation and analyte. It includes UV-Visible , Infrared, Raman, Mass, nuclear magneticresonance(NMR), Fluorimetry, Flamephotometry, Nepheloturbidimetry.

Electro analytical methods invoves the measurements of electrical properties such as voltage, current, resistance etc. it include potentiometry, condutometry, amperometry, electrogravimetry etc.

Separation methods include all types of chromatography like thin layer, paper,column, gas chromatography, HPLC,HPTLC.

Miscellaneous methods include Thermal analysis(based on heat of reaction), Kinetic techniques (based on the kinetics reaction of the analyte), Enzyme assay.

Hyphenated techniques include combinations of the above techniques(Chromatography and Electrophoresis) produce"hybrid" or "hyphenated" techniques. Hyphenated separation techniques refer to a combination of two (or more) techniques to detect and separate chemicals from solutions. Most often the other technique is some form of chromatography. The examples are LC-MS (or HPLC-MS), HPLC/ESI-MS,LC-NMR, LC-IR,LC-DAD, CE-MS,CE-UV, GC-IR and GC-MS.

ANALYTICAL METHOD VALIDATION

Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use.For pharmaceutical analytical methods guidelines from the United States Pharmacopeia (USP) International Conference on Harmonisation (ICH), and Food and Drug Administration (FDA) provide a frame work for performing such validation.

Validation (P.D.Sethi 2008)

It is a process involving confirmation or establishing by laboratory studies that a method/procedure/system/analyst give accurate and reproducible result for intended analytical application in a proven and established range.

Types of Validation

- Prospective validation
- Retrospective
- Concurrent

Prospective Validation

This is employed when historical data of the product is not available or is not sufficient and in-process and finished product testing are not adequate to ensure reproducibility or high degree of compliance to product likely attributes. Such validation is conducted prior to release of either new product or product made under revised /new manufacturing process where revision may effect the product characters.

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Retrospective Validation

This provides trend of comparative result i.e. review and evaluation of existing information for comparison when historical data is sufficient and readily available. Retrospective validation is acceptable provided specific test results generated by reliable analytical method on number of samples are available to allow statistical analysis. Simply pass/fail test results would not be accepted as part of retrospective validation -useful for trend setting.

Concurrent Validation

Based on information generated during implementation of a system. For this extensive testing and monitoring are performed as a part of initial run of the method.Concurrent validation verifies the quality characteristics of a particular batch and provide assurance that the same quality would be attained again when subsequent batches are manufactured and analysed under similar conditions.

Analytical parameters to be validated

- Accuracy
- Precision
- Selectivity (specificity)
- Linearity
- Range
- Sensitivity
- Limit of detection(LOD)
- Limit of quantification (LOQ)
- Ruggedness
- Robustness

Accuracy

It relates to the closeness of test results to true value i.e. measure of exactness of analytical method.It is expressed as % recovery by the assay of known/added amount of analyte in the linearity range.

One can design experiments for recovery of known or spiked samples (usually 10% of the claim) in presence of expected matrix, keeping the matrix constant. Accuracy can also be determined by comparing the results with those obtained using an alternative method which has already been validated.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

Accuracy (Trueness)

The accuracy of an analytical procedure express the closeness of the test results obtained by that method to the true value (a conventional true value or an accepted reference)

Accuracy is calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample, or as the difference between mean and the accepted true value, together with confidence intervals.

The ICH documents recommend that accuracy be assessed using a minimum of nine determinations over a minimum of three concentration level covering the specified range (i.e., three concentrations and three replicates of each concentration)

Precision

The precision of an analytical procedure is the degree of agreement among the individual test result when the method is applied repeatedly the multiple samplings of a homogenous sample under the prescribed conditions. Precision of an analytical procedure is usually expressed as the standard deviation or relative standard deviation(Coefficient of variation) of a series of measurements. Precision may be a measure of either the degree of reproducibility or repeatability of the analytical method under operating conditions.

Repeatability

It refers to the use of the analytical procedure within the laboratory over a short period of time using the same analyst with same equipment.

Reproducibility

It refers to the use of analytical procedure in different laboratories as in a collaborative study.

Intermediate precision

It expresses within laboratory variation , on the different days or different analysts or equipment within the same laboratory.

ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure (i.e., three concentrations) or a minimum of six determinations at 100% of the test concentration.)

Detection limit (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample , which can be detected but not necessarily quantitated as an exact value. Based on the standard deviation of the response and the slope, the detection limit of detection (LOD) may be expressed as **DL= 3.3 σ**/s where μ is the standard deviation of the response and S is the slope of the calibration curve (of the analyte).

Quantitation (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. It is a parameter used particularly for the determination of impurities and / or degradation products .The quantization limit expressed as the concentration of analyte (e.g., percentage parts per million) in the sample.

LOQ=10 **σ** /S

Where, **σ** - standard deviation of the response; S = slope of the calibration curve (of the analyte)

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Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample.

Range

The range of an analytical procedure is the interval between the upper and lower concentration (amount) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision and linearity.

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same sample under variety of conditions, such as different laboratories, analysts, instruments, lots of reagents, elapsed times, assay temperatures or days. Intermediate precision can be considered as ruggedness.

Robustness

The robustness of an analytical is a measure of its capacity to remain unaffected by small normal usage**.**

Statistical parameters

The precision and reproducibility of the analytical method was determined by repeating the analysis and the following statistical parameters were calculated.

Mean

The mean of the any distribution is a measure of centrality, but in case of the normal distribution, it is equal to the mode of the distribution. The mean, or average, is obtained by dividing the sum of observed values by the number of observations,n.

$$X = \sum x/n$$

Standard deviation

Standard deviation is a measure of data dispersion or variability. The standard deviation gives an idea that how close the entire set of data is to the average value. Data sets with small standard deviation have tightly grouped, precise data.SD is also called the root mean square deviation as it is the square of the sum of the squares of the differences between the values and the mean of those values.

$$SD = \sum (X - X)^2 / \sqrt{n-1}$$

Relative standard deviation

The relative standard deviation is also called as co efficient of variation. This is useful when the standard deviation is proportional to the magnitude of the measurement. It is defined as

$$RSD = SD / \bar{X}$$

$$\% RSD = SD / \bar{X} \times 100$$

Regression equation:

A regression is a statistical analysis assessing the association between two variables. It is used to find the relationship between two variables.

Regression equation(y) = mx+c

Where, m- the slope of the regression line

c- the intercept of the regression and the y axis.

Standard error:

The standard error (SE)

$$SE = SD / \sqrt{n}$$

An example of the equation for the standard error of the mean reveals that means constructed from very large sample sizes will be very stable,i.e.,non variable.

DRUG PROFILE

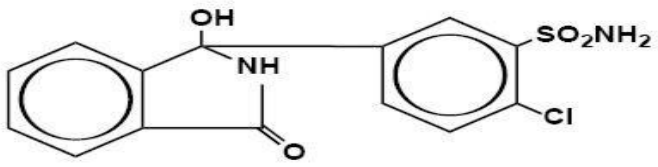
CHLORTHALIDONE:

Chlorthalidone is chemically (RS)-2-chloro-5-(3-hydroxy-1-oxo isoindoli-3-yl)benzene sulphonamide(fig:1) is widely used in antihypertensive 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 .

EMPERICAL FORMULA: $C_{14}H_{11}ClN_2O_4S$

MOLECULAR WEIGHT: 338.8 g/mol

STRUCTURE :



DESCRIPTION:

A White to yellowish- white, crystalline powder; almost odourless; tasteless.

SOLUBILITY:

Practically insoluble in water, in solvent ether and in chloroform; Soluble in alcohol and in alkali hydroxides.

MECHANISM OF ACTION:

Chlorthalidone increases the excretion of sodium, chloride, and water into the renal lumen by inhibiting sodium ion transport across the renal tubular epithelium. Its primary site of action is in the cortical diluting segment of the ascending limb of the loop of Henle. Thiazides and related compounds also decrease the glomerular filtration rate, which further reduces the drug's efficacy in patients with renal impairment (e.g. renal insufficiency). By increasing the delivery of sodium to the distal renal tubule, chlortalidone indirectly increases potassium excretion via the sodium-potassium exchange mechanism (i.e. apical ROMK/Na channels coupled with basolateral NKATPases). This can result in hypokalemia and hypochloremia as well as a mild metabolic alkalosis; however, the diuretic efficacy of chlortalidone is not affected by the acid-base balance of the patient being treated.

Initially, diuretics lower blood pressure by decreasing cardiac output and reducing plasma and extracellular fluid volume. Eventually, cardiac output returns to normal, and plasma and extracellular fluid volume return to slightly less than normal, but a reduction in peripheral vascular resistance is maintained, thus resulting in an overall lower blood pressure. The reduction in intravascular volume induces an elevation in plasma renin activity and aldosterone secretion, further contributing to the potassium loss associated with thiazide diuretic therapy.

INDICATION AND USAGE:

It is a diuretic drug used in the treatment of hypertension, edema, heart failure.

DOSAGE AND ADMINNISTRATION:

Hypertension

25-100 mg/day; usual range, 12.5-25 mg/day

Edema

50-100 mg/day or 100 mg PO every other day; not to exceed 200 mg/day

Heart failure

12.5-25 mg/day; not to exceed 100 mg/day

CONTRAINDICATION:

Advanced renal failure, hypersensitivity to sulfonamide.

DRUG INTERACTIONS:

Drug interactions with chlorthalidone are possible if it is combined with medications such as barbiturates, corticosteroids, narcotics, or alcohol. These interactions can increase the risk of certain side effects, such as low blood pressure, and may decrease the effectiveness of some drugs.

REVIEW OF LITERATURE

An extensive literature review was done for the official drug chlorthalidone. It was observed that various analytical methods inclusive of UV-Visible spectrophotometry, RP-HPLC methods have been reported.The present work aims to devise novel methods which has been reported till date.

Stephen M. Walters., ***et al.,*** **(1982)** described reversed-phase high performance liquid chromatographic method for the determination of chlorthalidone and clonidine hydrochloride combinations in tablets. Individual tablets or composite samples were sonicated in water, diluted with methanol, and filtered prior to chromatographing. Chlorthalidone, formulated at 15 mg/tablet, was chromatographed on octadecylsilyl-bonded, 5 to 6-µm, spherical silica with 50% methanol in water mobile phase. Clonidine hydrochloride, formulated at 0.1 or 0.2 mg/tablet, was chromatographed on trimethylsilyl-bonded, 5 to 6-µm, spherical silica with 65% methanol in pH 7.9 phosphate buffer mobile phase. Both were determined with a spectrophotometric detector at 254 nm

Luis ML., ***et al.,*** **(1999)** determined simultaneously Chlorthalidone and spironolactone with the aid of univariate and multivariate calibration methods. Univariate calibration was performed by the zero-crossing and derivative ratio spectrum methods.

Alaa El-Gindy., ***et al.,*** **(2005)** reported the high performance liquid chromatographic (HPLC) method depends on the separation of each drug on a reversed phase, RP_{18} column. Elution was carried out with a mobile phase consisting of acetonitrile -5mM heptansulphonic acid sodium salt (20:80, v/v, pH 4.4). Quantitation was achieved with UV detection at 274 nm based on peak area.

Arshad Khuroo., ***et al.,*** **(2008)** carried out liquid chromatography–tandem mass spectrometry method for the simultaneous separation and quantitation of atenolol and chlorthalidone in human plasma using metoprolol and hydrochlorothiazide as internal standard. Following solid phase extraction, the analytes were separated by an isocratic mobile phase on a reversed-phase C_{18} column and analyzed by MS in the multiple reaction-monitoring mode (atenolol in positive and chlorthalidone in the negative ion mode)

Alaa El-Gindy., ***et al .,*** **(2008)** optimized and validated high-performance liquid chromatographic method for the determination of atenolol and chlorthalidone (CT) in human breast milk. The milk samples were extracted and purified using ACN and phosphoric acid for precipitation of proteins followed by removal of ACN and milk fats by extraction with methylene chloride.

Elshanawane, Abdalla A., ***et al.,*** **(2009)** developed a high-performance liquid chromatographic method for the simultaneous determination of 2 ternary mixtures containing amiloride hydrochloride, atenolol, hydrochlorothiazide, and chlorthalidone used in hypertension therapy. The use of cyanopropyl column results in satisfactory separation of both mixtures. The mobile phase consisted of 10 mM KH_2PO_4 buffer (pH 4.5) and methanol in a ratio of (75 25 v/v), at a flow rate of 1 mL/min. UV detector was operated at 275 nm.

Nada. S. Abdelwahab., ***et al.,*** **(2010)** developed determination of atenolol, chlorthalidone and their degradation products by TLC-densitometric and chemometric methods with application of model updating TLC-Densitometric one have been developed for the selective determination of Atenolol (ATE) and Chlorthalidone(CLT) along with their hydrolytic degradation products. The suggested methods have been used for the determination of the studied drugs in their pharmaceutical formulations and the results were statistically compared to the reported RP-HPLC method.

Mohamed S. Elgawish., ***et al.,*** **(2011)** reported and validated chromatographic method for the simultaneous quantification of atenolol and chlorthalidone in human plasma using hydrochlorothiazide as internal standard (IS). The method utilized proteins precipitation with acetonitril as the only sample preparation involved prior to reverse phase-HPLC. The analytes were chromatographed on Shim-pack cyanopropyl column with isocratic elution with 10 mM KH_2PO_4 (pH 6.0) – methanol (70:30, v/v) at ambient temperature with flow rate of 1 mL min^{-1} and UV detection at 225 nm.

Madhu Babu Kasimala., ***et al., (*****2012)** developed and validated RP-HPLC method for the simultaneous estimation of Azilsartan Medoxomil and Chlortalidone in pharmaceutical dosage forms. Isocratic elution at a flow rate of 0.9ml min-1 was employed on a symmetry C18 column at ambient temperature. The mobile phase consisted of Methonal: Water: Acetonitrile : 0.1% Ortho phosphoric acid 30:35:15:5(v/v/v/v). The UV detection wavelength was at 251 nm.

Akiful Haque.M, Nivedita.G., ***et al .,(2012)*** developed a simple, accurate, precise, economical and reproducible UV Spectrophotometric method for the simultaneous estimation of Atenolol and Chlorthalidone in bulk and in combined tablet dosage form. The stock solutions were prepared in methanol followed by further required dilutions with methanol. The absorbance maxima of Atenolol and Chlorthalidone were found to be 225nm & 284nm respectively. Beers law obeyed the concentration range of atenolol is 10 µg mL-1 to 60 µg mL-1 & chlorthalidone is 30 µg mL-1 to 140 µg mL-1.

Youseff RM., ***et al., (2013)*** performed the method for the simultaneous determination of amiloride hydrochloride, atenolol, and chlorthalidone using HPTLC and HPLC with photodiode array detector**.** Two stability-indicating chromatographic methods are described for simultaneous determination of amiloride hydrochloride (AMI), atenolol (ATE), and chlorthalidone (CHL) in combined dosage forms. The method was based on HPTLC separation of the three drugs followed by densitometric measurements of their bands at 274 nm.

Kreny E. Parmar.N., ***et al.,*** **(2013)** carried out reverse-phase HPLC method for simultaneous estimation of Telmisartan and Chlorthalidone in bulk and tablet formulations. Separation was performed on a C -18 column (250 × 4.6mm ID,5 µm) with Acetonitrile : Methanol (85:15v/v) , flow rate of 1.0ml/ min and UV detection at wavelength 242 nm.

Pradip Parikh., ***et al .,*** ***(*****2013**) developed and validated for the simultaneous estimation of Chlorthalidone (CHT) and Olmesartan Medoxomil(OLM) by the first-order derivative UV spectroscopic method. The quantification was achieved by the first-order derivative spectroscopic method at 239.40 nm and 275.60 nm over the concentration range of 5-25 µg/ml for estimation of Chlorthalidone and 10-50 µg/ml for Olmesartan Medoxomil in a combined tablet formulation

AIM AND OBJECTIVE OF THE STUDY

Chlorthalidone is an official drug of IP/BP and USP , Review of literature indicates that several methods have been reported for the estimation of chlorthalidone by UV-Visible spectrophotometric and RP-HPLC.

The aim of the present work is to develop and validated simple, novel,sensitive,highly specific,accurate and precise UV-Visible, Infrared spectrophotometry and spectrofluorimetry methods for the estimation of chlorthalidone in bulk and tablet dosage form which has not been reported till date.

The novel analytical methods comprises of :

- **UV Spectrophotometry**
  - Standard absorbance method
  - Area under the curve
  - First derivative spectroscopy
  - Second derivative spectroscopy
  - Q-Absorbance method

- **Infrared Spectrophotometry**
  - KBr Disc method using Internal standard.

Spectrofluorimetry

- Direct spectrofluorimetric method

MATERIALS AND METHODS

Ultraviolet spectroscopy is most frequently employed technical employed technique in pharmaceutical analysis. The ultraviolet region of the electromagnetic spectrum is used in the analysis which extends from 200-400nm .It involves transition of electrons of π orbital and lone pairs (n = non bonding) so UV spectroscopy is the most use for identifying conjugated system.

Choice of solvent (Sharma.YR,2010); (Chatwal and Anand,2007)

The most important requirement of the solvent are :

- it should solubilize the anlyte freely
- it should not itself absorb in the region of the analyte.
- it should not undergo association or dissociation with analyte

The absorption law (Sharma.YR,2010)

There are two laws which govern the absoption of light by the molecules they are,

- ❖ Lambert's Law
- ❖ Beer's Law

Lambert's Law

It states that when a beam of monochromatic radiation passes through a homogenous absorbing medium, the ratio of decrease of intensity of transmitted radiation with the thickness of absorbing medium is directly proportional to intensity of the incident radiation.
The law given by: $\mathbf{I = I_0\, 10^{-ax}}$

Where $\mathbf{I_0}$ **,** is the intensity of the incident light ; **I ,** is the intensity of transmitted light.

Beer's law

This law states that, when a beam of monochromatic radiation is passed through a solution of an absorbing substance, the rate of decrease of intensity of transmitted radiation with concentration of the absorbing solution is directly proportional to the intensity of the incident radiation,

The law is given by**; $I= I_0e^{-k'ex}$**

On combination of these two laws, the Beer's – lambert's law is formulated

$LogI_0/I = a.c.l = A$

Where,

A= Molar Extinction Coefficient; C=Concentration of solute in Moles/Litre.

L= path length of the sample (1cm); A= Absorbance

Quantitative analysis

The use of UV in quantitative analysis employs of comparing the absorbance of the standard and sample at selected wavelength(metryi,2008)

Assay of substance in single component sample (Beckett and Stenlake,2002)

The most important characteristics of photometric and spectrophotometric method are high selectivity and ease of convenience, quantitative analysis can be done using following methods.

- **Use of $A_{1\%, 1cm}$ values**

This method can be used for the estimation of formulation or raw material when reference standard not available, the use of standard $A_{1\%, 1cm}$ value avoid the need to prepare a standard solution of the reference substance in order to determine its absorption.

- **Use of calibration graph**

In this method a calibration curve is plotted using concentration (x- axis) Vs absorbance (y-axis) with the value of five or more standard solutions. A straight line is drawn through maximum number of points. This line is called line of best fit, by interpolating the absorbance of sample solutions on the calibration charts, the concentration of the drug amount and percentage purity can be calculated. This is used in the new method development for the estimation of analyte by UV-Visible spectrophotometry.

The amount present can be calculated using the formula

$$\text{Amount present} = \frac{\text{Sample abs} \times \text{dil. factor of standard} \times \text{wt of std} \times \text{Avg.wt}}{\text{Std abs} \times \text{dil. factor of sample} \times \text{wt of sample}}$$

- For area under curve, instead of absorbance, area is used
- For derivative spectrophotometry, amplitude of negative maxima is used instead of absorbance

Standard absorbance

Most organic compounds absorb UV or Visible light making them susceptible for quantification using spectrophotometers. The technique of UV spectrophotometry is the most frequently employed method in pharmaceutical analysis. It involves the measurement of the amount of ultraviolet (190-380nm) or visible (380-800nm) radiation absorbed by substance in solution. Absorption of light in both the UV and visible regions of the electromagnetic spectrum occurs when the energy of the light matches that required to induce in the molecule an electronic transition and its associated vibrational and rotational transitions.

The use of UV for quantitative analysis employs the method of comparing the absorbance of standards and samples at a selected wavelength. The analysis of mixtures of two or more components is facilitated by measuring the absorbance. Other applications include measurement of absorption of complexes to establish their composition. All chromogenic compounds are not suitable for quantitative measurements, i.e. the choice of the system and procedure depends largely on the chemistry of the species to be determined.

Criteria to select this procedure include

- ✓ Conformity to the Beer's Lambert's law
- ✓ A calibration graph showing linearity
- ✓ Stability of absorbance

First derivative spectroscopy

Derivative spectroscopy involves the transformation of absorption spectra in to first, second or higher order derivative spectra. In derivative spectroscop, the ability to detect and to measure minor spectral features is considerably enhanced. It can be used in quantitative analysis to measure the concentration of an analyte whose peak is obscured by a larger overlapping peak. It is useful in eliminating the matrix interference in the assay of many medicinal substances. Derivative spectrum is done by wavelength modulation with dual wavelength photometers and microprocessor controlled digital photometer.

Normal spectrum is zero order spectrum. The first derivative (D^1) spectrum is a plot of the ingredient of absorption curve (rate of change of absorbance with wavelength i.e. $dA^1/d\lambda^1 Vs\ \lambda$) against wavelength. It is characterized by a maximum, minimum and a cross over point at the λ_{max} of the absorption band (IP 1996).

Advantages

1. Accurate determination of λ_{max} is possible.
2. Increased resolution permits the selective determination of certain absorbing substances.
3. absorption bands can be recognized when there are two or more absorption bands overlapping at the same or slightly different wavelength.

To get the quantitative measurement, peak heights (in mm) are usually measured. The amplitudes of the negative and positive peaks adjacent to the cross over point. In this method, use is made of the fact that amplitude of positive or negative and positive peaks adjacent to the cross over point is directly proportional to the concentration.

Second derivative spectroscopy

Normal spectrum is a zero order spectrum. The primary spectrum obtained for the above was then derivatized for the second order. The second derivative spectrum(D^2) is a plot of curvature of the absorption spectrum against wavelength ($d^2 A/d\lambda^2$ Vs λ). The amplitude(D_L) of satellite peak of the second order curve was measured. The amplitude of the negative peak maximum corresponding to λ_{max} was measured.

Area under the curve

This method is applicable when there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths λ_1 and λ_2. The inbuilt software calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under the curve and concentration.

ULTRA-VIOLET SPECTROPHOTOMETRIC METHOD

Instruments employed

Shimadzu UV –Visible spectrophotometer, Model 1650 PC.

METHOD:1 STANDARD ABSORBANCE METHOD

Preparation of standard stock solution

100mg of standard chlorthalidone was accurately weighed & transferred into 100ml standard flask. Sufficient quantity of ethanol was added to dissolve the drug & the volume was made up with ethanol (1mg/ml).From the above standard stock solution different concentrations in the range of 40-160μg/ml were prepared at an interval of 20 μg/ml.

Preparation of sample solution

Five tablets were weighed and powdered. Accurately weighed tablet powder equivalent to 100mg of chlorthalidone was taken in a 100ml volumetric flask and shaken well with ethanol to dissolve the active ingredient and made upto volume to produce 1000 μg/ml . The solution was then filtered, first few ml of the filtrate was discarded and the filtrate was used for further analysis.

ESTIMATION OF CHLORTHALIDONE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY UV SPECTROPHOTOMETRY (Metreyi 2008)

Most organic compounds absorb UV or visible light making them susceptible for quantification using spectrophotometers. Ultra violet spectroscopy involves the measurement of light absorbed by the analyte present in the range of 200-400nm. The use of UV for quantitative analysis employs the method of comparing the absorbance of standards and samples at a selected wavelength.

The analysis of mixtures of two or more components is facilitated by measuring the absorbance. Other applications include measurement of absorption of complexes to establish their composition. All chromogenic compounds are not suitable for quantitative measurements. i.e. the choice of the system and the procedure depends largely on the chemistry of the species to be determined.

Criteria to select the procedure includes

- Conformity to the Beer-Lambert's law and plot calibration data for the range of concentration measured.
- Degree of selectivity of complexing agent includes the effect of other species likely to be present.
- Stability of absorbance with respect to time ,pH,ionic strength and temperature.

Establishment of various parameters

- Absorption maximum
- Beer's concentration
- Calibration graph
- Estimation of analyte in dosage form
- Recovery studies

Absorption Maximum

The standard stock solution was suitably diluted in ethanol to yield a concentration of 40µg/ml. This solution was scanned in the UV region between 200-400nm using ethanol as blank. It was found that chlorthalidone exhibited an intense maximum absorption at 275nm .

Beers's Concentration to confirm the linearity range

Aliquots of standard solution of chlorthalidone were suitably diluted to give various concentrations ranging of 40-160 µg/ml. The absorbance was measured at about 275nm was given in the **Table 1**.

Table 1: Absorbance of Chlorthalidone at 275nm

S.NO	Concentrations in (µg/ml)	Absorbance *
1	40	0.209
2	60	0.332
3	80	0.428
4	100	0.532
5	120	0.620
6	140	0.746
7	160	0.812

***** Each value is the mean of three readings

Calibration graph

A graph of absorbance against concentration was plotted .From the graph the Beer's law concentration for the analyte was found to be between 40-160 µg/ml**.(Fig-1)**

Figure-1: Calibration graph for chlorthalidone [standard absorbance method]

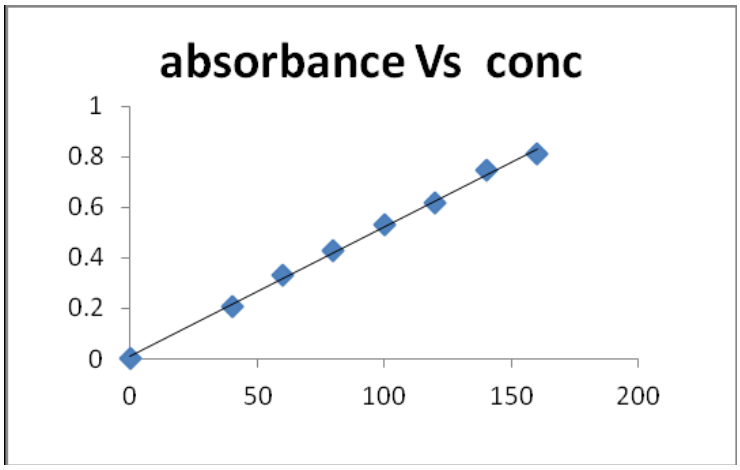
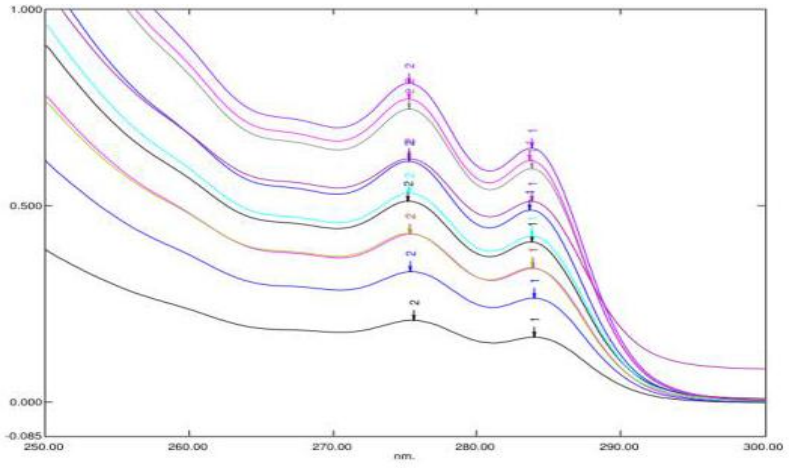


Figure-2: Overlain spectra of chlorthalidone



Analysis of Sample

The sample solution was further diluted with ethanol to the required concentration and the absorbance of the solution was then measured at 275nm using ethanol as blank. The amount of chlorthalidone was calculated using the formula and the results are tabulated in **Table 10.**

$$\text{Amount present} = \frac{\text{Sample conc} \times \text{Dil. factor of Std} \times \text{Wt. of Std}}{\text{Sample absorbance} \quad \text{Dil. factor of sample} \quad \text{Wt . of sample}} \times \text{Avg.Wt}$$

Recovery studies

To study the accuracy, precision and reproducibility of the proposed method, recovery studies were carried out by adding a known quantity of drug to preanalysed sample and the percentage recovery was calculated by using the formula and the results obtained are presented in **Table 11**.

$$\frac{\text{Amount found in sample} - \text{Actual amount of preanalysed of sample}}{\text{Amount of the standard added}} \times 100$$

METHOD 2 : AREA UNDER THE CURVE METHOD

(Beckett 1997, Niraimathi.V *et al*., (2010)

This method is applicable when there is no peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wave lengths between two selected wavelengths λ1 and λ2. The inbuilt software calculates the area bound by the curve and the horizontal axis .The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration.

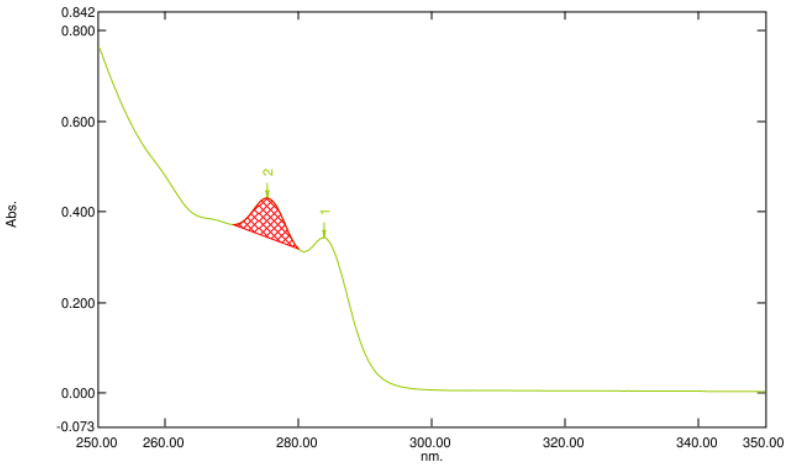
Establishment of various parameters

- Area under the curve
- Beer's concentration
- Calibration graph
- Estimation of analyte in dosage form
- Recovery studies

Area under the curve

The standard stock solution of chlorthalidone was suitably diluted to give varying concentrations ranging from 10-40 µg/ml .The solutions were scanned in the UV region between 200-400nm using ethanol as blank. The area under the curve between 270.2-280.0 nm was measured by using the inbuilt software.**(Fig-3)**

Fig -3: AUC spectrum of chlorthalidone



Linearity range

The AUC obtained for different concentrations of standard solution of chlorthalidone are presented in **Table 2.**

Table 2: AUC of chlorthalidone betweeen 270.2 and 280nm

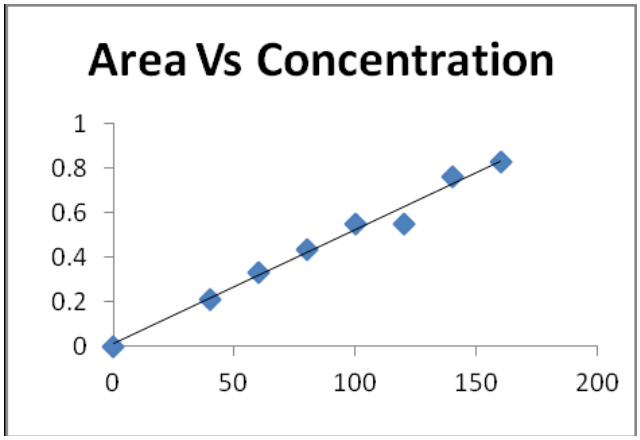
S. No	Concentration (in µg/ml)	Area*
1	40	0.212
2	60	0.333
3	80	0.437
4	100	0.549
5	120	0.550
6	140	0.759
7	160	0.827

*Each value is the mean of three readings

Calibration graph

A graph of AUC against concentration was plotted. From the graph it was found that the drug obeyed linearity in the range 40-160 µg/ml.

Fig 4: Calibration curve of chlorthalidone by AUC method



Analysis of sample

The sample solution was suitably diluted and was scanned in the spectrum mode and AUC was calculated in the wavelength range of 270.2 – 280 nm. The AUC so obtained was interpolated on the calibration graph and the concentration of sample determined the amount present per tablet was calculated and presented in the **Table 10.**

Recovery studies

To study the accuracy, precision and reproducibility of the proposed method, recovery studies were carried out on spiked sample by adding predetermined amount of standard drugs to the respective sample. About 20%, 50% and 100% of standard drug was added to the sample and the absorbance was measured against method blank. The percentage recovery was calculated and presented in the **Table 11.**

METHOD 3 : FIRST DERIVATIVE SPECTROPHOTOMETRY (Chatwal 2005)

Derivative spectrophotometer involves the transformation of absorption spectra into first, second or high order derivative spectra .In derivative spectroscopy , the ability to detect and to measure minor spectral features is considerably enhanced. It can be used in quantitative analysis to measure the concentration of an analyte whose peak is obscured by a large overlapping peak. It is useful in eliminating matrix interference in the assay of many medicinal substances.Derivative spectrum is done by wavelength modulation with dual wavelength photometers and microprocessor controlled digital photometer.

Normal spectrum is a zero order spectrum .The first derivative (D1) spectrum is a plot of the gradient of absorption curve (rate of change of absorbance with wavelength i.e .(d A/ d λ Vs λ) against wavelength .It is characterized by a maximum , minimum and a cross over point at the λ_{max} of the absorption band.

Advantages :

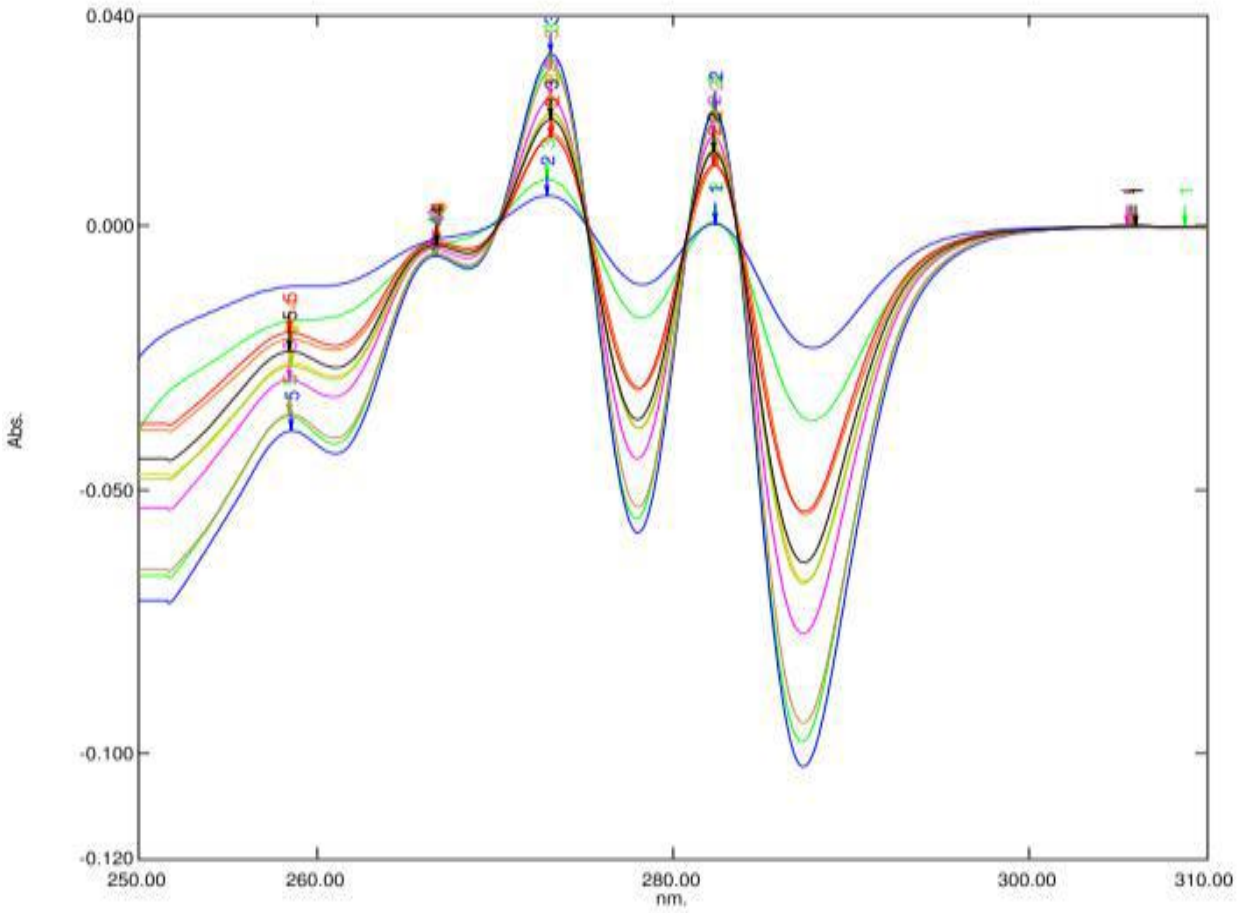
- Accurate determination of λ max is possible.
- Increased resolution permits the selective determination of certain absorbing substances
- Absorption bands can be recognized when there are two or more absorption bands overlapping at the same or slightly different wavelength.

  For quantification , peak heights (in mm) are usually measured. The amplitude is the distance from the maximum to the minimum at the λ max (which is the zero crossing in the spectrum) in first order. In this method, use is made of the fact the amplitude of positive peak adjacent to the cross over point is directly proportional to the concentration.

Establishment of optimum parameters

The standard stock solution of chlorthalidone was suitably diluted to give the various concentrations ranging from 40-160 µg/ml. These solutions were scanned between 200-400nm and the primary absorption spectra were recorded. The primary spectrum was then derivatized for the first order.**(Fig-5)**

Figure-5: Overlain spectra of first order derivative of chlorthalidone



The primary spectrum was then derivatized to the first order using derivative mode.The amplitude of the negative peak maximum at the zero crossing of the first order curve was measured in mm at 275nm and is presented in **Table 3.**

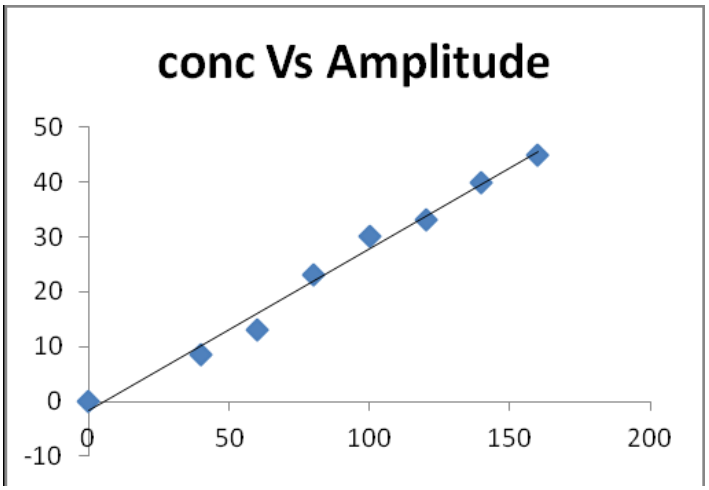
Table 3: Concentration versus Amplitude in mm (First Derivative)

Concentration (µg/ml)	First Derivative Amplitude (mm)*
40	8.5
60	13
80	23
100	30
120	33
140	40
160	45

Calibration graph

A graph was constructed by plotting amplitude against concentration and is shown in Fig.7 for first derivative.The Beer's Law was obeyed in the range of 40-160µg/ml for first derivative spectrophotometric method.

Figure 6: Calibration graph of chlorthalidone (First derivative method)



Analysis of sample solution

The sample solution was suitably diluted and scanned between 200-400nm using ethanol as blank and the primary spectrum obtained was derivatized for first order derivative. The amplitude (DL) of the peak maximum and minimum at the zero crossing of the first order curve (i.e. λ max of the fundamental spectrum) were measured in mm. The amount of drug present was found by interpolation on the calibration graph and the amount of drug present per tablet was calculated by using the formula and the assay results are given in the **Table 10.**

$$\text{Amount present} = \frac{\text{Sample concentration x Dilution factor x Avg. Wt}}{\text{Wt. of tablet powder taken}}$$

Recovery studies

To study the accuracy, precision and reproducibility of the proposed method, recovery studies were carried out by adding a known quantity of drug to preanalysed sample and the percentage recovery was calculated by using the formula and the results obtained are presented in **Table 11**.

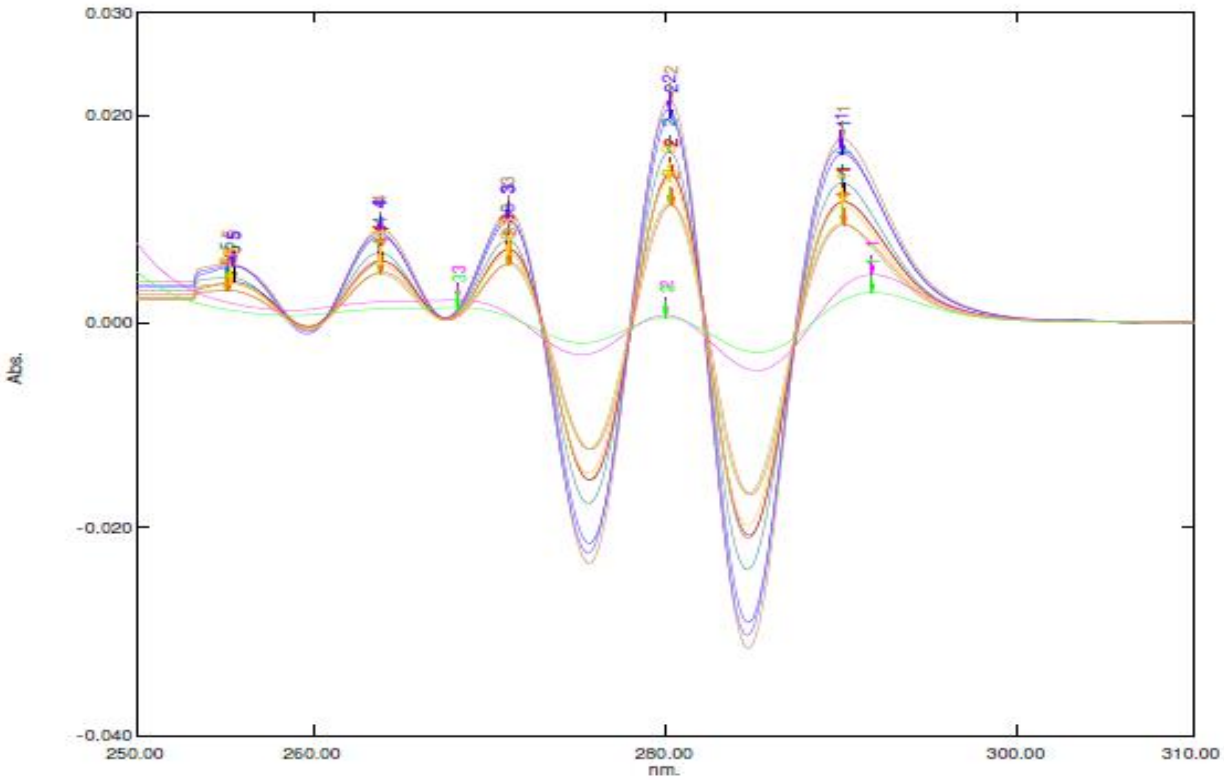
METHOD 4 : SECOND DERIVATIVE SPECTROPHOTOMETRY (Beckett 1997)

Normal spectrum is a zero order spectrum. The primary spectrum obtained was then derivatized to zero order. The second derivative spectrum (D2) is a plot of curvature of the absorption spectrum against wavelength (d^2 A/ $d\lambda^2$Vs λ). The amplitude (D_L) of long wave peak satellite of the second order curve was measured. The amplitude of the negative peak maximum corresponding to λ max of fundamental spectrum was measured.

Establishment of optimum parameters

The standard stock solution of chlorthalidone was suitably diluted to give the various concentrations ranging from 40-160 µg/ml. These solutions were scanned between 200-400nm and the primary absorption spectra were recorded . The primary spectrum was then derivatized for the second order **(Fig-7).**

Fig-7: Overlain spectra of second derivative of chlorthalidone



The primary spectrum obtained for the above was then derivatized to the second order. The amplitude of the negative peak maximum was measured in mm at 275nm and is presented in **Table 4.**

Table 4: Concentration versus Amplitude in mm (Second Derivative)

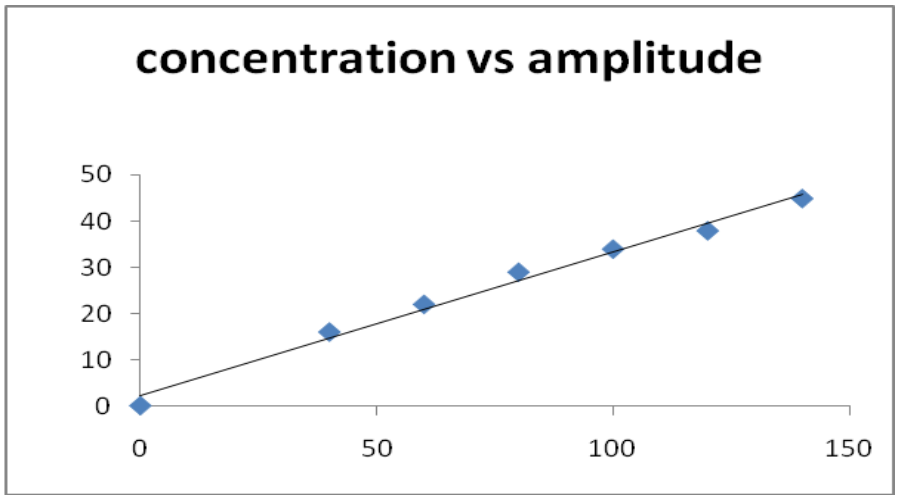
Concentration (µg/ml)	Second Derivative Amplitude (mm)*
40	14
60	16
80	22
100	27
120	31
140	38
160	42

* Each value is the mean of five readings

Calibration graph

A graph was constructed by plotting amplitude against concentration and is shown in Fig. for first derivative. The Beer's Law was obeyed in the range of 40-160µg/ml for second derivative spectrophotometric method.

Fig 8: **Calibration graph of chlorthalidone by second derivative spectrophotometry**



Analysis of sample solution

The sample solution was suitably diluted and scanned between 200-400nm using ethanol as blank and the primary spectrum obtained was derivatized for second order derivative. The amplitude (DL) of long wave peak satellite of the second order curve (i.e. λ max of the fundamental spectrum) were measured in mm. The amount of drug present was found by interpolation on the calibration graph and the amount of drug present per tablet was calculated by adopting the formula.The assay results are given in the **Table10.**

$$\text{Amount present} = \frac{\text{Sample conc x Dilution factor x Avg. Wt}}{\text{Wt. of tablet powder taken}}$$

The assay results are given in the **Table 10.**

Recovery studies

To study the accuracy, precision and reproducibility of the proposed method, recovery studies were carried out by adding a known quantity of drug to pre analyzed sample and the percentage recovery was calculated by using the formula and the results obtained are presented in **Table 11**.

METHOD 5: Q – ABSORBANCE METHOD

Q – absorbance method depends on the property that, for a substance which obeys Beer's law at all wavelength, the ratio of absorbances at two wavelength is a constant value independent of concentration or pathlength (Beckett , 1997). The wavelengths selected for this method are 275 nm and 284 nm. The difference in absorbances between these two wavelengths were calculated. The values obtained by the proposed method are presented in **Table 5.**

Fig-9: Calibration curve of chlorthalidone by Q-absorbance method

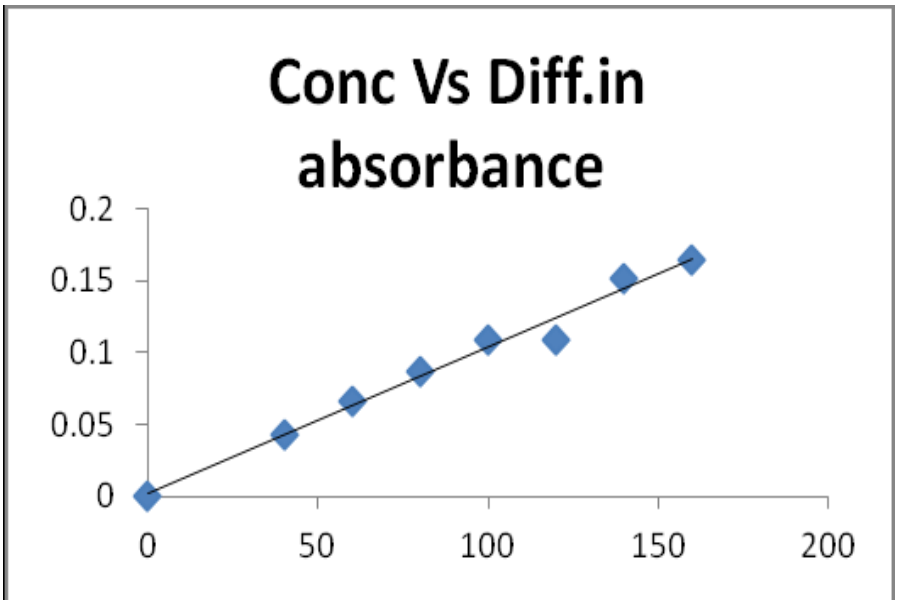


Table 5: Difference in absorbance at 275nm and 284 nm

S.NO	Concentration (µg/ml)	Absorbance at 275 nm	Absorbance at 284 nm	Difference in Absorbance
1	40	0.209	0.166	0.043
2	60	0.332	0.266	0.066
3	80	0.428	0.341	0.087
4	100	0.532	0.423	0.109
5	120	0.620	0.501	0.109
6	140	0.746	0.594	0.152
7	160	0.812	0.647	0.165

Establishment of optimum parameters

The standard stock solution of chlorthalidone was suitably diluted to give the various concentrations ranging from 40-160 µg/ml. These solutions were scanned between 200-400nm and the primary absorption spectra were recorded .It was found that chlorthalidone exhibited an intense maximum absorption at about 275nm. The two wavengths chosen for this method are 275nm -284nm respectively.

Analysis of sample solution

The sample solution was suitably diluted and scanned between 200-400nm using ethanol as blank and the absorbance of the solution was then measured at 275m and 284nm using ethanol as blank .The difference in absorbances between these two wavelengths was calculated. The amount of chlorthalidone was calculated using the formula.

$$\text{Amount present} = \frac{\text{Sample concentration}}{\text{Sample absorbance}} \times \frac{\text{Dil. factor of Std}}{\text{Dil. factor of sample}} \times \frac{\text{Wt.of Std}}{\text{Wt.of sample}} \times \text{Avg. Wt}$$

The results are given in the **Table 10.**

Recovery studies

To study the accuracy, precision and reproducibility of the proposed method, recovery studies were carried out by adding a known quantity of drug to pre- analysed sample and the percentage recovery was calculated by using the formula and the results obtained are presented in **Table 11**.

QUANTITATIVE INFRARED SPECTROPHOTOMETRY

Quantitative infrared spectrophotometry (Herida Regina Nunes Salgado., et al 2012) involves the measurement of amount of infrared radiation absorbed by substance in the pellet. The wavelength 25 to 2.5 micron or wavenumbers from 400cm^{-1} to 4000cm^{-1} is considered to be infrared region. The use of quantitative infrared spectroscopy for quantitative analysis employs the method of comnparing the absorbance of reference standards and samples at a selected wavelengths. Various infrared quantitative methods include baseline method, compressed disc method (internal standard method), reflectance method and GC-FTIR.

Different types of infrared quantitative methods

1.Baseline method

2.Compressed disc method

3.Reflectance disc method

- Attenuated total reflectance (ATR)
- Speccular reflectance
- Diffused reflectance

4.GC-FTIR

Internal standard method

Pellets from the disc technique can be used in quantitative measurement. Uniform pellets of similar weight are essential however for quantitative analysis. Disadvantage of measuring pellet thickness is overcome by using the internal standard method. KSCN makes an excellent internal standard which is used in KBr disc for quantitative measurement, KSCN is intimately mixed and ground to give a uniform concentration , usually 0.1 -0.2% W/W of KBr. KBr/KSCN disc will give a characteristic absorption band at 2068cm^{-1}.

The advantages of quantitative infrared spectroscopy are

- Simple, easy sample preparation
- No prior extraction of drug from dosage form.
- Reduced time of analysis .
- Drugs presenting solubility problems with more appropriate solvent could be prepared in powder form(KBr) for obtaining the pellets.
- Time of pellet preparation is shorter than solution preparation.
- Excipients present in pharmaceutical preparation did not interfere with the results obtained because those do not present specific absorption bands used to identify the analysed drug in powder form.

QUANTIFICATION BY IR SPECTROSCOPY

METHOD 6: KBr DISC METHOD USING INTERNAL STANDARD

INSTRUMENTATION

All spectral measurements were made on ABB-IR instrument (model no: MB 3000) with KBr press.(model no: M 15)

MATERIALS AND METHODS

All the chemicals used throughout the experiment were of highest purity of (IR grade).

- Potassium bromide (KBr)
- Internal standard: Potassium thiocyanate (KSCN)
- Bulk material: sample of chlorthalidone was gifted from Madras Pharmaceuticals.
- Dosage form: Chlorthalidone tablets was purchased from local market.

Method

Calibration of the standard :

Potassium thiocyanate was used as an internal standard which was preground, dried, and then reground with dry KBr to make a concentration of about 0.2% by weight of potassium thiocyanate. The final mixture was stored over phosphorus pentoxide. Five different concentration of standard and KBr-KSCN were prepared by mixing known weights of the standard substance with a known weight of the KBr-KSCN mixture and then grinded by using agate mortar & pestle under IR lamp. A standard calibration curve was constructed using ratio of absorbances and concentration.

Table 6: Concentration of KBr/KSCN mixture and standard

KBr/KCN (in mg)	50	50	50	50	50
Standard (in mg)	0.0	0.5	1.0	1.5	2.0

The discs were prepared by using KBr press and the infrared spectrum was recorded in absorbance mode; the calibration curve was obtained by plotting the ratio of the IR absorption at 2067.54 cm-1 (prominent band) and 1704.95 cm-1 against the concentration of the substance .

Fig-10: IR spectra of standard chlorthalidone with KBr disc method using internal method

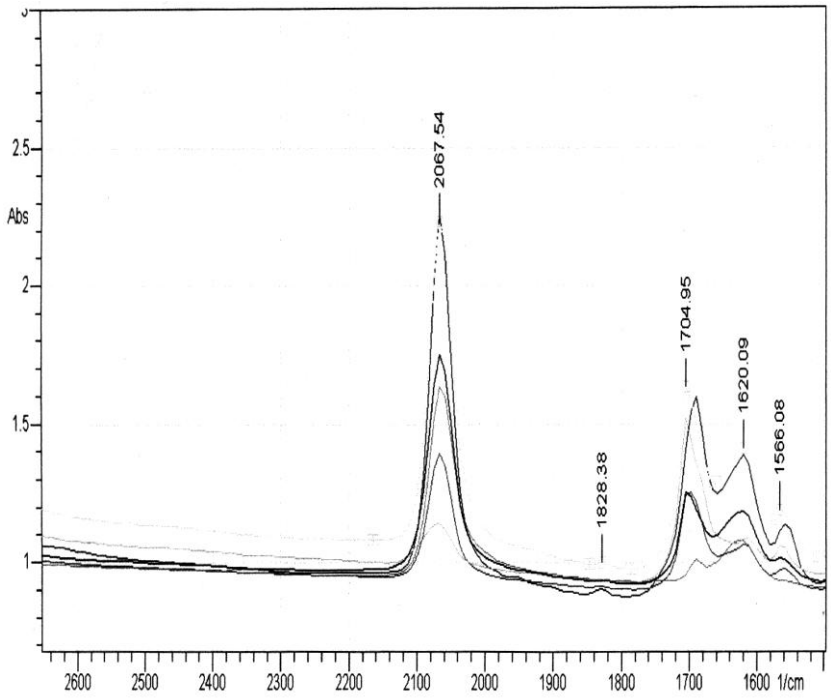


Table 7: Concentration Vs Ratio of absorbance

concentration(in mg)	**Ratio of absorbances ($2067.54cm^{-1}$ /$1704.95cm^{-1}$)**
0.5	0.75
1	1.112
1.5	1.613
2	2.15

*Each value is the mean of three readings.

Assay

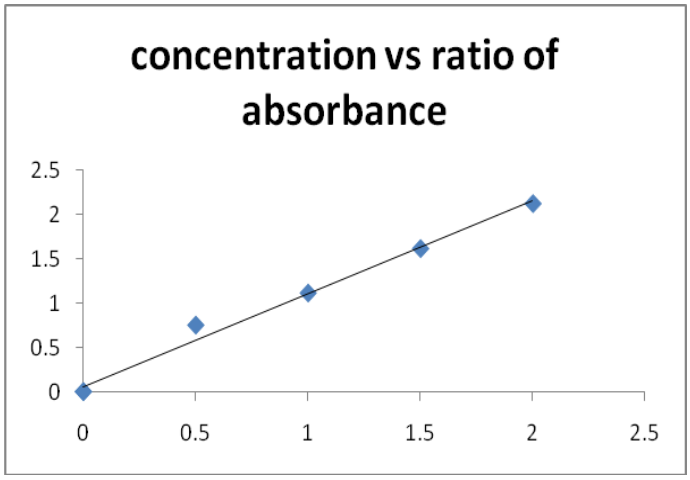
10 tablets of Chlorthalidone were weighed and ground to fine powder. Accurately weighed tablet powder equivalent to 10mg of chlorthalidone is dissolved in 100ml of ethanol to make a concentration of 100mcg/ml. From that 10 ml of solution which was equivalent to 1mg was taken in a porcelain dish and evaporated. Then it was mixed with the KBr/KSCN mixture and then homogenized by using agate mortar & pestle under IR lamp. The final powder was transferred to KBr press to form a disc and the infrared spectrum in absorbance mode was recorded.

The sample peak area was interpolated on the respective linearity chart of the chlorthalidone and the concentration was determined. .The amount of drug present in each tablet was calculated and the assay results are presented in the **Table 13.**

Calibration graph

A graph was constructed by plotting the ratio of absorbances against concentration and is shown in (**Fig-11)**. It was observed chlorthalidone obeyed Beer's law in the concentration range of 0.5-2.0mg.

Fig- 11: Calibration graph for chlorthalidone



Recovery studies

The recovery studies were carried out on spiked sample by adding predetermined amount of standard drugs to the respective sample about 50% and 100% of standard drug was added to the sample and the absorbance was measured against method blank. The percentage recovery was calculated and presented in the **Table 14.**

QUANTITATIVE SPECTROFLUORIMETRY

Quantitative spectrofluorimetric assays involves dilution, extraction and chromatographic separation, chemical reaction of sample and finally the determination of the intensity of fluorescence ni9s carried out. Fluorescence intensity is directly proportional to the concentration of the substance Chlorthalidone was found and obey linearity at low concentration(mcg or ng/ ml) but in high (concentration mg/ml) it does not obey linearity.

Establishment of various parameters

- Emission spectrum
- Fluorescence spectrum
- Calibration spectrum
- Estimation of analyte in dosage form
- Recovery studies

Instrumentation

The fluorimetric measurements were made on Jobin Yvon flurolog 3-11- spectrofluorimeter with Data max/ Grams/31 software.

Preparation of standard stock solution

A standard stock solution was prepared by dissolving 100mg of chlorthalidone in 100ml standard flask and the volume was made up with ethanol to produce 1000mcg/ml.

Preparation of sample solution

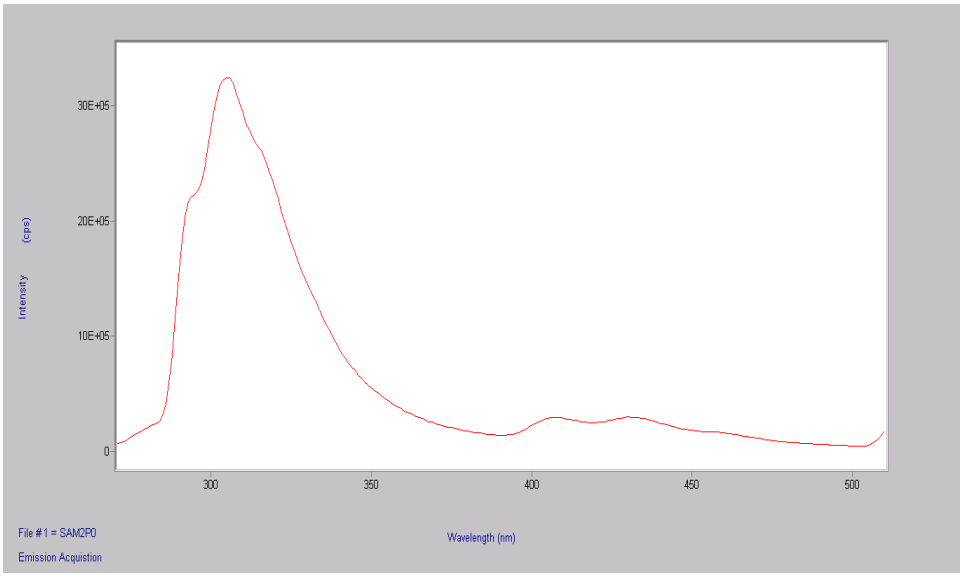
The average weight 10 tablets of chlorthalidone was weighed and finely powdered. The powder equivalent to 100 mg of chlorthalidone was taken in a 100 ml volumetric flask and made up to the volume to produce 100mcg/ml with ethanol. The solution was filtered, first few ml of the filtrate was discarded and the filtrate was used for further analysis.

METHOD 7: DIRECT SPECTROFLUORIMETRIC METHOD

Emission spectrum

The standard stock solution was suitably diluted in ethanol to yield a concentration of 0.8mcg/ml. This solution was scanned in the spectrofluorimeter between 250-700nm using ethanol as blank. It was found that chlorthalidone exhibited an intense maximum absorption at about 305nm.

Fig-12: Flourescence spectra of chlorthalidone at 305 nm



Fluorescence concentration to confirm the linearity range

Aliquots of standard solution of chlorthalidone were suitably diluted to give varying concentrations ranging from 0.4-2.0 µg/ml. the solution were scanned in the range of 250-700nm and the relative fluorescence was measured an emission wavelength of 305nm with excitation wavelength of 258nm. A calibration graph was obtained by plotting fluorescence intensity versus concentration.

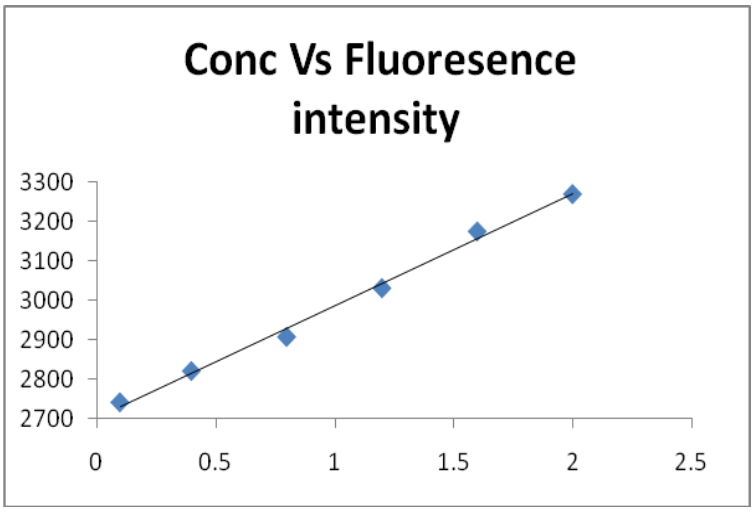
Table 8: Fluoresnce intensity of chlorthalidone at 305nm

S.NO	Concentration (in mcg/ml)	Fluorescence intensity (mcps)*
1	0.4	2819.7
2	0.8	2906.7
3	1.2	3030.7
4	1.6	3205.0
5	2.0	3298.4

Calibration graph

A graph of fluorescence intensity against concentration was plotted. From the graph the fluorescence concentration for the analyte was found to be between 0.4-2.0µg /ml. **(Fig-13)**

Fig-13: Calibration graph of Chlorthalidone by spectrofluorimetry



Analysis of sample

Weighed 10 tablets of chlorthalidone and ground to fine powder. Accurately weighed tablet powder was taken in a 100 ml volumetric flask and shaken with ethanol to dissolve the active ingredient and made up to volume to produce 100µg/ml. The solution was then filtered, first few ml of the filtrate was discarded and the filtrate was used for further analysis. The sample solution was suitably diluted to get a concentration between 0.4-2.0µg/ml and the same procedure was adopted. The fluorescence intensity obtained for the sample was then interpolated on the calibration graph and the concentration of chlorthalidone in the sample was then determined. The spectrum for this method is shown in **(Fig-12).**

Recovery studies:

The recovery studies were carried out on spiked samples by adding predetermined amount of standard drug to the respective sample. About 50 and 100% of standard drug was added to the sample and the fluorescence intensity was measured. The percentage recovery was calculated and represented in **Table 17.**The recovery study was performed to confirm the precision and accuracy of the above said method.

RESULTS AND DISCUSSION

Table 9: Optical characteristics of chlorthlidone by UV spectrophotometry

S. No	Parameters	UV spectrophotometry	AUC Method	First Derivative spectroscopy	Second Derivative Spectroscopy	Q-absorbance Method
1	Wavelength range (nm)	275	275	275	275	275
2	Linearity range (µg/ml)	40-160	40-160	40-160	40-160	40-160
3	Molar absorptivity ($Lmol^{-1}cm^{-1)}$	6106.667	-	-	-	-
4	Sandell's sensitivity (µgcm-2/ 0.001 A unit)	0.189322	-	-	-	-
5	Regression equation (y=mx+c)	0.005219x+ 0.008162	0.005018x +0.0222071	0.309821x+ 3.4821	0.333929x+9.10714	0.001019x+ 0.002175
6	Slope (m)	0.005219	0.005018	0.309821	0.333929	0.001019
7	Intercept (c)	0.008162	0.022071	3.4821	9.10714	0.002175
8	Correlation coefficient	0.998831	0.98657	0.9929	0.97810	0.99196

*Each value is the mean of three readings .

The results obtained by various spectrophotometric methods are presented in **Table10.**

Table 10: Results of analysis of formulation and statistical parameters for Chlorthalidone by Various spectrophotometric methods

S.NO	Methods	Label claim (mg)	Amount found by proposed method (mg)*	% Label claim	SD	SE	RSD
1	UV spectrophotometry	12.5	12.6	100.8	0.5139	0.010564	0.9659
2	AUC Method		12.5	100	0.1888	0.03930	0.3438
3	First Derivative Spectrophotometry		12.50	100	12.48	1.7561	0.416
4	Second Derivative Spectrophotometry		12.49	99.99	12.22	1.6325	0.4525
5	Q-absorbance method		12.50	100	0.02846	0.008096	0.2611

*Each value is the mean of three readings .

The results obtained for recovery studies performed for different spectrophotometric methods are presented in **Table 11.**

Table 11: Recovery studies for chlorthalidone by various spectrophotometric methods

S.NO	Method	Label claim(in mg)	Amount of drug added (%)	Amount of drug recovered (%)	% Recovery
1	UV spectrophotometry	12.5	20 50 100	20 49 98.69	100 98.27 98.69
2\	AUC Method	12.5	20 50 100	19.77 49.13 98.69	98.99 98.27 98.69
3	First Derivative Spectrophotometry	12.5	20 50 100	20 49.5 98.69	100 99 98.69
4	Second Derivative Spectrophotometry	12.5	20 50 100	20 49.13 98.06	100 98.27 98.06
5	Q-absorbance method	12.5	20 50 100	20 49.19 98.69	100 98.27 98.69

*Each value is the mean of three readings .

In **Standard absorbance method**, chlorthalidone showed an absorption maximum at 275nm and was subjected for quantification. The drug obeyed Beer's law in the range of 40-160µg/ml. The regression equation was found to be 0.005219x+ 0.008162. The molar absorptivity obtained was 6106.667 ($Lmol^{-1}$ cm^{-1}). The correlation coefficient was found to be 0.9988 which shows a good linearity between concentration and absorbance. The percentage recovery obtained was found to be 100.81 % ,which indicates the accuracy of the method. The results of the analysis of formulation from the (Table) show that the proposed method is in good agreement with the labeled amount of drug.

In **AUC method,** the normal spectra were subjected to AUC mode. The AUC wavelengths from 270.2 nm to 280.0 nm were selected to estimate the amount of chlorthalidone present in tablet dosage form.The area under the curves were noted and the calibration curve was plotted. The correlation coefficient was noted and the calibration curve was plotted. The area under the curves were noted and the calibration graph was plotted. The correlation coefficient was found to be 0.9865 which shows a good linearity between concentration and area.

The **First derivative spectroscopy** method is simple, accurate, rapid and reproducible. When the first derivative method is applied to chlorthalidone estimation,it produced good results without any interference from excipients. The recovery studies were done ,the values begin to 98.69 to 100.0 indicating the accuracy of the proposed method. The regression equation was 0.309821x+ 3.4821. The correlation coefficient was found to be 0.9929 . The RSD was found as 0.416 proving the precision of the method.

When the **Second derivative spectroscopy** was applied to chlorthalidone estimation, it produced good results without any interference from excipients. The recovery studies done ,where the value was 98.06 to 100.0 indicating the accuracy of the proposed method. The regression equation was 0.333929x+9.10714 . The correlation coefficient was found to be 0.9781. The RSD was found as 0.4525 proving the precision of the method.

The **Q-absorbance method** is based on the criteria that the ratio of absorbances at any two wavelengths is a constant value independent of concentration or pathlength. In this method the wavelengths selected are 275 and 284 nm . The ratio of absorbance at these wavelengths is constant for all the concentrations. The linearity range is40-160µg/ml. To study the accuracy of the developed method, recovery study was carried out using standard addition method. The results that was no interference of excipients.

Table 12: Optical characteristics of chlorthalidone by infrared spectrophotometry method

Parameters	**IR spectroscopy quantification method**
Beer's law limit	0.5-2.0
Regression equation	1.0206x+0.0984
Slope	1.0206
Intercept	0.0984
Correlation coefficient	0.9942
Standard deviation	0.5274

*Each value is a mean of 3 determinations

The results obtained by infrared Spectrophotometric method are presented in **Table13.**

Table 13: Results of analysis of formulation and statistical parameters for chlorthalidone by using infrared spectrophotometric method (IR)

METHOD	**Label Claim(in mg)**	**Amount of drug found by proposed method (in mg)**	**% Label claim**	**SD**	**RSD**
KBr disc method using Internal standard	12.5	12.25	98.0	0.5274	0.4742

*Each value is a mean of 3 determinations

The results obtained for recovery studies performed for infrared spectrophotometric method are presented in **Table 14.**

Table 14: Recovery study for chlorthalidone by Infrared spectrophotometric method

Method	Label claim(in mg)	Amount of drug added(in mg)	Amount of drug recovered	%recovery
IR quantification method	12.5	1.5	1.4463	96.42
		2.0	1.9064	95.32

*Each value is a mean of 3 determinations

Infrared spectroscopic method is simple, accurate, rapid and reproducible. It produced good results without any interferences from excipients. The recovery studies were done, where the value was 95.32 to 96.42 indicating the accuracy of the proposed method. The regression equation was 1.0206x+ 0.0984 . The correlation coefficient was found to be 0.9942. The RSD was found to be 0.4742 proving the precision of the method.

Table 15: Optical characteristics of Chlorthalidone by Spectrofluorimetric method

S.NO	Parameters	Spectrofluorimetric method
1	Excitation wavelength(nm)	258
2	Emission wavelength (nm)	305
3	Fluorescence concentration (in mcg/ml)	0.4-2.0
4	Regression equation (y=mx+c)	313.92x+2675.39
5	Slope(m)	313.92
6	Intercept (c)	2675.39
7	Correlation coefficient	0.9938

*Each value is the mean of three readings

The results obtained by spectrofluorimetric method are presented in the **Table 16**.

Table 16: Results of analysis of formulation and statistical parameters for chlorthalidone by using spectrofluorimetric methods.

S.NO	**Method**	**Label claim**	**Amount found by proposed method(mg)***	**%Label claim**	**SD**	**SE**	**RSD**
1	Direct Spectrofluorimetric method	12.5mg	12.56mg	100.48	178.68	25.64	0.0589

*Each value is the mean of three readings

The results obtained for recovery studies performed for spectrofluorometric method are presented in **Table 17.**

Table 17: Recovery study for chlorthalidone by Spectrofluorimetric method

S.NO	Method	Label claim	Amount of drug added(mcg)*	Amount of drug recovered(mcg)*	% Recovery
1	Direct fluorimetric method	12.5mg	1.8 2.4	1.85 2.35	102.7 97.91

*Each value is the mean of three readings

The **direct spectrofluorimetric method** adopted simple, accurate,sensitive, rapid highly specificity and reproducible. It produces good results without any interferences from excipients. The recovery studies were done, where the value was 97.91 to 102.7 % indicating the accuracy of the proposed method. The regression equation was 313.92x+2675.39.The correlation. coefficient was found to be 0.9938. The RSD was found to be 0.0589 proving the precision of the method.

SUMMARY AND CONCLUSION

The present work entitled **" Quantification of Chlorthalidone in bulk and Pharmaceutical dosage form by UV, IR spectrophotometry and spectrofluorimetry "** comprises of the following novel methods which have not been reported till date.

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- **UV Spectrophotometry**
    - Standard absorbance method
    - Area Under curve method
    - First Derivative Spectrophotometry
    - Second Derivative method Spectrophotometry
    - Q-absorbance method
- **Infrared Spectrophotometry**
    - KBr Disc method using Internal standard
- **Spectrofluorimetry**
    - Direct spectrofluorimetric method

The Ultraviolet method involves the determination of chlorthalidone by different methods like standard absorbance method ,area under curve, derivative spectroscopy and Q-absorbance method. The drug obeyed Beer's law at the concentration of 40 -160 µg/ml. The correlation coefficient was found to be 0.998 for all the methods. The low percentage RSD value shows that the methods developed are not affected by the presence of sample matrix (or) devoid of interference by the excipients.

The quantitative infrared spectrophotometric method is a novel method where KSCN has been used as the internal standard. The drug obeyed Beer's law in the concentration range of 0.5mg-2.0mg. The drug showed good linearity as indicated by correlation coefficient value 0.9942. The low percentage RSD value shows that the methods developed are not affected by the presence of sample matrix (or) devoid of interference by the excipients.

The quantitative spectrofluorimetric methods for estimation of Chlorthalidone is also a novel method. Chlorthalidone was found to exhibit fluorescence in the concentration range of 0.4 - 2.0µg/ml by direct fluorimetric method. Chlorthalidone showed good linearity as indicated by correlation coefficient value of 0.9938 respectively. Thus the methods were precise, sensitive, highly specific and accurate.

Therefore all the methods developed and validated could be used for routine analysis and are devoid of interference by sample excipients.

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UV SPECTROPHOTOMETRIC METHODS FOR THE ESTIMATION OF CHLORTHALIDONE IN BULK AND ORAL DOSAGE FORM

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ABSTRACT

Simple, precise and economical spectrophotometric methods (Method A ,Method B and Method C and Method D) have been developed for the estimation of chlorthalidone bulk as well as in tablet dosage form . MethodA involves the determination of chlorthalidone by standard absorbance method at 275nm and the Beer's concentration range was found to be 40-160µg/mL. Method B involves the determination of chlorthalidone by Area Under Curve(AUC) method and the linearity was established. Method C involves the determination of chlorthalidone by first order derivative method. In Method D the difference between two absorbances at 275nm and at 284 (Q-absorbance) were used for quantification .The results of analysis have been validated statistically by ANOVA method and by recovery studies.

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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 UV spectrophotometric method for the estimation of chlorthalidone .So the purpose of the described method is to develope 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, UV spectrophotometric 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.

MATERIALS AND METHODS

1. Bulk material: Sample of chlorthalidone gifted from Madras pharmaceuticals.
2. Dosage form: Chlorthalidone tablet was purchased from local market.
3. Ethanol

EXPERIMENTAL [9]

Instrumentation

All spectral and absorbance measurements were made on Shimadzu UV-Visible spectrophotometer -1650.

Preparation of standard stock solution

100mg of standard chlorthalidone was accurately weighed & transferred into 100ml standard flask. Sufficient quantity of ethanol was added to dissolve the drug & the volume was made up with ethanol (1µg/mL).From the above standard stock solution different concentrations in the range of 40-160µg/mL were prepared at an interval of 20 µg/mL.

Preparation of sample solution

Five tablets were weighed and powdered. Accurately weighed tablet powder equivalent to 100mg of chlorthalidone was taken in a 100ml volumetric flask and shaken well with ethanol to dissolve the active ingredient and made upto volume to produce 1000 µg/mL . The solution was then filtered, first few ml of the filtrate was discarded and the filtrate was used for further analysis.

METHOD A - STANDARD ABSORBANCE METHOD

Various aliquots of standard solutions ranging from 40-160 µg/mL concentrations of chlorthalidone were scanned at 275 nm and the absorbance was noted using ethanol as blank. Graph was plotted by taking concentrations on X-axis and absorbances on Y-axis. Chlorthalidone obeys Beer's law in the range of 40-160 µg/mL. The absorbance obtained for the sample was then interpolated on the calibration graph(Fig.2) and the concentration of chlorthalidone in the sample was then determined. The spectrum for this method is shown in (Fig-3).

METHOD B – AREA UNDER CURVE [10]

The standard stock solution of chlorthalidone was suitably diluted to give varying concentrations ranging from 40-160 µg/mL. The solutions were scanned in the range of 200-400nm. The area under the curve between 270.2 - 280 nm was measured by using the inbuilt software. The inbuilt software calculates the area bound by the curve and the horizontal axis .The horizontal axis is selected by entering the wavelength range over which the area has to be calculated . The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration (Fig.4) , Table.1. The AUC spectrum is shown in Fig.5.

METHOD C: FIRST DERIVATIVE SPECTROPHOTOMETRY[11]

The first derivative (D^1) spectrum is a plot of the gradient of absorption curve (rate of change of absorbance with wavelength (i.e,$dA/d\lambda$ Vs λ) against wavelength.The standard stock solution of chlorthalidone was suitably diluted to give varying concentrations ranging from 40-160µg/mL. The solutions were scanned in the range of 200-400nm and the primary spectrum was recorded.The primary spectrum was then derivatized to the first order using derivative mode. The amplitude of the positive peak maximum at the zero crossing of the first order curve was measured in mm at 275nm. A calibration graph was obtained by plotting concentration versus amplitude. The sample solution was suitably diluted to get a concentration between 40-160µg/mL and the same procedure

was adopted .The amplitude obtained for the sample was then interpolated on the calibration graph (Fig.6), Table.2 and the concentration of Chlorthalidone in the sample was then determined. The overlain spectra for this method is shown in (Fig.7) .

METHOD D: Q-ABSORBANCE METHOD[12]

Q-absorbance method depends on the property that, for substance which obeys Beer's law at all wavelength ,the ratio of absorbances at two wavelengths is a constant value independent of concentration or path length .The difference in absorbance between these two Wavelengths were calculated. The linearity chart was constructed using difference in absorbance and concentration. The difference in absorbance of sample was then interpolated on the calibration graph (Fig .8) and the concentration of chlorthalidone was then determined. The values obtained by proposed methods are presented in Table 3.

RECOVERY STUDIES

The recovery studies were carried out on spiked samples by adding predetermined amount of standard drugs to the respective sample. About 20%, 50% and 100% of standard drug was added to the sample and the absorbance was measured. The percentage recovery was calculated. The recovery study was performed at two levels to confirm the precision and accuracy of the above said method.

RESULTS AND DISCUSSION

The optical characteristics such as absorption maxima ,Beer's law limits,molar absorptivity and Sandell's sensitivity are furnished in the Table- 4 . The regression characteristics like slope (b), intercept (a),correlation co-efficient (r) and standard error (SE) were calculated and the results are summarized in the Table- 4 . The percentage recovery of the four methods lies between 99 - 100 %w/w. The correlation coefficient for the four methods was found to be 0.999 & 0.998 and the recovery studies indicate that there is no interference of other ingredients present in the formulation. Thus, these four methods are simple, precise, accurate, less time consuming, specific, sensitive and could be used for routine analysis. The amount , % label claim and % recovery studies obtained by the proposed methods as shown in Table -5

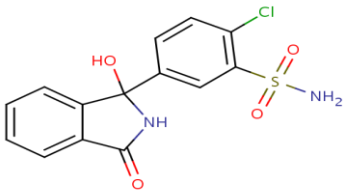

Figure 1: Structure of chlorthalidone

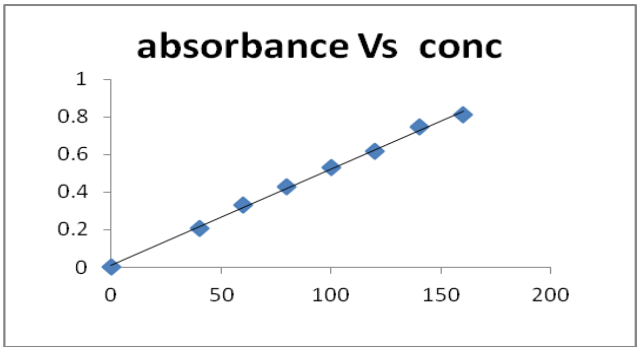

Figure 2: Calibration graph for chlorthalidone [standard absorbance method

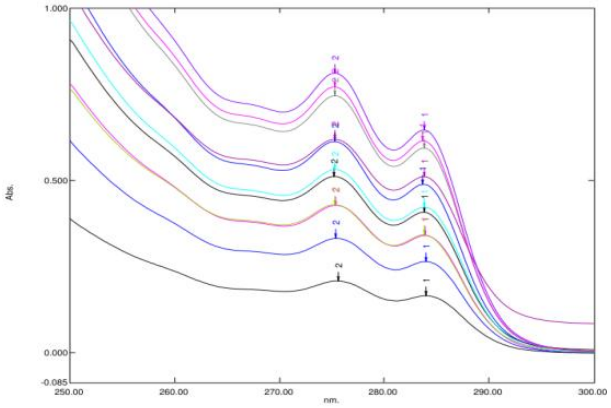

Figure 3. Overlain spectrum of chlorthalidone

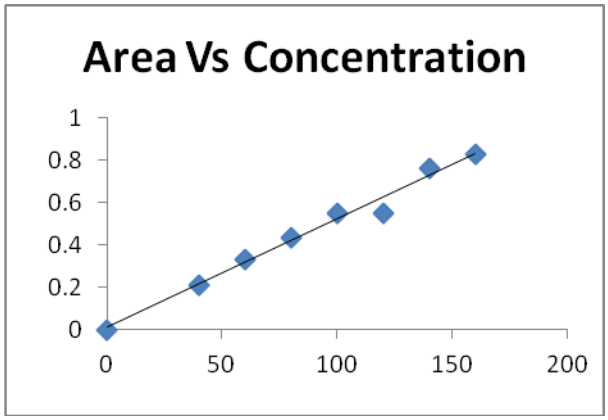

Figure 4: Calibration graph for chlorthalidone (AUC method)

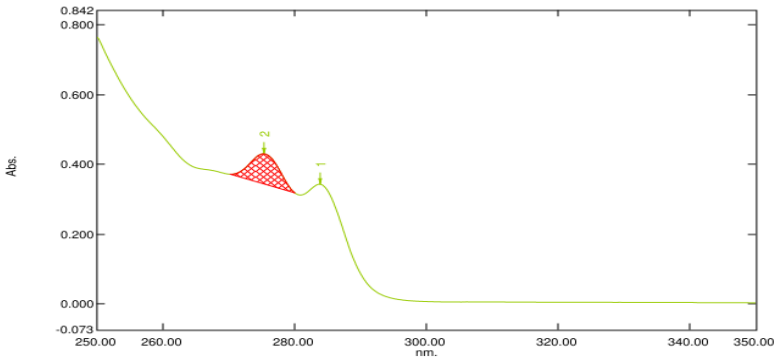

Figure 5: AUC spectrum of chlorthalidone

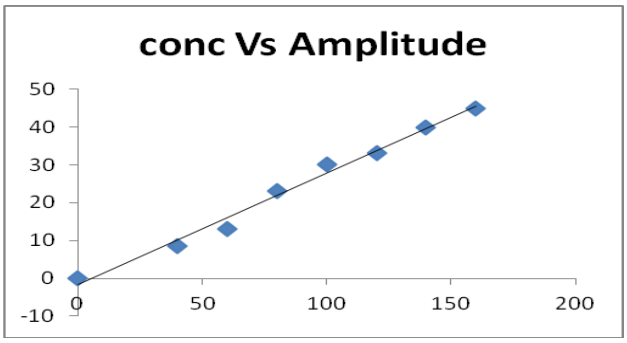

Figure 6: Calibration graph for chlorthalidone (First derivative method)

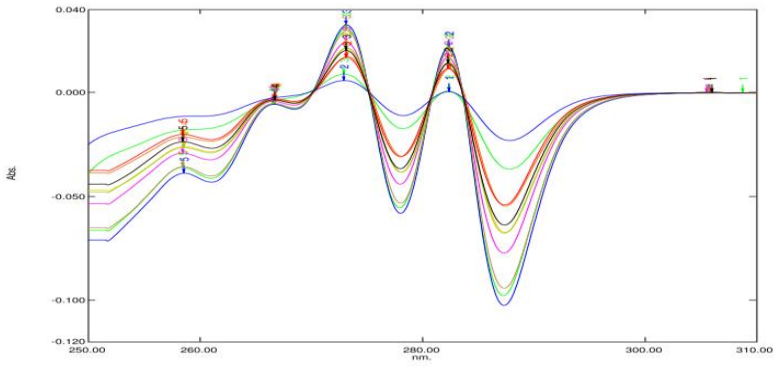

Figure 7: Overlain spectrum of chlorthalidone (First derivative)

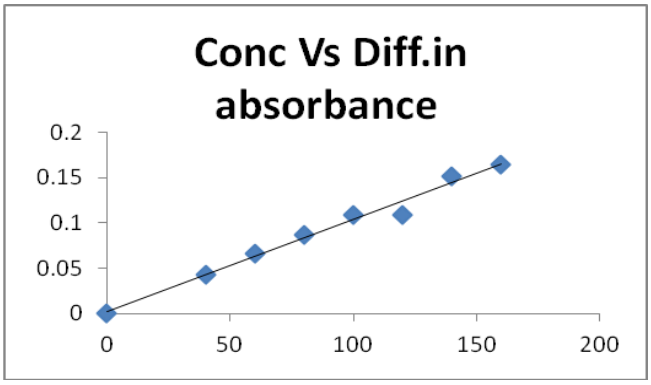

Figure 8: Calibration graph for chlorthalidone (Q-absorbance method)

TABLE 1: Area Vs Concentration

Sl. NO.	Concentration (in µg/mL)	Area*
1	40	0.212
2	60	0.333
3	80	0.437
4	100	0.549
5	120	0.550
6	140	0.759
7	160	0.827

* Each value is the mean of five readings.

TABLE 2: Concentration Vs Amplitude [FIRST DERIVATIVE]

Sl. NO.	Concentration in µg/mL)	First derivative Amplitude (mm)*
1	40	8.5
2	60	13
3	80	23
4	100	30
5	120	33
6	140	40
7	160	45

* Each value is the mean of five readings.

TABLE 3: Difference in absorbance at 275 and 284nm

PARAMETERS	METHOD A	METHOD B	METHOD C	METHOD D
λ max (nm)	275	275	275	275
Beer's Law limits (µg/ml)	40-160	40-160	40-160	40-160
Molar absorptivity (L mol^{-1} cm^{-1})	6106.667	-	-	-
Sandell's sensitivity (µgcm^{-2} /0.001 absorbance unit)	0.189322	-	-	-
Regression equation (*Y)	0.005219 x +0.008162	0.005018 x + 0.022071	0.309821 x + 3.48214	0.001019 x + 0.002175
Slope (m)	0.005219	0.005018	0.309821	0.001019
Intercept (c)	0.008162	0.022071	3.48214	0.002175
Standard Deviation [SD]	0.5139	0.1888	12.48	0.02846
Correlation co-efficient (r)	0.998831	0.98657	0.99290	0.99196
Standard error	0.010564	0.039307	1.756112	0.008096

* Each value is the mean of five readings.

TABLE 4: Optical characteristics for proposed methods

Conc. in mcg/ml	Absorbance at 226nm	Absorbance at 262nm	Difference in absorbance
40	0.209	0.166	0.043
60	0.332	0.266	0.066
80	0.428	0.341	0.087
100	0.532	0.423	0.109
120	0.620	0.501	0.109
140	0.746	0.594	0.152
160	0.812	0.647	0.165

Table 5: Recovery Studies

Sl. no	Method	Label claim	Amount Drug added (%)	Amount Drug Recovered (%)	% Recovered
			20	20	100
1	UV spectrophotometry		50	49	98.27
			100	98.69	98.61
			20	19.77	98.99
2	AUC		50	49.13	98.27
		12.5mg	100	98.69	98.69
			20	20	100
3	First Derivative		50	49.5	99
	spectrophotometry		100	98.69	98.69
			20	20	100
4	Q -Absorbance		50	49.19	98.27
	Method		100	98.69	98.69

Table 5: ANOVA calculation

ANOVA calculation for AUC method				
Source of variation	SS	df	MS	F- ratio
Between sample	-1.693	5	0.3386	3.203
Within sample	3.173	30	0.1057	
ANOVA calculation for FIRST DERIVATIVE method				
Source of variation	SS	df	MS	F- ratio
Between sample	-9382.14	4	-2345.5	4.746
Within sample	14823.7	30	494.12	
ANOVA calculation for Standard absorbance method				
Source of variation	SS	df	MS	F- ratio
Between sample	-1.74	4	-0.435	-4.14
Within sample	3.176	30	0.105	

CONCLUSION [13]

The proposed methods are simple,accurate ,precise and selective for estimation of chlorthalidone in bulk and pharmaceutical dosage form. These methods are economical ,rapid and do not require any sophisticated instruments contrast to chromatographic method.Hence it can be effectively applied for the routine analysis of chlorthalidone in bulk and pharmaceutical dosage form.

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IR QUANTICATION OF CHLORTHALIDONE IN BULK AND ORAL DOSAGE FORM

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Abstract

Simple and sensitive Infrared spectrophotometric method have been developed for the estimation of chlorthalidone in tablet dosage form and the Beer's concentration range was found to be 0.5mg-2.0mg. The correlation coefficient for the method was found to be 0.9942 and the developed method was analyzed for specificity, linearity of response, precision and accuracy. Thus the proposed method could be adopted for routine analysis of bulk drug and its formulation.

Keywords: Infrared spectroscopy (IR), Potassium thiocyanate(KSCN), Potassium bromide disc.

Introduction[1-4]

Chlorthalidone is (RS)-2-chloro-5-(3-hydroxy-1-oxo isoindoli-3-yl) benzene sulphonamide and is widely used in antihypertensive 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 .Literature survey revealed that few sophisticated analytical methods have been reported for the estimation of chlorthalidone. The present work aims to devise a novel method using Infrared spectrophotometry (IR) which has not been reported till date.

Materials and Methods

All the chemicals used throughout the experiment were of highest purity of (IR grade).

- Potassium bromide (KBr)
- Internal standard: potassium thiocyanate (KSCN)
- Bulk material: Gift sample of chlorthalidone was obtained from Madras pharmaceuticals.
- Dosage form: Chlorthalidone tablets was purchased from local market.

Instrumentation

All spectral measurements were made on ABB-IR instrument (model no: MB 3000) with KBr press (model no:M 15)

Method

Standard preparation: Calibration of the standard: Potassium thiocyanate was used as an internal standard which was preground, dried, and then reground with dry KBr to make a concentration of about 0.2% by weight of potassium

thiocyanate. The final mixture was stored over phosphorus pentoxide. Stock of standard was prepared in alcohol and diluted with ethanol to give a concentration of 100mcg/ml. From this aliquot quantity required were pipette out to meet the required concentration and evaporated in a porcelain dish. To the residue known quantity of KBr-KSCN was added mixed and homogenized using agate mortar& pestle under IR lamp (Table-1). The discs were prepared by using KBr press and the infrared spectrum was recorded in absorbance mode(Fig1).Ratio of absorbance was taken from the two wave numbers (2067.54cm^{-1} /1704.95cm^{-1}). A standard calibration curve was constructed using ratio of absorbance versus concentration and presented in Table-2, (Fig 2).

Table-1: Concentration of KBr/KSCN mixture and standard.

KBR/KSCN (in mg)	50	50	50	50	50
Standard in) (in mg)	0.0	0.5	1.0	1.5	2.0

Fig.1: IR Spectra of standard Chlorthalidone with internal standard (KBr/KSCN).

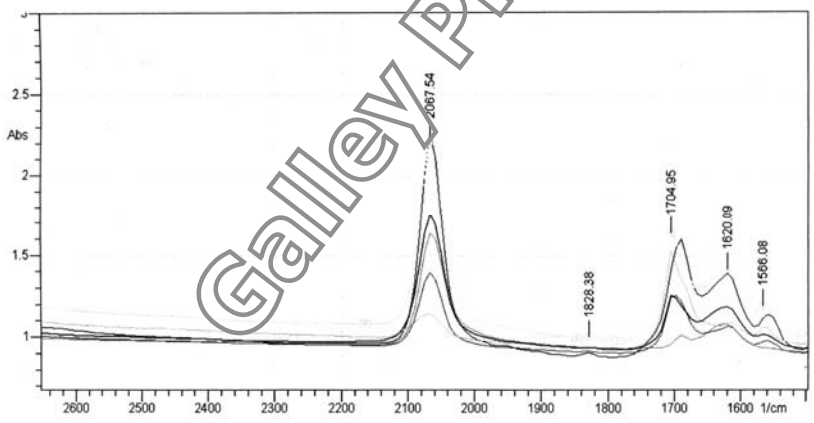
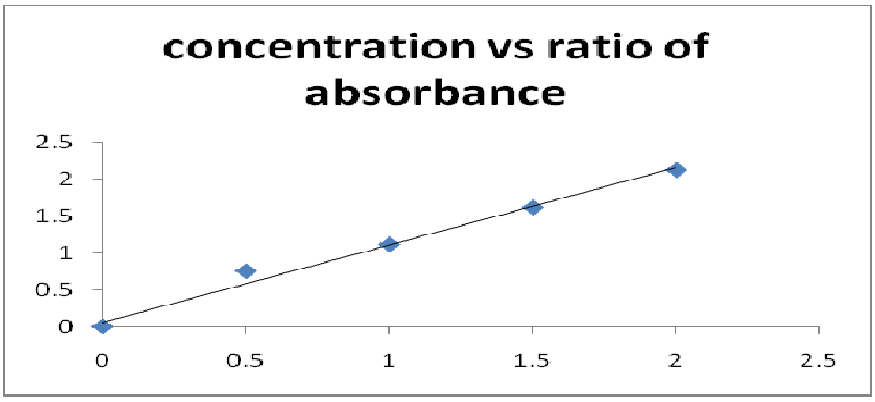


Table-2: Concentration Vs Ratio of absorbance.

concentration(in mg)	Ratio of absorbances (2067.54cm^{-1} /1704.95cm^{-1})
0.5	0.75
1	1.112
1.5	1.613
2	2.15

Fig.2 Calibration graph for chlorthalidone



Sample preparation

10 tablets of Chlorthalidone were weighed and ground to fine powder. Accurately weighed tablet powder equivalent to 10mg of chlorthalidone is dissolved in 100ml of ethanol to make a concentration of 100mcg/ml. From that 10 ml of solution which was equivalent to 1mg was taken in a porcelain dish and evaporated. Then it was mixed with the KBr/KSCN mixture and then homogenized by using agate mortar & pestle under IR lamp. The final powder was transferred to KBr press to form a disc and the infrared spectrum in absorbance mode was recorded. The sample peak area was interpolated on the respective linearity chart of the chlorthalidone and the concentration was determined.

Recovery Studies

The recovery studies were carried out on spiked samples by adding predetermined amount of standard drugs to the respective sample. About 50 and 100% of standard drugs were added to the sample and the absorbance was measured. The percentage recovery was calculated. The recovery study was performed at two levels to confirm the precision and accuracy of the above said method.

Results and Discussion

Chlorthalidone was found to obey Beer's law in the concentration range of 0.5mg-2.0mg. chlorthalidone good linearity as indicated by correlation coefficient value of to 0.9942. The optical parameters of chlorthalidone are presented in table-3. The percentage of the drug in the formulation was calculated and presented in table-4. The results of the analysis showed that the amount of drug present in the formulation was in good agreement with the label claim of the formulation. The accuracy of the proposed method was determined by recovery study. The recovery studies were carried

out on spiked samples at two levels 50%, 100%. The percentage recovered were found to be in the range of 95-100% represented in table-5. The IR quantification process does not involve prior extraction and is independent of drug materials solubility.

Table-3: Optical parameters of chorthalidone by IR spectrophotometry.

Parameters	IR spectroscopy quantification method
Beer's law limit	0.5-2.0
Regression equation	1.0206x+0.0984
Slope	1.0206
Intercept	0.0984
Correlation coefficient	0.9942
Standard deviation	0.5274

Table-4: Result of tablet Assay and statistical parameters for chlorthalidone by IR spectrophotometry.

Method	**Label Claim(in mg)**	**Amount of drug found by proposed method (in mg)**	**% Label claim**	**SD**	**%RSD**
KBr disc method using Internal standard	12.5	12.25	98.0	0.5274	47.42

*The given value is a mean of 3 determinations

Table-5: Recovery study for chlorthalidone by infrared spectrophotometric method.

Method	Label claim(in mg)	Amount of drug added(in mg)	Amount of drug recovered	%recovery
IR quantification method	12.5	1.5	1.4463	96.42
		2.0	1.9064	95.32

Conclusion

The percentage recovery of the method lies between 95- 100 %. The correlation coefficient for the method was found to be 0.9942 and the recovery studies indicates that there is no interference of other ingredients present in the formulation. Thus the method is simple, precise, accurate, less time consuming and could be used for routine analysis.

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