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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF HYDROCHLOROTHIAZIDE CONTENT USING UV- SPECTROSCOPIC TECHNIQUE

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ABSTRACT
A Simple, definite, informal, rapid, precise and accurate UV Spectrophotometric analytical method have been developed and Validated for estimation of Hydrochlorothiazide formulation drug. Hydrochlorothiazide showed the absorption maxima in at 271.0 nm and was linear for a range of 5µg/ml-25µg/ml with correlation coefficient of 0.9995. The validation for the proposed analytical method was performed by using precision and accuracy-recovery studies. The analytical method showed good Intra precision (Repeatability) with relative standard deviation 0.563% and Inter precision with relative standard deviation is 0.634% which is within 2. The percentage of accuracy-recovery for three different levels i.e. 50%, 100% and 150% was found to be 50.1%, 99.5% and 151.1% respectively. The proposed analytical method was validated for the parameter Specificity, Precision, Linearity & range, Ruggedness, Accuracy and recovery. Hence projected analytical method for estimation of Hydrochlorothiazide formulation drug using UV spectrophotometer in pharmaceutical can be functional for the routine excellent analytical study.

Keywords: Validation, UV Spectrophotometer, λ max, Hydrochlorothiazide.

1. INTRODUCTION
UV visible spectrophotometric method is being used frequently in pharmaceutical analysis due to easy and time saving structures, which includes measurement of the amount of ultraviolet or visible radiation absorbed by a formulation in solution by an instrument which measures the ratio or a function of the ratio of the intensity of two beams of light in UV Visible region. UV spectrophotometric method for sample analysis of a component measures absorbance difference between total absorbance of the solution in the sample cell and that of the solution in the reference blank cell.
Hydrochlorothiazide is a thiazide organic compound which works by preventing the reabsorption of sodium from the distal convoluted tubules. The sodium takes water with it from blood that decreases the amount of fluid flowing through vessels which lowers blood pressure. Hydrochlorothiazide drug is used for controlling hypertension and edema. Diuretics are being used efficiently in the management of hypertension to achieve and maintain optimal blood pressure, and typically prescribed due to their effectiveness, minor price and minor side effects profile [1].

The IUPAC name of Hydrochlorothiazide is 6-chloro-1, 1-dioxo-3, 4-dihydro-2*H*-1, 2, 4-benzothiadiazine-7 sulfonamide, having molecular formula $C_7H_8ClN_3O_4S_2$ and Molecular weight 297.74g/mol. It is official in United States pharmacopoeia [2] and European/British pharmacopoeia [3] with assay method by chromatographic technique and potentiometric titration respectively. Literature survey revealed that very few analytical methods are available including titrimetric, spectrophotometric and chromatographic HPLC [4-11].
In the present work, a simple, informal, accurate and precise method for estimation of Hydrochlorothiazide content in formulation drug substance pure form was introduced. All those reported estimation methods either took a long time for analysis or employ mobile phases with pH adjustment of buffer solutions for sample preparation, which is monotonous and anomalous [4-11], especially for routine estimation of quality control samples of assay test study. Hence, it was needed to build up a simple, informal, fast, Economical and precise UV spectrophotometric technique for the direct estimation of Hydrochlorothiazide formulation drug [12-15].
The current research work deals with the development of

UV Spectrophotometric technique and its validation as per International Conference on Harmonization (ICH) guideline [16-18]. The developed method was found to be simple, informal, specific, stable, fast, accurate, precise, reliable, economical and time saving by UV spectrophotometric technique for the estimation of Hydrochlorothiazide content in drug substance.

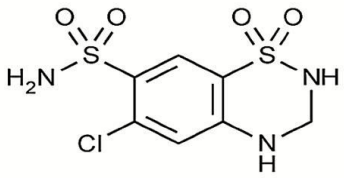

Fig. 1: Chemical structure of Hydrochlorothiazide

2. MATERIAL, METHODS AND RESULTS

2.1. Instrumentation and material

Double beam U.V. visible spectrophotometers having U.V. matched quartz cells with path length 1cm using Make- Elico, Model SL 210 with Spectra treat software were used to measure absorbance of the resulting solution. Standard and sample of Hydrochlorothiazide were gifted from Omicron Pharmaceuticals, Gujarat. All the chemicals, solvents and reagents i.e. Ethanol, Methanol and Water used, were of analytical grade and purchased from Merck Ltd, India, Qualigens and S.D. Fine Chem Ltd.

2.2. Method Development

2.2.1. Preparation of Diluent Solution

About 300 ml of water was transferred to the 1000 ml volumetric flask, then slowly added about 5.0 g of sodium hydroxide with constant stirring. About 100 ml of Ethanol was added with stirring and mixed well, then with constant stirring slowly added methanol up to mark to make volume 1000 ml. the solution was used as diluent.

2.2.2. Preparation of Standard Solution

About 200 mg of Hydrochlorothiazide was dissolved in 20ml ethanol, then added diluents with intermittent shaking and made up the volume up to 200 ml, further transferred 2 ml of solution to 200 ml volumetric flask. Made the volume up to mark to get a concentration of 10µg/ml.

2.2.3. Selection of wavelength for analysis of Hydrochlorothiazide

The standard solution of Hydrochlorothiazideconcentration10µg/ml was scanned at 200 nm to 400 nm with diluents as the blank to detect maximum wavelength (Fig. 2).

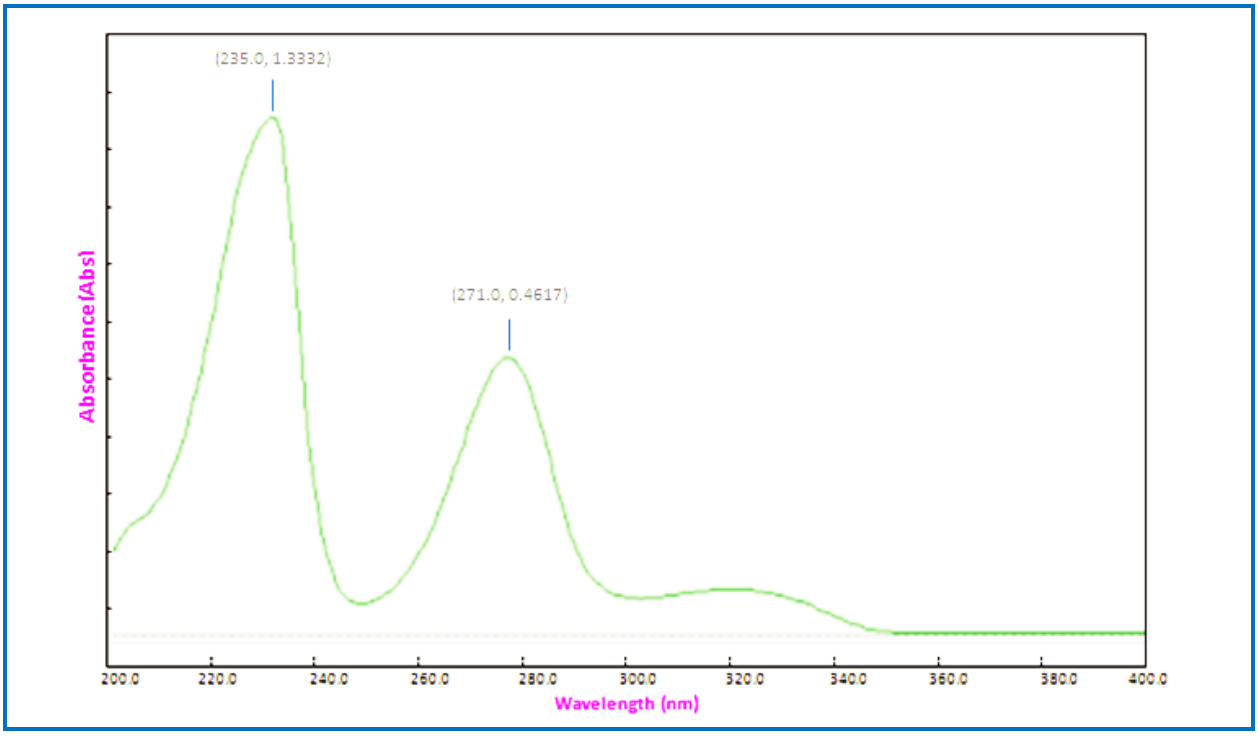

Fig. 2: Estimation of maxima of Hydrochlorothiazide

From the Fig. 2, spectra of Hydrochlorothiazide wavelength maxima identified for estimation were 271.0 nm (λmax).

2.2.4. Validation of proposed Analytical Method

The developed estimation method was validated as per International Conference on Harmonization ("ICH") guidelines referenced for Validation of analytical procedures [16-18]. Analysis of variance was used to ensure the validity and performance efficiency of the proposed analytical methods for Hydrochlorothiazide estimation.

2.2.5. 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. Specificity was done by scanning of diluent solution and standard solution of Hydrochlorothiazide having concentrations 15 µg/ml in spectrophotometric range from 200 nm to 400 nm to check specific absorption maxima at predefined wavelength i.e. 271.0nm and solution stability study performed to evaluate the solution stability at different time interval up to 26 hrs.

2.2.6. Instrument Precision

Instrument precision was assessed to make sure the suitability of the developed proposed analytical method with reverence to capability of instrument constancy to provide the precise wavelength maxim when scanned the standard solution of Hydrochlorothiazide having concentrations 10 µg/ml in the UV range from 200 nm to 400 nm. To check specific absorption maxima at predefined wavelength 271.0 nm with reproducible absorption detection, six separated standard preparations were scanned / analyzed according to the proposed method of analysis. The % RSD due to Hydrochlorothiazide concentration for the six standards was found 0.954%. The % RSD due to Hydrochlorothiazide concentration for the instrument precision meets the requirements. Results are tabulated in the Table 1.

2.2.7. Linearity and Range

The linearity of an assay method is its proficiency to carryout test results, which are directly comparative to the concentrations of drug in samples of the accessible range. Linearity indicates the make use of single-point calibrations. The correlation coefficient of the regression line was found 0.9995.

Five levels of five different concentrations, standard solution of Hydrochlorothiazide having concentrations 5 µg/ml, 10µg/ml, 15 µg/ml, 20 µg/ml and 25 µg/ml, in the range relative to the working concentrations, were prepared and measured as per projected analytical method of analysis. A linear regression curve was constructed, the correlation coefficient (R2) and estimation assessment calculated. The correlation coefficient (R2) for Hydrochlorothiazide obtained is 0.9995. The plot is a straight line and the results are tabulated in the Table 2 and curve is shown in the fig. 3.

Table 1: Instrument Precision

Standard Number	Absorbance @271.0 nm	% RSD
Standard Preparation -1	0.4655	0.954% (Limit < 2%)
Standard Preparation -2	0.4619	
Standard Preparation -3	0.4690	
Standard Preparation -4	0.4588	
Standard Preparation -5	0.4569	
Standard Preparation -6	0.4633	
Average Absorbance	0.4626	

Table 2: Linearity and range

Standard Concentration (µg/ml)	Absorbance @271.0 nm	Correlation coefficient
5	0.2376	0.9995 (Limit ≥0.999)
10	0.4688	
15	0.7085	
20	0.9643	
25	1.2172	

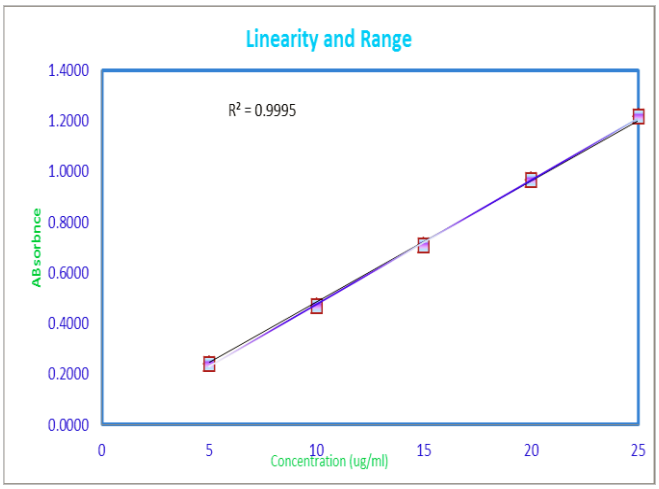

Fig. 3: Linearity and range of Hydrochlorothiazide

2.2.8. Analytical Method Precision

The precision of an analytical procedure expresses the degree of agreement among individual test results when the method is applied to multiple sampling of a homogenous sample.

2.2.8.1. Procedure for analysis of Sample

About 200 mg of Hydrochlorothiazide was dissolved in 20 ml ethanol, then added diluent with intermittent shaking and made up the volume up to 200 ml, further transferred 2 ml of solution to 200 ml volumetric flask.

2.2.8.2. Intra Precision (Repeatability)

This parameter concludes the repeatability of Hydrochlorothiazide assay results under the same operating conditions over a short period of time. The % RSD due to Hydrochlorothiazide concentration for the six samples was found to be 0.563%. Six separated sample preparations were analyzed according to the proposed method of analysis. The % RSD due to Hydrochlorothiazide concentration for the assay meets the requirements. Results are summerized in the table 3.

Table 3: Intra precision (repeatability) results

Sample Number	Hydrochloro-thiazide % Assay content	% RSD of Six Assay content
Sample Preparation -1	99.7	0.563% (Limit < 2%)
Sample Preparation -2	100.0	
Sample Preparation -3	99.0	
Sample Preparation -4	99.6	
Sample Preparation -5	98.6	
Sample Preparation -6	100.1	
Average % Assay	99.5	

2.2.8.3. Inter Precision (Repeatability)

This parameter concludes the intermediate repeatability of Hydrochlorothiazide assay results below the same operating conditions test performed on a different day, using different makes of reagents and solvents. The %RSD due to Hydrochlorothiazide concentration for the six samples was found to be 0.634%. Six separated sample preparations were analyzed according to the proposed method of analysis. The % RSD due to Hydrochlorothiazide concentration for the assay convenes the requirements. Results are summerized in the Table 4.

2.2.9. Ruggedness

Ruggedness of the proposed analytical method was assessed by performing the analysis on different days, different makes of reagents and solvents. The respective test assay results of Hydrochlorothiazide having concentration as 10µg/ml was well-quantitated. The result is expressed as shown in table 3 and 4. The developed analytical method for estimation of Hydrochlorothiazide was found to be robust as revealed in table 5.

Table 4: Inter Precision (Repeatability) Results

Sample Number	Hydrochloro-thiazide % Assay Content	% RSD of Six Assay content
Sample Preparation -1	99.7	0.634% (Limit<2%)
Sample Preparation -2	100.6	
Sample Preparation -3	100.4	
Sample Preparation -4	100.3	
Sample Preparation -5	98.9	
Sample Preparation -6	99.6	
Average % Assay	99.9	

Table 5: Ruggedness

Precision	% RSD of Assay (Six Preparation of each)	Limit For Ruggedness
Intra Precision	0.563	NMT 2%
Inter Precision	0.634	
% RSD of Overall 12 assays content	0.625	

2.2.10. Accuracy

This parameter determines the accuracy of the assay results below the same operating conditions test.

Hydrochlorothiazide sample was analyzed for the accuracy with composed known quantity of samples of Hydrochlorothiazide at 50%, 100%, 150% concentration levels and analyzed as per the method stated in proposed analytical method respectively. Three estimations were performed over each concentration levels respectively. Results are shown in tables 6-8.

The %RSD due to recovery of Hydrochlorothiazide at 50%, 100%, 150% concentration levels was found to be 50.1%, 99.5% and 151.1% respectively.

Nine sample preparations were analyzed as per projected analytical method of analysis. The % RSD due to Hydrochlorothiazide concentration for the assay meets the requirement and accuracy of recovery is inside 98.0% to 102%. Results are tabulated in the tables 6-8.

Table 6: Accuracy and recovery results @ 50 % concentration level

Accuracy@50% level	Recovery of Hydrochlorothiazide % Assay content	%Recovery 98.0%-102.0%	% RSD
Sample Preparation -1	49.5	100.2	1.070% (Limit< 2%)
Sample Preparation -2	50.3		
Sample Preparation -3	50.5		
Average % Assay	50.1		

Table 7: Accuracy and recovery results @ 100 % concentration level

Accuracy@100% level	Recovery of Hydrochlorothiazide % Assay content	% Recovery 98.0%-102.0%	% RSD
Sample Preparation -1	100.1	99.5	0.803% (Limit< 2%)
Sample Preparation -2	98.6		
Sample Preparation -3	99.9		
Average % Assay	99.5		

Table 8: Accuracy and recovery results @ 150 % Concentration level

Accuracy@150% level	Recovery of Hydrochlorothiazide % Assay content	% Recovery 98.0%-102.0%	% RSD
Sample Preparation -1	151.6	100.8	0.766% (Limit< 2%)
Sample Preparation -2	152.0		
Sample Preparation -3	149.8		
Average % Assay	151.1		

2.2.11. Solution Stability

Solution stability of the Hydrochlorothiazide solution was performed up to 26 hrs with set of different time interval and found the bench top solution is stable showing cumulative % RSD of different time interval is 0.913 which is less than the 2. Hence the Hydrochlorothiazide bench top solution is found to be stable up to 24 hrs at room temperature and recommended 24hrs solution stability.

3. DISCUSSION

The projected analytical method for estimation of Hydrochlorothiazide discussed in the present work provides a simple, informal, fast, stable, accurate, precise, reliable, economical, time saving and convenient method for the estimation analysis of Hydrochlorothiazide using UV Spectrophotometer λmax = was 271.0 nm. In the developed analytical method, the linearity was observed 0.9995 in the concentration range of 5 µg/ml -25 µg/ml.

Method precision for the Hydrochlorothiazide at concentrations level 10µg/ml was found in the range of 98.6%-100.6%. Accuracy of the proposed analytical method was established by recovery studies and the results were expressed as percent recovery and were found in the range of 99.5%-100.8%. Values of standard deviation and coefficient of variance was satisfactorily indicating the accuracy of the analytical methods. Intra-day and Inter-day precision studies were carried out by analyzing the sample of Hydrochlorothiazide different time interval on the same day and on different days respectively. Standard deviation and coefficient of variance for Intra-day and Inter-day precision studies was found to be less than 2 indicating precision of the proposed method.

4. CONCLUSION

Based on the significances of projected analytical method development and analytical validation studies test results, it was found that, the projected analytical method for estimation of Hydrochlorothiazide using UV Spectrophotometer is precise, reproducible, accurate, simple, informal, fast, stable, time saving and economical. This projected analytical method can be actively engaged for routine quality control estimation of Hydrochlorothiazide formulation drug in pharmaceutical analysis.

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Conflict of interest

The authors certify that there is no conflict of interests with any financial organization regarding the material discussed in the paper.

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