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ESTIMATION OF HYDROCHLOROTHIAZIDE IN BULK AND TABLET DOSAGE FORMS BY AREA UNDER CURVE SPECTROPHOTOMETRIC METHOD

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ABSTRACT

A simple, accurate and precise area under curve method was developed for spectrophotometric estimation of hydrochlorothiazide in pure and tablet dosage forms. "The area under two points on the mixture spectra is directly proportional to the concentration of the component of interest" is the AUC curve. The area selected for estimation of hydrochlorothiazide was between 260 to 280 nm. The method represented regression coefficient (R^2 = 0.999) at concentration rang 5-25 µg/ml. Estimation of the drugs was found up to 100 % representing the accuracy of the method. The recovery of the hydrochlorothiazide was found up to 100 %. Validation of the proposed method was carried out for its accuracy, precision and specificity according to ICH Q2 (R1) guidelines. The developed methods can be successfully applied in routine work for the estimation of hydrochlorothiazide in its pharmaceutical dosage form.

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INTRODUCTION [1-6]

Hydrochlorothiazide (HCTZ), 6-chloro-3,4-dihydro-2H-1,2,4- benzothiadiazine-7-sulfonamide 1,1-dioxide, which is widely used in antihypertensive pharmaceutical preparations, reduces active sodium reabsorption and peripheral vascular resistance. The review of the literature revealed that no method is yet reported for the simultaneous estimation of both the drugs in combined dosage forms. The AUC method is simple, accurate and results of analysis have been validated statistically and by recovery studies. Hydrochlorothiazide produced its significant prolongation of half-life due to decrease in hydrochlorothiazide elimination. The official monographs describe the procedure for individual assay of hydrochlorothiazide, and hydrochlorothiazide combination.
Present work describes to simple, accurate, reproducible, rapid and economical method for estimation of HCTZ in tablet formulation.

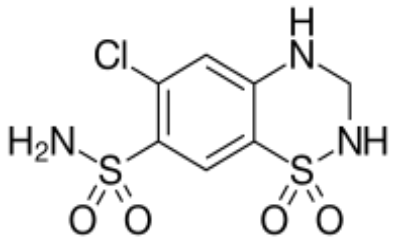

Figure 1: The chemical structure of hydrochlorothiazide.

MATERIALS AND METHODS

CHEMICALS

Hydrochlorothiazide was obtained gift sample of Sun pharma Pharmaceuticals Ltd., Mumbai, India. Tablet of hydrochlorothiazide 25mg (AQUAZIDEH 25mg) was procured from local pharmacy. Ethanol (S.D. Fine Chemicals, Mumbai, India) was used. All chemicals and reagents were of analytical reagent (AR) grade.

INSTRUMENT USED

A Shimadzu (Kyoto, Japan) model UV-1800 double beam UV-Visible spectrophotometer attached with computer operated software UV probe 2.0 with spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells was used to measure absorbance of the resulting solutions. Analytical balance, Mettler Toledo (Model JL1503-C)

PREPARATION OF STANDARD AND SAMPLE STOCK SOLUTION

Transfer an accurately weighed quantity of hydrochlorothiazide (10 mg) to a separate 100 ml volumetric flask, dissolved and diluted up to the mark with ethanol to get standard solution having concentrations 100 µg/ml of Successive dilutions were carried out to get 10 µg/ml of hydrochlorothiazide which was scanned in the UV-region i.e. 400 to 200 nm. In UV–Spectrophotometric method, two wavelengths 260 nm and 280 nm were selected for estimation of area under curve (AUC) of hydrochlorothiazide.
For Sample solution, hydrochlorothiazide tablet was taken, (label claim = 25mg), 10 mg which was transferred into 10 ml volumetric flask and make up the volume with ethanol. 1 ml from above solution was taken into 10 ml volumetric flask and make up volume with ethanolto get a final concentration of 100 µg/ml.

AREA UNDER CURVE [7]

The AUC (area under curve) method was applicable where 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 the two selected wavelengths λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The above mentioned spectrums were used to calculate AUC. Thus, the calibration curve can be constructed by plotting concentration versus AUC.

METHOD VALIDATION[8-10]

The method was validated as per the ICH guideline

1. Linearity

Standard solution of hydrochlorothiazide (1.25, 2.5, 3.75, 5 and 6.25 ml) was pipette out in to a separate series of 25 mlVolumetric flask. The volume was adjusted to the mark with distilled water and mixed. The area under curve for solutions was measured between 260 to 280 nm against distilled water as blank. From using this area, the 'X' value of the drug was determined at the selected AUC range.

2. Precision

Precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analyses solution (5-25 µg/ml) on the same day. The %RSD of six determinations was calculated. Intermediate precision of the method was checked by repeating studies on two different days. The %RSD of determinations was calculated

3. Sensitivity

The sensitivity of the method was determined in terms of limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were calculated by using the formula, LOD = 3.3 × σ/S and LOQ = 10 × σ/S, where σ is standard deviation of regression line and S is slope of corresponding regression line.

4. Recovery

To study the accuracy of the proposed methods and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition.

ASSAY PROCEDURE

Twenty tablets (hydrochlorothiazide) containing 10 mg of hydrochlorothiazide weighed, average weight calculated and triturated to fine powder and then weight equivalent 10 mg of hydrochlorothiazide transferred into 100ml volumetric flask and dissolved in water and diluted up to the mark with water to get a solution containing of 100 µg /ml from the 2.5 ml was transferred to 25 ml volumetric flask and diluted up to the mark with water to get hydrochlorothiazide solution containing 10 µg /ml of hydrochlorothiazide.

RESULTS AND DISCUSSION

The area under the curve spectra for hydrochlorothiazide was recorded at the wavelength of 260-280 nm.

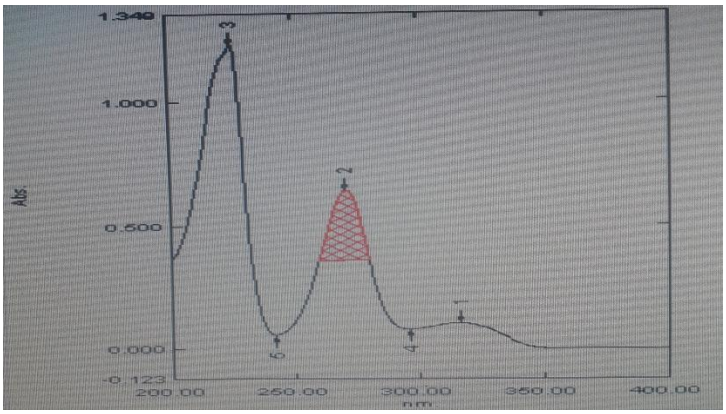

Figure 2: area between 260-280 nm selected for hydrochlorothiazide (10µg/ml).

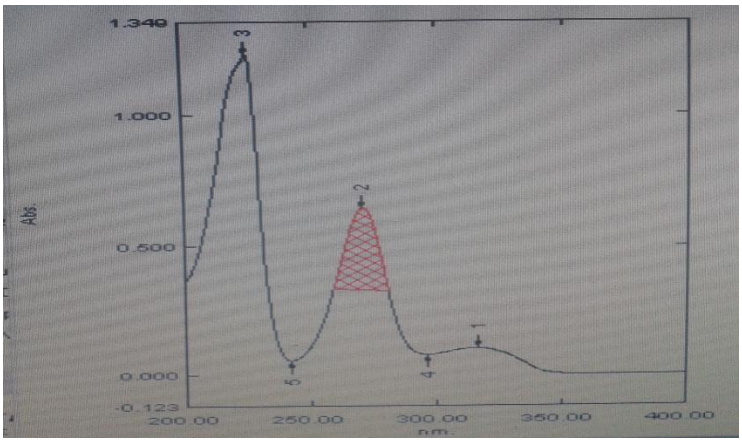

Figure 3: area between 260-280 nm selected for hydrochlorothiazide tablet formulation (10µg/ml).

Table 2: calibration curve data of hydrochlorothiazide (5-25 ug/ml).

Concentration	AUC
5	1.582
10	3.642
15	5.987
20	8.166
25	10.640

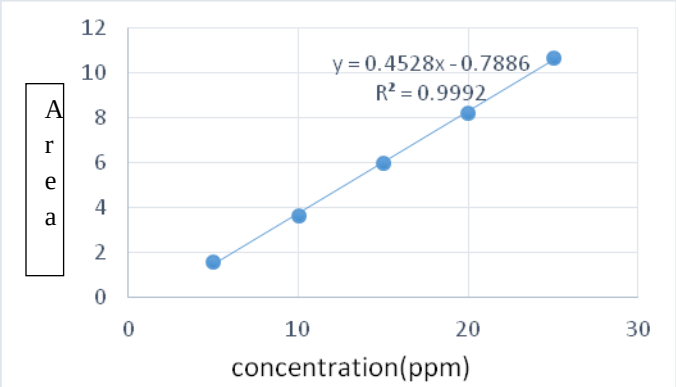

Figure 3: calibration curve of hydrochlorothiazide.(5-25ug/ml).

Linearity and range

Under the experimental conditions described, the graph obtained for area under the curve spectra showed linear relationship (Figure 3). Regression analysis was made for the slope, intercept and correlation-coefficient values. The regression equations of calibration curves were y = 0.4528x-0.7886(r2 =0.9992) at 260-280 nm for area under the curve spectrophotometry. The range was found to be 5- 25 µg/ml for area under the curve spectrophotometric method (Table 1).

Table 2: Regression Analysis Data for hydrochlorothiazide by the Area under Curve Method.

Parameter	AUC
wavelength range(nm)	260-2800nm
Concentration range (µg/ml)	5-25
Slope (m)	0.4528
Intercept (c)	-0.7886
Correlation coefficient (r2)	0.9992

Precision

To determine the precision of the method, a hydrochlorothiazide solution at a concentration 10 µg/ml was analyzed each six times for area under the curve spectrophotometric method. Solutions for the standard Curves were prepared fresh everyday (Table 3).

Table .3: Results of Intra and Inter Day Precision.

Parameter	± S.D. *	%RSD *
Interday	0.06	1.68
Intraday	0.05	1.40

*n=6

Sensitivty

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations LOD = 3 x σ / S and LOQ = 10 x σ /S, where σ is the standard deviation of intercept, S is the slope. The LOD and LOQ were found to be 0.03µg/ml and 0.01 µg/ml respectively for area under the curve method

Recovery

To study the accuracy of the proposed methods and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. This study was performed by addition of known amounts of hydrochlorothiazide to reanalyzed solutions of commercial dosage form (Table 4).

Table 4: Data of Recovery Studies.

Level of Mean Recovery (%)	% Mean Recovery	SD*	% RSD
80%	99.32	0.5755	0.5794
100%	99.60	0.5478	0.55
120%	99.75	0.7018	0.7035

(*n=3).

ANALYSIS OF THE MARKETED FORMULATION

There was no interference from the excipients commonly present in the injectable. The drug content was found to be 98 % for area under the curve spectrophotometric method. It may therefore be inferred that degradation of hydrochlorothiazide had not occurred in the marketed formulations that were analyzed by this method. The low % R.S.D. value indicated the suitability of this method for routine analysis of hydrochlorothiazide (Table 5).

Table .5 Assay Results for the Estimation of hydrochlorothiazide in Pharmaceutical Formulation.

Parameter	Labelled claim(mg/tab)	Amount found(mg/tab)	% labelled claim
AUC	10	9.80	98%

SUMMARY DATA OF VALIDATION PARAMETER

Table. 6: summery data of validation parameter.

SR. NO.	PARAMETER	AUC METHOD
1.	Linearity range	5-25
2.	Regression equation	Y= 0.4528x-0.7886.
3.	Correlation co-efficient	R2=0.9992.
4.	LOD (µg /ml)	0.0353
5.	LOQ (µg /ml)	0.0116
6.	Precision	
	Intra day	1.40%RSD
	Inter day	1.68%RSD
7.	% recovery	0.5794-0.7035%RSD

CONCLUSION

No any spectrophotometric methods have been described for AUC estimation of hydrochlorothiazide Therefore, simple, fast and reliable area under curve spectrophotometric method was developed for the routine analysis of hydrochlorothiazide. The developed method can be concluded as accurate, sensitive and precise and can be easily applied to the pharmaceutical formulation.

REFERANCE

1. Satoskar R S. Pharmacology and pharmacotherapeutics. Popular Prakash an, New Delhi, Revised 21st edition, 587-588, 2008.
2. Michael E Swartz, Ira S Krull. Analytical method development and validation. Marcer Dekker Inc, NY, 17:25-26, 1997.
3. Christian G D. Analytical chemistry, Sixth edition, John Wiley and Sons Inc, NJ, USA, 1- 2:604- 620, 2003.
4. Skoog, Holler, Nieman. Principles of Instrumental Analysis, 5th edition, Thomson Asia Pvt Ltd, Singapore, 300-325, 2004.
5. Chatwal G R, Anand S K. Instrumental methods of chemical analysis, 5th edition, Himalaya Publishing House, Mumbai, 1.1-1.5,2.108-2.109, 2005.
6. Ishikawa-machi, Hachioji-shi. Instruction Manual User's System Guide UV-530 Visible Spectrophotometer (Double Beam), Jasco, Japan, 2967-5, 2000.
7. Willard H H, Merritt Jr Lyne L, Dean John A, Settle, Jr Frank A,Instrumental Methods of Analysis, 7th edition, CBS Publishers and Distributors pvt. Ltd, New Delhi, 2-5, 1998.9.
8. Connors K A. A textbook of pharmaceutical analysis, 3rd edition, John Wiley and Sons, USA, 221-224, 1982.
9. Beckett A H, Stenlake J B. Practical Pharmaceutical Chemistry, 4th edition, CBS Publishers and Distributors, New Delhi, 2:275-295, 2002.
10. ICH, Q2A. Text on validation of analytical procedures: methodology, International Conference on Harmonization, Geneva, 1-8, 1996.

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