

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC ESTIMATION OF HYDROCHLOROTHIAZIDE IN BULK AND TABLET DOSAGE FORM USING AREA UNDER CURVE METHOD.

Pawar Seemarani^{1*} & Tamboli Ashpak¹.

Department of Pharmaceutical chemistry, Sahyadri College of Pharmacy, Methwade,
Sangola-413307, Solapur, Maharashtra, India¹.

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ABSTRACT

A simple, precise, accurate, and economical UV visible spectrophotometric method has been developed for estimation of Hydrochlorothiazide drug by AUC method. The standard and sample solutions were prepared by using double distilled water as a solvent. Quantitative determination of the drug was performed at wavelength range 222-228 nm. The linearity was established over the concentration range of 0.2-10 µg/ml for Hydrochlorothiazide with correlation coefficient value of 0.9979. Precision studies showed that % relative standard deviation was within range of acceptable limits. The mean percentage recovery was found to be 99.39%. The proposed method has been validated as per ICH guidelines.

Keywords: -Hydrochlorothiazide, UV visible spectrophotometry, AUC, Method Validation.

No: of Tables: 4

No: of Figures: 4

No: of References: 5

INTRODUCTION

Hydrochlorothiazide (HCTZ) (Fig.1) is a chemically 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiazidine-7- sulfonamide-1, 1-dioxide.(Fig.1) is a diuretic, which inhibits active chloride reabsorption at the early distal tubule via the Na-Clco-transporter, resulting in an increase in the excretion of sodium, chloride, and water¹. It has been used in the treatment of several disorders including edema, hypertension, diabetes insipidus and hypoparathyroidism.^[1]Literature survey revealed several analytical methods UV-

spectrophotometry^[2], HPLC^[3], have been reported in bulk, pharmaceutical dosage form for determination of Hydrochlorothiazide. Hence an attempt has been made to develop new UV-spectrophotometry (AUC) method for estimation of Hydrochlorothiazide in bulk and pharmaceutical formulations with good accuracysimplicity, precision and economy.

Molecular Formula: C₇H₈N₃S₂O₄Cl

Molecular weight: 297.75g/mole

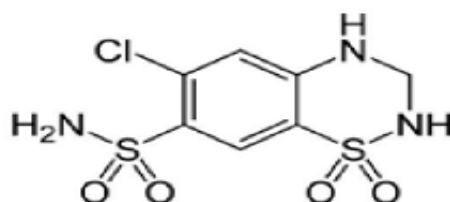


Fig. 1: Chemical structure of Hydrochlorothiazide.

MATERIALS AND METHODS:-

Apparatus and instrumentation

A shimadzu 1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. Single Pan Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath (Spectra lab UCB 40, India). Calibrated volumetric glassware (Borosil®) was used for the validation study.

Materials

Reference standard of Hydrochlorothiazide API was supplied as gift sample by Marksan Pharmaceutical Ltd., Verna, Goa. Tablet sample with label

claim 100 mg per tablet were purchased from local market Pune.

Method development

Determination of Wavelength Range

For the selection of analytical wavelength range for area under curve method, 10 µg/ml solution of Hydrochlorothiazide was scanned in the spectrum mode from 400 nm to 200 nm against methanol as blank. Wavelength range was selected around wavelength maxima (224 nm). Different working standards were prepared between 0.2-10 µg/ml. Various wavelength range were tried and final wavelength range between 222-228 nm was selected on the basis of linear relationship between area and corresponding concentration (Figure 2).

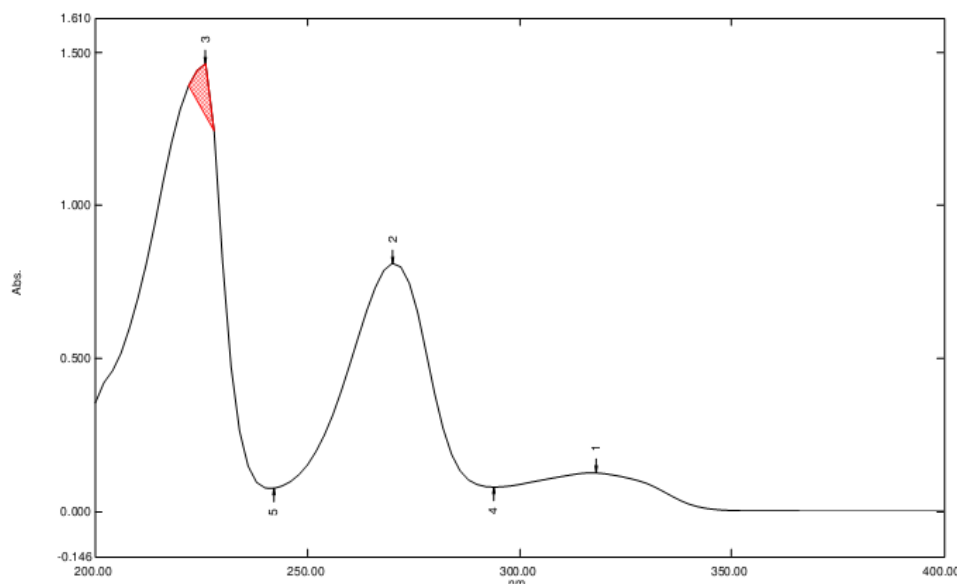


Fig. 2 UV AUC spectrum of Hydrochlorothiazide (10µg/ml)

Area under curve (Area calculation)

Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ_1 and λ_2 representing start and end point of curve region. The area under curve between λ_1 and λ_2 was calculated using UV probe software. In this study area was integrated between wavelength ranges from 222 to 228 nm.

$$\text{Area calculation: } (a+\beta) = \int_{\lambda_2}^{\lambda_1} Ad\lambda$$

Where, a is area of portion bounded by curve data and a straight line connecting the start and end point, β is the area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ_1 and λ_2 are wavelength range start and end point of curve region^[7].

Preparation of standard solution

The standard stock solution of Hydrochlorothiazide was prepared by accurately weighing & transferring, 10 mg of API to 100 ml of volumetric flask. The drug was dissolved with sonication in 50 ml of methanol and volume was made up to the mark by using distilled water. Then take from that 0.1ml and add to 10ml volumetric flask and make up with distilled water to get final standard stock solution (10µg/ml) was further diluted with distilled water to obtain 02-10 µg/ml Hydrochlorothiazide solutions.

Calibration curve for Hydrochlorothiazide.

The dilutions were made from Standard Stock solution to get concentration of 02, 04, 06, 08, 10 µg/ml respectively. These solutions were scanned from 400 to 200 nm and area under curve (AUC) values was integrated in the range of 222-228 nm. The calibration curve was plotted between areas under curve values against concentration (Fig. 3).

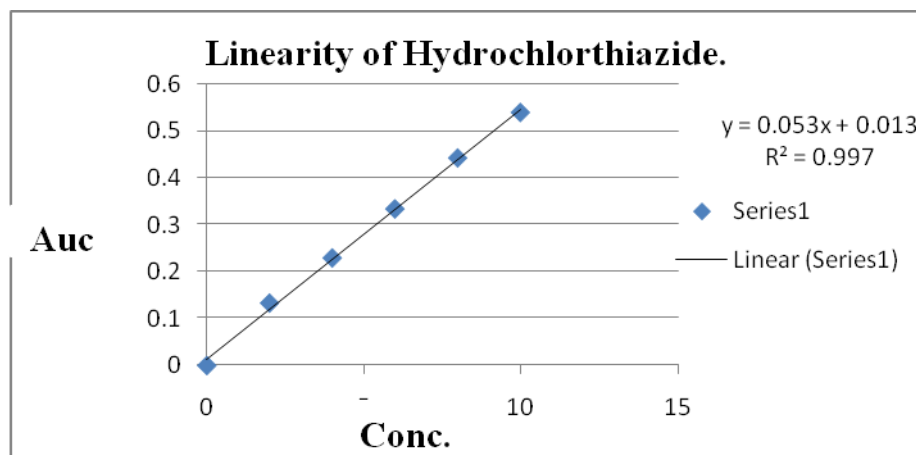


Fig.3 Linearity of Hydrochlorothiazide.

Assay of tablet formulation

Twenty tablets each containing 100 mg of Hydrochlorothiazide were weighed crushed to powder and average weight was calculated. Powder equivalent to 10 mg of Hydrochlorothiazidehydrochloride was transferred in 100 ml of volumetric flask. A 50 ml of methanol was added

andsonicated for 15 minutes. Then solutionwas further diluted up to the markwith methanol. The solutionwas filtered using Whatmannfilter paper no. 41, first 5 ml of filtrate was discarded. This solution was further diluted to obtain 10µg/mL solution with water, subjected for UV analysis using methanol as blank. This procedure was repeated three times (Table 1).

Table 1: Assay of tablet dosage form

Sr.No.	Sample Solution Concentration (µg/ml)	Amount found (%)	Mean % found	%RSD
1	10	95.27		
2	10	98.21	97.41	1.9226
3	10	98.75		

*n=3, % RSD = % Relative Standard Deviation.

Method validation [4-5].

The above method was validated for various parameters such as Accuracy, Linearity, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH guideline.

Accuracy

The accuracy for the analytical method was evaluated at 80%, 100% and 120% levels of 20µg/ml standard solution. Area under curve (AUC) was measured in wavelength range 222-228 nm and results were obtained in terms of percent recovery. Three determinations at each level were performed and % RSD was calculated for each level

Table 2: Accuracy results for Hydrochlorothiazide.

Accuracy level	Sample conc (µg/ml)	Std. conc	Total amount. Added (µg/ml)	% Recovery	Mean % Recover y	% RSD
80	10	08	18	99.47		
100	10	10	20	98.18	99.39	1.1888
120	10	12	22	100.54		

Precision

The precision of an analytical procedure expresses the closeness of an agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions intraday precision was studied by

integrating area of standard solution of 8µg/ml concentration at six independent series in the same day. Inter-day precision studies were performed by integrating area of standard solution of 8µg/ml concentration on three consequent days. The %RSD Was calculated.

Table 3: Precision results for Hydrochlorothiazide.

Parameter	Intra day	Inter-day
Sample sol conc	08	08
AUC (mean)	0.445567	0.4409
%RSD	0.657146	0.257605

Linearity and Range

The linearity was determined by using working standard solutions between 02-10 µg/ml. The areas under curve (AUC) of

these solutions were recorded. Calibration curve of area under curve to concentration plotted on excel sheet and linear regression was performed. The correlation coefficient, regration Equation was calculated. (Fig. 3)

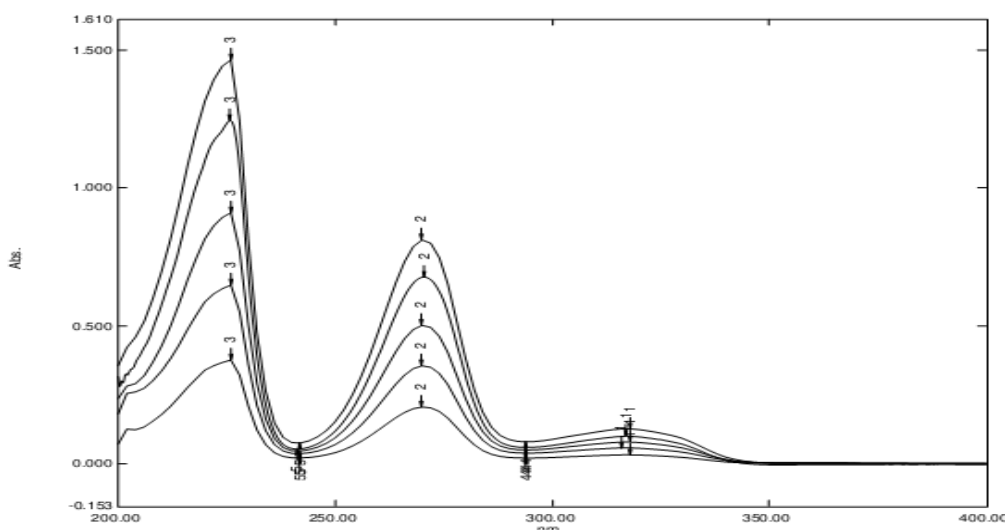


Fig.4 Overlay of Hydrochlorothiazidespectra at diff. Concentration.

Limit of Detection and Limit of Quantification:

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula

$$LOD = 3.3 \sigma / S$$

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula

$$LOQ = 10 \sigma / S$$

Where, σ is standard deviation of the response and

Table 4: Summary of validation parameters

Parameter	Result
λ range	222-228nm
Regression Equation (y=mx+c)	Y = 0.0533x+0.0133
Measured wavelength	224nm
Linearity range	2-10 μ g/ml
Slope	0.0533
Intercept	0.0133
Correlation coefficient (R ²)	0.9979
Limit of Detection (LOD) μ g/ml	0.240246
Limit of Quantitation (LOQ) μ g/ml	0.728018
Accuracy (Mean % Recovery)	99.39
Precision (%RSD)	0.6571

RESULTS AND DISCUSSION

The UV visible spectroscopic method for the Hydrochlorothiazide by area under curve was found to be simple, accurate, economical and reproducible. The drug concentrations were found to be linear in the range of 02-10 μ g/ml and the correlation coefficient value of 0.9985 indicates that developed method was linear. For Precision the percent relative standard deviation (% RSD) was found to be 0.6571 while, intra-day and inter-day precision results in terms of

S is the slope of the calibration curve.

LOD& LOQ of Hydrochlorothiazide was found to be 0.240246 μ g/ml &0.728018 μ g/ml respectively.

Five sets of known concentrations (02-10 μ g/ml) were prepared and scanned. By using these spectras, regression equations were obtained. By taking average of slopes and standard deviation of y-intercept, LOD and LOQ were calculated. The values of LOD and LOQ are given in table 4.

percent relative standard deviation values were found to be 0.6571 and 0.2576 respectively thus the method is observed as precise. The accuracy of the method was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The values of standard deviation were satisfactory and the recovery studies were close to 100%. The % RSD value is ≤ 2 indicates the accuracy of the method. The Limit of Detection and Limit of Quantitation values were found to be 0.240246 μ g/ml &0.728018 μ g/ml

respectively. The result of the analysis for pharmaceutical formulation by the developed method was consistent with the label claim, highly reproducible and reliable. The validation parameters are summarized in Table 4. The method can be used for routine quality control analysis of Hydrochlorothiazide in bulk and pharmaceutical formulations.

CONCLUSION

The UV spectroscopic AUC method for the analysis of Hydrochlorothiazide was found to be simple, precise, and accurate, can be used for assay of bulk drug and pharmaceutical dosage formulations.

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