Research Article

UV-Spectrometric Method Development and Validation of Hydrochlorothiazide in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

Validation is a documented program that provides a high degree of assurance that a facility or operation will consistently produce product meeting a predetermined specification. The present research work is mainly emphasized to establish a novel, simple, economic, rapid and accurate method for the estimation of hydrochlorothiazide drug by UV-spectrophotometric method. The method was successfully developed by using distilled water as a mobile phase and the maximum absorbance was found to be at 272 nm. To confirm the developed method's appropriateness, it was validated as per ICH guidelines. This method obeyed Beers-Lambert's law over a concentration range of 5 to 17.5 µg/ml. The method was confirmed to be accurate with good recovery value and precise showing % RSD value within acceptance criteria. Due to its rapidness, accuracy, precision, sensitivity, ruggedness and more affordable mobile phase confirmed that this method can be undoubtedly applicable for routine quality control analysis of hydrochlorothiazide in bulk and pharmaceutical dosage form.

Keywords: Hydrochlorothiazide, validation, accuracy, linearity, diuretic.

INTRODUCTION

Pharmaceutical analysis plays a vital role in the quality assurance and quality control of bulk drugs. Analytical chemistry involves separating, identifying, and determining the relative amounts of components in a sample matrix. Pharmaceutical analysis is a specialized branch of analytical chemistry. Hydrochlorothiazide (3,4-dihydro-2H-1, 2, 4-benzothiadiazine-1,1-dioxide), which is classified as a BCS class IV drug, is commonly prescribed as the first-line diuretic for treating hypertension and peripheral edema. It falls under the class of diuretics, specifically in the thiazide sub-class. Hydrochlorothiazide works by directly inhibiting the sodium chloride co-transporter located on the apical membrane of the distal convoluted tubules in the kidney. It is soluble in water, sodium hydroxide, n-butylamine[1-3]. The structure of hydrochlorothiazide is illustrated in Figure 1.

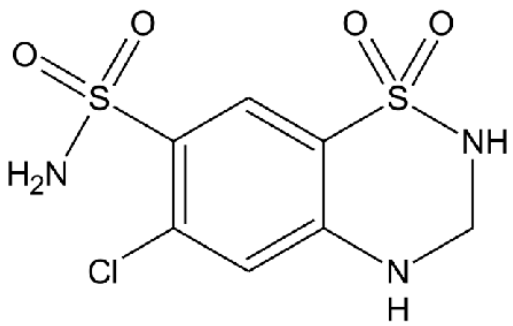

Figure 1: Structure of hydrochlorothiazide

Literature survey revealed that there are many methods like RP-HPLC[5-10] for the estimation of single drug and in the combination with ramipril and telmisartan. But there are only few methods developed for the estimation by simple UV-Spectrophotometric method. Hence, it felt necessary to develop a novel, economical, rapid and accurate method by using distilled water as a mobile phase. So, that it can be readily applicable for analyzing the retest period of bulk drug and can be routinely applicable for quality control analysis of pharmaceutical dosage forms[4-6].

MATERIALS AND METHODS

Chemicals used

Hydrochlorothiazide active pharmaceutical ingredient (API) was procured as a gift sample from Suralabs, Hyderabad, Telangana, and Tablet (Aquazide) was purchased from Local Pharmacy, Hyderabad, Telangana, India.

Instrumentation

UV 3000+ double beam Spectrophotometer (LABINDIA), digital weighing balance, ultra sonicator from UV Scientifics, Hyderabad, Telangana, India.

Solubility studies

Table 1: Solubility studies

Solvent	Solubility
Ortho phosphoric acid	Slightly soluble
Dimethyl sulfoxide	Not soluble
Dimethyl formamide	Soluble
Ethanol	Soluble
Distilled water	Soluble

Preparation of stock solution: 0.1g of hydrochlorothiazide was accurately weighed and transferred it into a 100ml volumetric flask, then the volume was adjusted by adding distilled water as the diluent.

Preparation of working stock solution: 0.25ml of the stock solution was pipetted out into a separate 25ml volumetric flask, where it was then diluted to the mark with distilled water and mixed thoroughly[7,8].

Method development[9-13]

For the present method development, the absorbance of the prepared working stock solution of hydrochlorothiazide was measured by scanning it with UV-spectrophotometer across a wavelength range of 200-400nm. Using mobile phase as distilled water and the observed λ_{max} was found to be 272 nm.

Method validation

To assure that the developed analytical method is appropriate for use, it was validated for various parameters according to ICH Guidelines.

Linearity

Preparation of hydrochlorothiazide working standard stock solutions for linearity (5ppm working standard solution): 5ml of working stock solution was pipetted out in to a 10ml volumetric flask and diluted to volume by using distilled water as diluent to obtain 5µg/ml of hydrochlorothiazide.

7.5ppm working standard solution: 7.5ml of working stock solution was pipetted out into a 10ml volumetric flask and diluted to volume by using distilled water as diluent to obtain 7.5µg/ml of hydrochlorothiazide.

10ppm working standard solution: 10ml of working stock solution was pipetted out into a 10ml volumetric flask and diluted to volume by using distilled water as diluent to obtain 10µg/ml of hydrochlorothiazide.

12.5ppm working standard solution: 12.5ml of working stock solution was pipetted out into a 10ml volumetric flask and diluted to volume by using distilled water as diluent to obtain 12.5µg/ml of hydrochlorothiazide.

15ppm working standard solution: 15ml of working stock solution was pipetted out into a 10ml volumetric flask and diluted to volume by using distilled water as diluent to obtain 15µg/ml of hydrochlorothiazide.

17.5ppm working standard solution: 17.5ml of working stock solution was pipetted out into a 10ml volumetric flask and diluted to volume by using distilled water as diluent to obtain 17.5µg/ml hydrochlorothiazide.

Procedure: The method's linearity over the range of 5-17.5 µg/ml was carried out by pipetting out of each standard solution into the volumetric flasks and measured the absorbance in UV spectrophotometer. A graph of concentration versus absorbance was plotted, and the correlation was calculated by regression analysis.

Accuracy

Accuracy was verified by calculating the recovery of the samples at concentration levels of 10ppm, 12.5ppm, and 15ppm, corresponding to spiking levels of 80%, 100%, and 120% respectively.

Procedure: For all these preparations, the absorbance was measured at 272 nm against a blank. Subsequently, the amount found, percentage recovery, and mean percentage recovery values were calculated.

Precision

Intra-day precision: Intra-day precision was carried out by measuring the absorbance of a 12.5ppm solution for five times under the same operating conditions within a short period of time. The % RSD value was then calculated.

Inter-day precision: Inter-day precision was performed and measured the absorbance of 12.5ppm concentration solution of hydrochlorothiazide for five times on the different days under same operating conditions and the % RSD value was calculated.

Assay determination of hydrochlorothiazide

The prepared standard and sample solutions of hydrochlorothiazide were assayed in five replicate samples. The spectra were recorded, and the percentage assay was calculated by using the formula;

$$\% \text{ Assay} = \frac{\text{Assay sample/Assay standard} \times \text{Concentration standard}}{\text{Concentration sample}} \times 100$$

Limit of detection (LOD)

Limit of detection was calculated by using the formula:

$$\text{Limit of detection} = \frac{3.3 \times \text{SD of intercept}}{\text{Slope}}$$

Limit of quantification (LOQ)

Limit of qualification was calculated by using the formula:

$$\text{Limit of quantification} = \frac{10 \times \text{SD of intercept}}{\text{Slope}}$$

RESULTS AND DISCUSSION

Method development

After the trials and errors, distilled water was adopted as a mobile phase and it has shown the maximum absorbance at the λ_{max} of 272 nm.

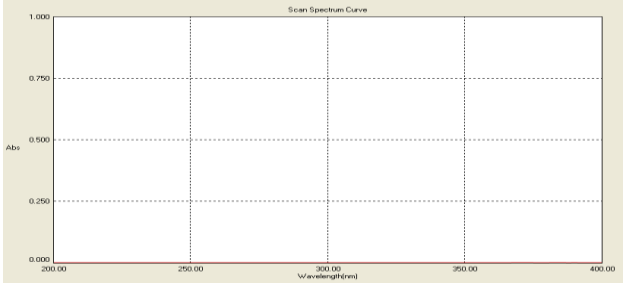

Figure 2: Blank spectrum

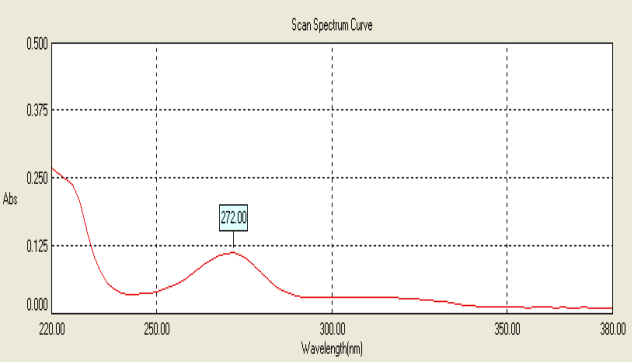

Figure 3: λ_{max} of Hydrochlorothiazide (272nm)

This condition was then finalized and proceeded for method validation. The blank spectrum and absorbance spectrum of hydrochlorothiazide was showed in Figure 2 & 3 respectively.

Method validation

Linearity

From the linearity graphs, it was confirmed that the method is exhibiting linearity over the range of 5 to 17.5 µg/ml. The correlation coefficient (r^2) was obtained as 0.999, which is fulfilling the validation criteria. The plotted graph and linearity data are presented in Figure 4 and Table 2. The overlain spectrum of linearity was shown in Figure 5.

Table 2: Linearity

λ_{max}	272 nm
Concentration (µg/ml)	**Absorbance**
5	0.049
7.5	0.069
10	0.089
12.5	0.110
15	0.127
17.5	0.146

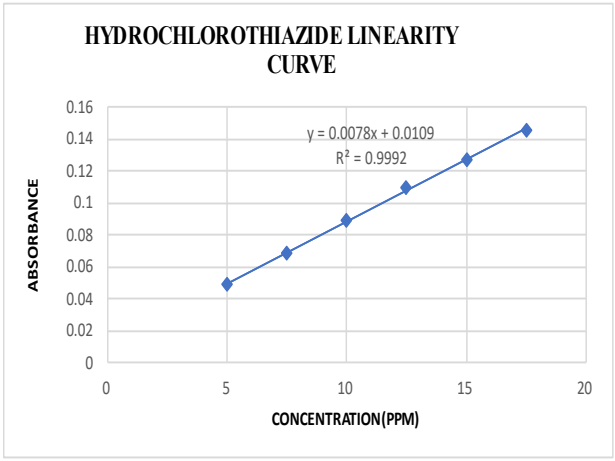

Figure 4: Calibration curve of hydrochlorothiazide

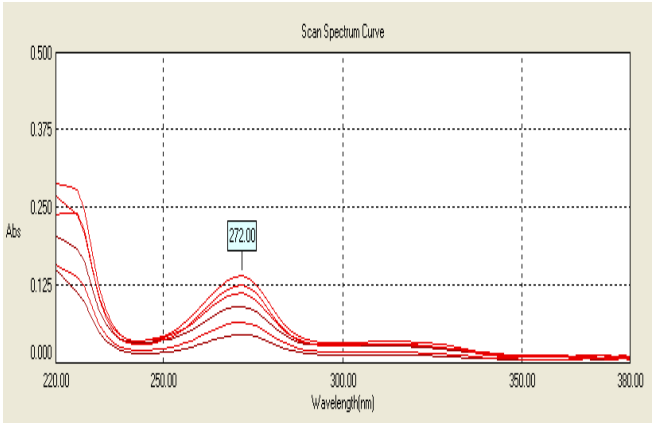

Figure 5: Overlain spectrum of linearity

Accuracy

The measured percentage average recovery at the levels of 80%, 100%, 120% was found to be 98.2%, 99.04% and 99.6%. The data is reported in Table 3.

Table 3: Accuracy data

Spiking level	Absorbance	Amount added (ppm)	Amount found (ppm)	Percentage recovery	Mean percentage recovery standard deviation
80%	0.185	10	9.8	98.2	
80%	0.185	10	9.8	98.2	
80%	0.185	10	9.8	98.2	**98.95%**
100%	0.205	12.5	12.38	99.04	**SD=0.5753**
100%	0.205	12.5	12.38	99.04	**% RSD=0.581**
100%	0.205	12.5	12.38	99.04	
120%	0.225	15	14.94	99.6	
120%	0.225	15	14.94	99.6	
120%	0.225	15	14.94	99.6	

Precision: The measured percentage relative standard deviation of intermediate precision and intra-day (Day-1 & Day-2) was found to be 0.447, 0.447 and 0.7312 respectively, which are within the specified limits. Accordingly, it confirmed the method precision. The data is reported in Tables 4, 5 and 6.

Table 4: Intra-day precision data

S. No.	Absorbance
1	0.110
2	0.109
3	0.110
4	0.110
5	0.109
Mean	0.1096
Standard deviation	0.00049
% RSD	0.447

Table 5: Inter-day precision data

S. No.	Absorbance	
	Day-01	Day-02
1	0.109	0.11
2	0.109	0.108
3	0.110	0.109
4	0.110	0.110
5	0.110	0.110
Mean	0.1096	0.1094
Standard deviation	0.00049	0.0008
% RSD	0.447	0.7312

Assay of hydrochlorothiazide table dosage form

The assay of pharmaceutical dosage form was calculated and the spectra is displayed in Figure 6. % Purity was found to be 100% w/w.

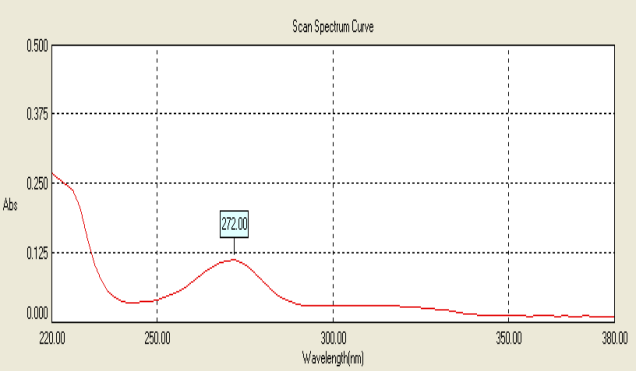

Figure 6: Assay of hydrochlorothiazide

LOD and LOQ

The parameters LOD and LOQ were determined by using formula. LOD and LOQ values are illustrated in Table 6.

Table 6: LOD and LOQ

Drug	LOD	LOQ
Hydrochlorothiazide	1.41 µg/ml	4.27 µg/ml

The present research work is mainly focused on development of a novel, rapid, accurate and UV-Spectrophotometer method by using double beam UV-Visible Spectrophotometer. The analytical method was developed using distilled water. The absorbance was measured over the range 200-400 nm and the λ_{max} was found to be 272 nm. The developed method obeyed Beer's-Lambert's law showing good linearity over a range of 5 to 17.5µg/ml. The developed method was found to be accurate and precise showing good recovery value and having % RSD with in the acceptance criteria. Thus, the developed method can be used routinely for quality control analysis of hydrochlorothiazide in the bulk drug.

CONCLUSION

A simple, economic, rapid, precise and accurate UV-Spectrophotometric method was developed & validated for the estimation of hydrochlorothiazide in active pharmaceutical ingredient. All the validation parameters were found to be within the acceptance criteria. The obtained Assay value and recovery values proved that, the present developed method can be applied for routine quality control analysis in laboratories for checking the retest period and purity of pharmaceutical dosage forms. The result obtained from the validation parameters met the ICH Q2 and USP requirement as well as obeys Beer's law.

DECLARATIONS

Author contributions

All authors contributed to experimental work, data collection, drafting or revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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Competing interest statement

All authors declare that there is no conflict of interests regarding publication of this paper.

Ethical approval

Not required.

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