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UV- SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF CHLORTHALIDONE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A rapid, simple, selective, sensitive, precise and specific UV Spectrophotometric method has been developed for the determination of Chlorthalidone in bulk and (200-400nm) in 1cm quartz cell in a double beam UV Spectrophotometer. The spectrophotometric detection was carried out at an absorption maximum of 227 nm using Methanol as solvent. The detector response for the Chlorthalidone was linear over the selected concentration range 2-10µg /ml with a correlation coefficient of 0.999 and equation for the regression curve was found to be *y=0.071x+0.182*. The accuracy was between 97-100%. The precision (%RSD) among six samples preparation was 0.160%. The LOD and LOQ was 0.251 and 0.3200µg/ml respectively. Statistical analysis proved that the methods are repeatable and specific for the determination of the said drug. These methods can be adopted in the routine assay analysis of Chlorthalidonein API and pharmaceutical dosage form.

KEYWORDS

Chlorthalidone, UV Spectrophotometer, Methanol and Method Validation.

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INTRODUCTION

Chlorthalidone (CTD) (first introduced in Switzerland in 1959) is a sulphanylbenzophenone derivative [2-chloro-5-(1-hydroxyl-3-oxo-2, 3-dihydro-1H-isoindol-1-yl)benzene-1-sulfonamide][9]. It is a diuretic agent used in the treatment of oedema associated with congestive heart failure Compared with other medications like thiazide class[7]. Chlorthalidone has a longer duration of action but a similar diuretic effect at maximal therapeutic doses

Chlorthalidone analysis as anti-hypertensive drug is of great interest, since hypertension is very common disorder, particularly in the past middle age[6]. Accordingly, the development and validation of new analytical methods for estimation of anti-hypertensive drug are required Literature survey reveals that there are various analytical methods for estimation of Chlorthalidone[5,8].

Chlorthalidone is a sulphanylbenzophenone derivative [2-chloro-5-(1-hydroxyl-3-oxo-2, 3-dihydro-1H-isoindol-1-yl)benzene-1-sulfonamide][9].

Chlorthalidoneit is a white to yellowish-white crystalline powder which is practically insoluble in water, in ether and in chloroform; soluble in methanol; slightly soluble in alcohol[4].

The present work is a simple, sensitive, accurate and precise Spectrophometric Method for the estimation of Chlorthalidonein bulk and its Pharmaceutical Dosage Forms with the help of Methanol.

MATERIAL AND METHODS

Instruments

For weighing, a calibrated weighing balance (Shimadzu) of 1mg sensitivity was used. A Systronic UV-visible double beam spectrophotometer- 2201 was used. All the glass wares and were made of borosilicate and were calibrated.

Chemicals

API- Chlorthalidone pure drug was gifted by Aadhaar life sciences Pvt Ltd. Solapur.

Chlorthalidone12.5 mg strength were purchased from the local pharmacy in Solapur under commercially available brand name CTD (12.5mg) (Ipac laboratories Ltd.), Methanol LR was used in this study.

UV Spectroscopic Method

Solvent Selection

Chlorthalidone is soluble in Methanol so, methanol is used as the solvent.

Preparation of Standard Stock Solution

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

Determination of Absorption Maxima

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

Preparation of Calibration Curve

For the preparation of calibration curve, the concentration of2-10µg/ml were prepared by pipetting out 0.2, 0.4, 0.6, 0.8 and 1 ml of the 100µg/ml solution into 10 ml volumetric flasks and made up the volume with Methanol.

The absorbance of each solution was measured at 227 nm against Methanol as blank. Calibration curve of the Chlorthalidone was plotted by taking the absorbance obtained on the y-axis and concentration of the solution on the x-axis. The curve showed linearity in the range of 2-10µg/ml with correlation coefficient 0.999.

Quantitative Analysis of Tablet Dosage Form

20 Tablets was accurately weighed, and reduced to fine powder. An accurately weighed powder sample equivalent to 12.5 mg of Chlorthalidone was transferred to 100 ml volumetric flask and methanol was added in it. Sonicate it for 20 min. The volume was then made up to the mark using same solvent. The resultant solution was filtered through 0.45 µ membrane filter. This solution was filtered through filter paper to remove some un- dissolved excipients. The filtrate was having concentration 125µg/ml for CHL. After filtration, from this 1ml was taken and diluted to 10 ml with Methanol which gives 12.5µg/ml solution and Absorbance of this sample solutions was recorded at 227nm ((λmax of CHL) and concentration of drug in the sample were determined the absorbance of the solution was measured at 227 nm.

Method Validation

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

Linearity

0. 2, 0.4, 0.6, 0.8, 1ml of standard CHL solution was transferred into a series of 10 ml volumetric flasks. The volume was made up to the mark with Methanol to obtain the concentration of 2, 4, 6, 8, 10µg/ml. Then absorption of these solutions was recorded and the graph was plotted of absorption against concentration. The correlation coefficient (r^2) of least square linear regression of CHL was calculated.

Range

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

Accuracy

Recovery study was carried out by the standard addition method by adding a known amount of CHL to the pre-analyzed sample at three different concentration levels that is 80%, 100%, 120% of assay concentration and percent recovery were calculated. 0.5ml of tablet solution was transferred to 4 different 10 ml volumetric flasks separately and 0.8, 1, 1.2ml of 100µg/ml standard solution was added respectively and the volume was made up to the mark with Methanol. Absorbances were noted for these samples. Standard deviation and %RSD was calculated. Accuracy is reported as % recovery, which was calculated from the expression as equation given below:

% Recovery = Observed value / True value×100

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same sample under the prescribed conditions. The precision of the method was determined in terms of repeatability and intra-day and inter-day precisions. Intra-day and inter-day precision (Intermediate Precision)

Intraday precision was determined by analyzing the drugs at concentrations (6µg/ml) and each concentration for three times, on the same day. Inter-day precision was determined similarly, but the analysis being carried out daily, for two consecutive days.

Repeatability

Repeatability of the method was determined by analyzing six samples of same concentrations of the drug (6µg/ml). Absorbance of each was measured.

Robustness

The robustness of the developed method is its capacity to remain unaffected by small changes in altered conditions. To determine the robustness of the method, the wavelength of analysis was deliberate and the assay was evaluated. The effect of detection wavelength was studied at ±5 nm.

Ruggedness

Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.

Limit of Detection

Detection limit was determined based on the standard deviation of absorbance of same concentration that is a standard solution of CHL (6µg/ml) and LOD calculated by LOD = 3.3(SD/S) Where, SD- standard deviation; S= slope of the curve

Limit of Quantification

Quantification limit was determined based on the standard deviation of peak area of same concentration that is standard solution CHL (6µg/ml) prepared six times and LOQ calculated by LOD = 10(SD/S) Where, SD= standard deviation; S= slope of Curve.

RESULTS AND DISCUSSION

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

Linearity

The linearity of this method was determined at ranges from 2-10 µg/ml for Chlorthalidone. The regression equation was found to be *Y=0.071x +0.182*, R^2=0.999.

The linearity for Chlorthalidone was found to be linear in the range of 2-10µg/ml with R^2= 0.999 and the straight line equation as *y= 0.0474x+0.0401*.

Accuracy

The accuracy of the analytical method for Chlorthalidone was determined at 80%, 100% and 120% levels of standard solution. Absorbance was measured at 227 nm and results were expressed in terms of % recoveries.

Precision

The precision (measurement of intra-day, inter-day, repeatability) results showed good reproducibility with the relative standard deviation (% RSD) below 2.0%. This indicated that method was highly precise.

Ruggedness (6µg)

Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.

Preliminary Analysis of Chlorthalidone

Preliminary analysis of Chlorthalidone such as description, solubility was performed.

UV-spctrophotometry for Chlorthalidone

Chlorthalidone being UV absorbing has been successfully employed for its quantitative determination by UV Spectrophotometric method. Being soluble in Methanol, stock solutions and working standards were prepared in Methanol. The maximum wavelength of absorption of drug was determined by taking scan of the drug solution in the UV region (200-400 nm). The correlation of the standard curve for the drug was 0.999. The accuracy was from 97-100%at 227nm. The proposed method showed absorption maxima at 254nm and obeyed Beer's law in the concentration range of 2-10µg/ml. The limit of detection (LOD) was found to be 0.2514µg/ml and limit of quantification (LOQ) to be 0.7620µg/ml respectively. All statistical data prove validity of the proposed method, which can be applied for routine analysis of Chlorthalidone.

Assay of Capsule formulation

Amount of drug present in Tablet formulation was calculated using equation at 227 nm, and *y=0.0714x + 0.1821* and amount of Chlorthalidone were found to be 102% of label claim respectively. This method can be employed for routine analysis of Chlorthalidone.

Summary and conclusion

Summary of UV Spectrophotometeric Method of Chlorthalidone.

Table No.1: Results obtained in the determination of CHL in dosage form

S.No	formulation	Label claim	Amount taken	Amount found	Assay%
1	CTD(12.5)	12.5mg	12.5 µg/ml	12.78µg/ml	102%

Table No.2: Linearity table

S.No	Conc.	Absorbance
1	2	0.328
2	4	0.461
3	6	0.610
4	8	0.76
5	10	0.892

Table No.3: Table for accuracy

S.No	Level of % Recovery	Amount present (µg/ml)	Amount of standard drug added (µg/ml)	Amount recovered (µg/ml)	% Recovery
1	0	10	0	0	0
2	80	10	8	17.59	97.72%
3	100	10	10	19.8	99%
4	120	10	12	21.98	99.90%

Intra-day Precision

Table No.4: Intra-day precision

S.No	Concentration (µg/ml)	Absorbance	SD	% RSD
1	6	0.61		
2	6	0.611		
3	6	0.612	0.000983	0.160
4	6	0.612		
5	6	0.61		
6	6	0.610		
		$\bar{y}$ = 0.610833		

Inter-day Precision

Table No.5: Inter-day precision study

S.No	Concentration (µg/ml)	Absorbance	SD	%RSD
1	6	0.610		
2	6	0.612		
3	6	0.612	0.001225	0.200
4	6	0.611		
5	6	0.610		
6	6	0.610		
		$\bar{y}$ = 0.6115		

Repeatability

Table No.6: Repeatability study

S.No	Concentration (µg/ml)	Absorbance	SD	%RSD
1	6	0.60		
2	6	0.60		
3	6	0.601	0.005441	0.900
4	6	0.601		
5	6	0.611		
6	6	0.611		
		$\bar{y}$ = 0.604		

Limit of Detection

Table No.7: For Limit of Detection

LOD (µg/ml)	0.2514 µg/ml

Limit of Quantification

Table No.8: For Limit of Quantification

LOQ (µg/ml)	0.7620 µg/ml

Robustness (6µg)

Table No.9: Robustness study

S.No	Wavelength (nm)	Absorbance	SD	%RSD
1	227	0.61		
2	228	0.61	0.005774	0.9518
3	229	0.60		
		$\bar{y}$ =0.606667		

Table No.10: For Ruggedness

S.No	Analyst-1		
	Concentration (µg/ml)	**Absorbance**	**Statistically analysis**
1	6	0.61	Mean = 0.606667
2	6	0.61	SD = 0.005774
3	6	0.60	% RSD = 0.9518
Analyst-2			
4	6	0.610	Mean = 0.611333
5	6	0.612	SD=0.001155
6	6	0.612	%RSD=0.1889

Table No.11: For Summary

S.No	Parameters	Values
1	Beer's Law limit (µg/ml)	2-10
2	Absorption maxima (nm)	227
3	Standard regression equation	0.0714x + 0.1821
4	Correlation coefficient (R^2)	0.999
5	Accuracy	97-100%
6	Precision (%RSD) Repeatability	0.160
7	LOD (µg/ml)	0.2514
8	LOQ (µg/ml)	0.7620
9	Robustness (%RSD)	0.9518
10	Ruggedness	0.9512 and 0.1889
11	Assay (%)	102%

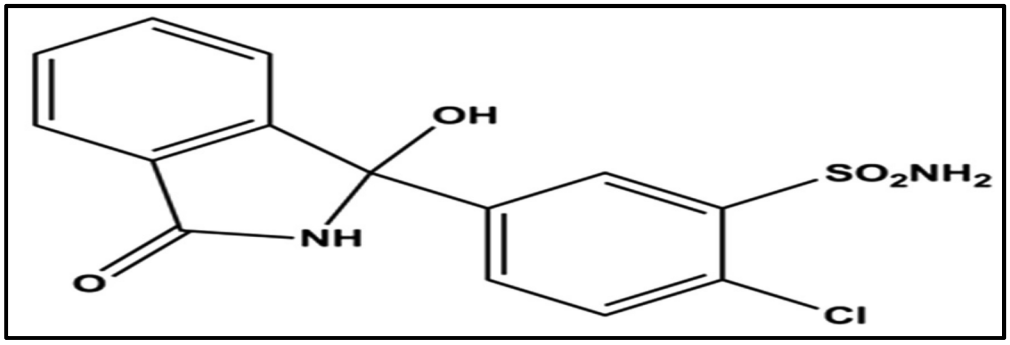

Figure No.1: Chemical structure of Chlorthalidone

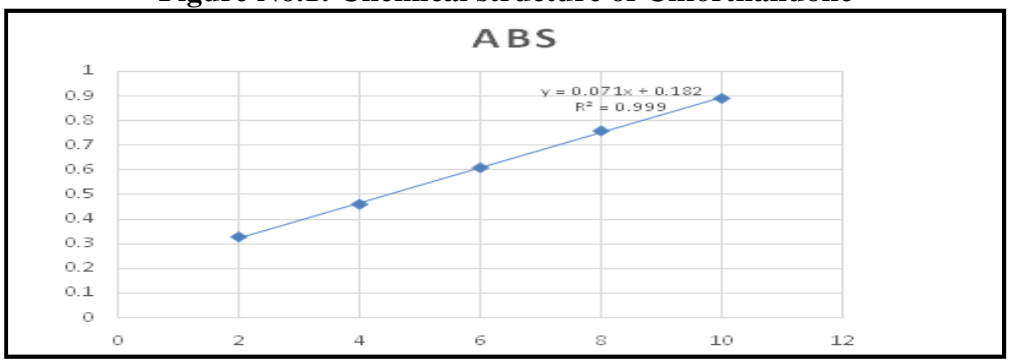

Figure No.2: Calibration curve of Chlorthalidone (CHL)

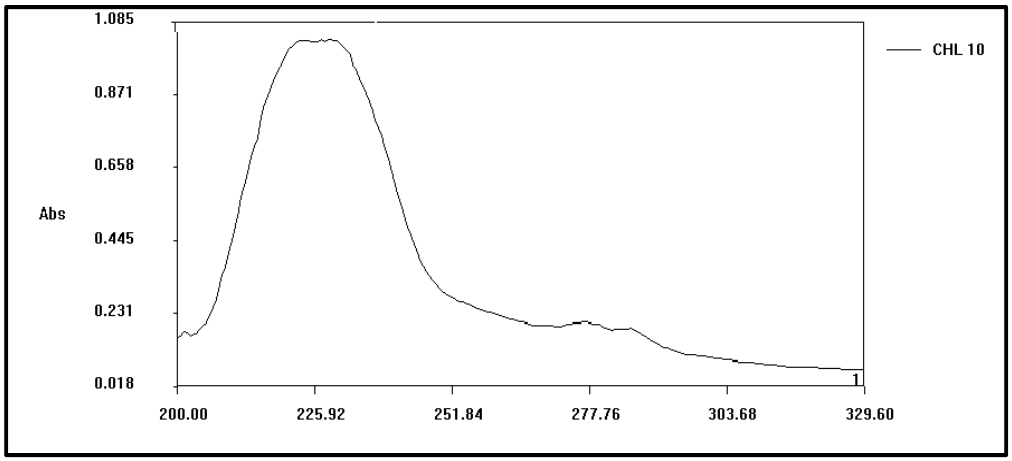

Figure No.3: Wavelength of maximum absorption of Chlorthalidone

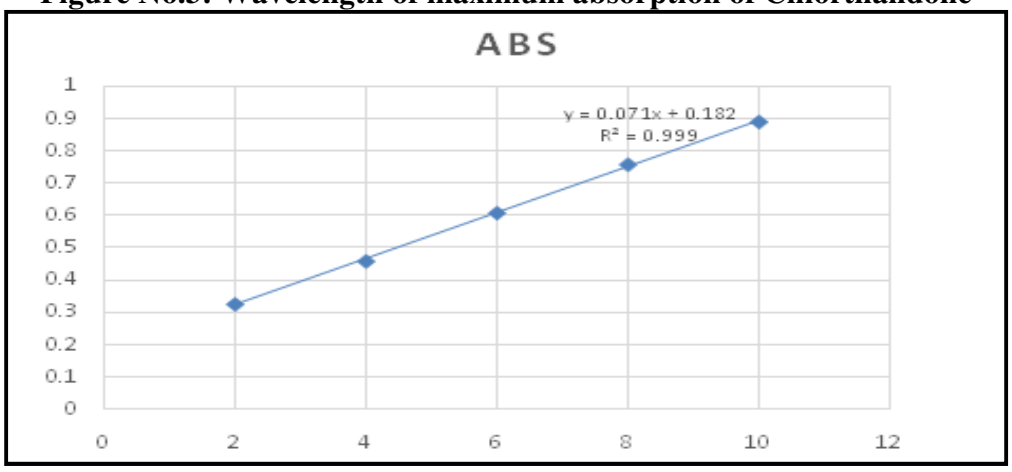

Figure No.4: Linearity graph of Chlorthalidone

CONCLUSION

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

ABBREVIATIONS

UV-Ultra Violet
API- Active Pharmaceutical Ingredient
CHL- Chlorthalidone

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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