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# Development and Validation of UV Spectrophotometric Method for the Determination of Hydrochlorothiazide in Pure and Formulations

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**ABSTRACT**

A simple, efficient, rapid, and accurate UV-spectrophotometric method has been developed and validated for the estimation of hydrochlorothiazide in pure and its formulation. Hydrochlorothiazide showed the absorption maxima in at 270 nm and was linear for a range of 2-10 µg/ml with a correlation coefficient of 0.996. The validation of the above-proposed method was done by carrying out precision and accuracy studies. The analytical method showed good intra precision (repeatability) with relative standard deviation 0.24% and inter precision with a relative standard deviation is 0.68% which is less than 2. The percentage recovery at three different levels i.e. 80%, 100% and 120% was found to be 95.01%, 99.33% and 101.66% respectively. The methods were successfully applied for assays of the studied drugs in their pure and tablet forms. Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH) for the determination hydrochlorothiazide in bulk and in tablet dosage form. The developed method was found to be accurate, precise and selective for determination of hydrochlorothiazide in tablet dosage form.

## INTRODUCTION

Hydrochlorothiazide chemical name is 2H-1,2,4-benzothiadiazine-7-sulfonamide, 6-chloro 3,4-dihydro-1,1-dioxide. Its molecular weight is 297.7 g/mol. It is slightly soluble in water and sparingly soluble in acetonitrile. Hydrochlorothiazide belongs to a class of drugs called thiazide diuretics, anti-hypertensive. Hydrochlorothiazide binds to and inhibits the enzyme carbonic anhydrase. It is frequently used alone or in combination with other medications for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, hypoparathyroidism, and edema and prevention of kidney stones and used in the treatment of osteoporosis [1-3].

A thiazide diuretic is always considered the prototypical member of this class. It reduces the reabsorption of electrolytes from the renal tubules. This results in increased excretion of water and electrolytes, such as sodium, potassium, chloride, and magnesium. It has been used for the treatment of several disorders including edema, hypertension, diabetes insipidus, and hypoparathyroidism. Hydrochlorothiazide is suddenly used for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis. It is also used for the prevention of kidney stones in those who have high levels of calcium in their urine [3,4].

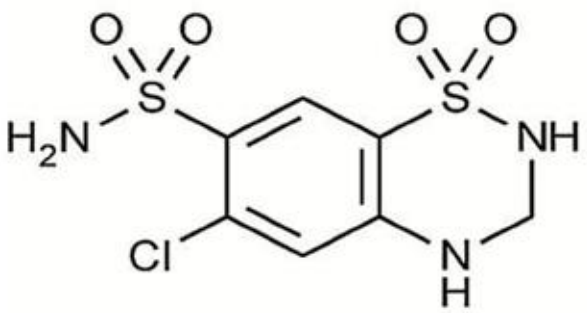


**Figure 1: Chemical structure of hydrochlorothiazide**

The aim of this work was the development of a simple, sensitive, and accurate analytical method for the determination of hydrochlorothiazide. The developed method was able to determine the content of the cited drugs in commercial tablets.

## MATERIALS AND METHODS

### Instruments

UV-visible spectrophotometer with UV Win software, weighing balances and matching quartz cells with a 1 cm cell path length were utilized along with the mentioned equipment, which had an automatic wavelength accuracy of 0.1 nm.

### Chemicals

Pharmaceutical grade hydrochlorothiazide (API) was procured as gift sample. The marketed pharmaceutical dosage form of hydrochlorothiazide tablets (Hydrazid 12.5mg) was purchased from local pharmacy, Hyderabad, Telangana, India. All chemicals and reagents were of analytical grade.

### Solvent selection

Several trails were done to find out the ideal solvent for dissolving the drug. The solvents such as double distilled water, and ethyl acetate was tried based on the solubility of the drug.

### Selection of detection wavelength [5-7]

Appropriate volume 1 ml of standard stock solution of hydrochlorothiazide was transferred into a 10 ml volumetric flask, diluted to a mark with ethyl acetate to give concentration of 10 µg/ml. The resulting solution was scanned in the UV range (200-400 nm).

### Preparation of stock solution

A precisely weighed, 10 mg of hydrochlorothiazide was transferred to 10 ml volumetric flask (clean and dry). Then few ml of ethyl acetate was added and dissolved the drug by vigorous shaking. The volume was then made up to the mark with ethanol to obtain the stock solution of 1000 µg/ml.

### Preparation of working standard solution

From stock solution 1 ml was pipetted out and further diluted to 10 ml with ethyl acetate to get the solution having the concentration of 100µg/ml.

### Preparation of calibration curve

From the working standard solution, pipetted out 2ml, 4ml, 6 ml, 8ml, and 10ml was diluted

to 10 ml using ethyl acetate to produce 2, 4, 6, 8 and 10 µg/ml solutions respectively. The absorbance of the solutions at the $\lambda_{max}$ of 270 nm using ethyl acetate as blank was measured. The calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis. The curve shows linearity in the concentration range of 2-10 µg/ml. The correlation co-efficient ($r^2$) was found to be 0.996.

**Assay of pharmaceutical formulation [8,9]**

20 Tablets of Hydrochlorothiazide marketed formulations were weighed and powdered. A quantity of tablet powder equivalent to 50mg of hydrochlorothiazide was transferred to 100 ml volumetric flask and volume was made up to the mark with ethyl acetate. The absorbance of the resulting solution was measured at 270 nm and the amount of hydrochlorothiazide was computed from its calibration plot.

**Analytical method validation [10-13]**

These current validation characteristics describe the validation parameters stated by the International Conference on Harmonization (ICH) guidelines.

**Linearity**

The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity can be assessed by performing single measurements at several analyte concentrations. A linearity correlation coefficient above 0.996 is acceptable for most methods, especially for major components in assay methods. The range of an analytical procedure is the interval between the upper and lower concentrations of analyte in the sample.

**Precision**

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple samples of the same homogenous sample under prescribed conditions. Precision was determined by intra-day and inter-day study. The repeatability of the method was evaluated by carrying out the assay 3 times on the same day and intermediate precision was evaluated by carrying out the assay on 3 consecutive days for the sample solution. The percent relative standard deviation (%RSD) was calculated.

**Accuracy (Recovery studies)**

The accuracy of the analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted true value. Accuracy studies were performed at three different levels (80%, 100% and 120%) by standard addition method and the samples were analyzed in triplicate by the proposed method. Known amount of standard sitagliptin at 80%, 100% and 120% of the predetermined sample was added to a pre-quantified tablet sample.

**Ruggedness**

Method ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, sources of reagents, chemicals, solvents, and so on. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method.

**Robustness**

The concept of robustness of an analytical procedure has been defined by the ICH as "a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters". The most important aspect of robustness is to develop methods that allow for expected variations in the separation parameters. For the determination of a method's robustness, parameters such as variation in detector wavelength are varied within a realistic range and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range. The absorbance was measured and the assay was calculated for six times.

**LOD and LOQ**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

$$\mathbf{LOD = 3.3 \times \sigma/S}$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = Standard deviation of the response, and

S = Slope of the calibration curve

## RESULTS AND DISCUSSION

The developed UV technique is precise, specific, accurate and stability-indicating. The developed method was validated based on ICH guidelines. Statistical analysis proves that the method is repeatable and selective for the analysis of hydrochlorothiazide as a bulk drug and in pharmaceutical formulations.

The method discussed in the present work provides a simple, stable, rapid, accurate, precise, reliable, less expensive, timesaving and convenient method for the analysis of hydrochlorothiazide using UV spectrophotometry. $\lambda_{max}$ selected for quantitation was 270 nm. In the developed analytical method, the linearity was observed at 0.996 in the concentration range of 2-10 µg/ml.

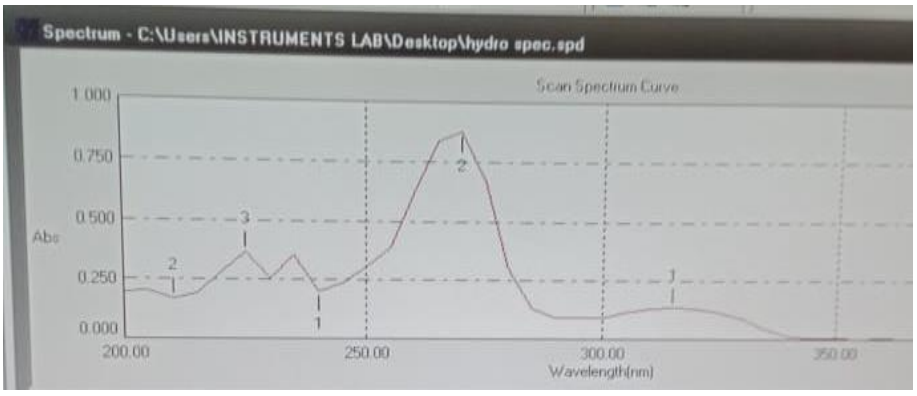


**Figure 2: Absorption maxima of hydrochlorothiazide**

**Table 1: Linearity of hydrochlorothiazide**

| Concentration (µg/ml) | Absorbance |
|---|---|
| **2** | 0.209 |
| **4** | 0.427 |
| **6** | 0.634 |
| **8** | 0.829 |
| **10** | 0.987 |

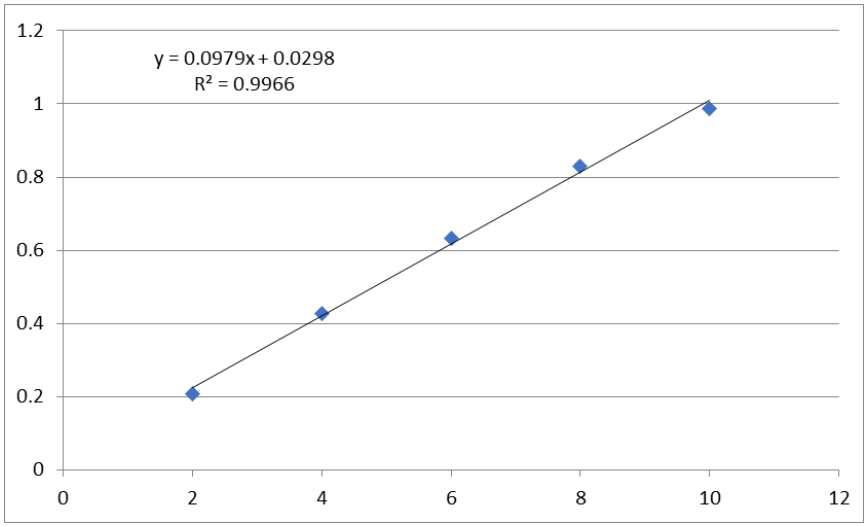


**Figure 3: Linearity**

**Table 2: Precision**

| S. No. | Concentration (µg/ml) | Absorbance (intraday) | Absorbance (interday) |
|---|---|---|---|
| 1 | 10 | 0.849 | 0.923 |
| 2 | 10 | 0.849 | 0.927 |
| 3 | 10 | 0.851 | 0.931 |
| 4 | 10 | 0.851 | 0.931 |
| 5 | 10 | 0.852 | 0.932 |
| 6 | 10 | 0.852 | 0.932 |
| **Mean** | | **0.8505** | **0.9275** |
| **Std. dev.** | | **0.002121** | **0.006364** |
| **% RSD** | | **0.2495** | **0.686** |

**Table 3: Accuracy**

| S. No. | Level of adding | Amount added (µg/ml) | Amount recovered (µg/ml) | Percentage recovery |
|---|---|---|---|---|
| 1 | 80 | 1.2 | 1.14 | 95.01 |
| 2 | 100 | 1.5 | 1.49 | 99.33 |
| 3 | 120 | 1.8 | 1.83 | 101.66 |

**Table 4: Robustness**

| S. No. | Wavelength | Absorbance |
|---|---|---|
| 1. | 268 nm | 0.853 |
| 2. | 270 nm | 0.869 |
| 3. | 272 nm | 0.793 |

**Table 5: Ruggedness**

| S. No. | Analyst | %RSD |
|---|---|---|
| 1 | Analyst-1 | 0.931 |
| 2 | Analyst-2 | 0.932 |

**Table 6: Assay of formulation**

| Drug | Labelled amount | Mean | SD | % Assay | % RSD |
|---|---|---|---|---|---|
| Hydrochlorothiazide | 12.5mg | 98.98 | 0.190919 | 99.12 | 0.192 |

**Table 7: LOD and LOQ of hydrochlorothiazide**

| | | |
|---|---|---|
| Hydrochlorothiazide | LOD | 0.741µg/ml |
| | LOQ | 2.24 µg/ml |

A simple, efficient, rapid, and accurate UV-spectrophotometric method has been developed for the estimation of hydrochlorothiazide in bulk and its formulation. Accuracy of the proposed method was ascertained by recovery studies and the results were expressed as percent recovery and were found in the range of 95.01%-101.66%. Values of standard deviation and coefficient of variance was satisfactorily indicating the accuracy of both the methods. Intra-day and inter-day precision studies were carried outby analyzing the sample of hydrochlorothiazide at 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.

## CONCLUSION

The developed UV method is suitable for the estimation of hydrochlorothiazide as it extracts the drug from the formulation and determines the percent content with good %RSD. So, this method is fast, accurate, precise, and sensitive hence it can be employed for routine quality control of tablets containing three drugs in industries. Validation of the proposed procedures was carried out according to the ICH guidelines. This method can be used for the routine determination of hydrochlorothiazide in commercial formulations.

## Competing interest statement

Declared None

## Sponsorship

No

## REFERENCES

1. Duarte JD, Cooper-DeHoff RM. Mechanisms for blood pressure lowering and metabolic effects of thiazide and thiazide-like diuretics. Expert Rev Cardiovasc Ther. 2010; 8(6):793-802.
2. P. Pickkers, R. S. Garcha, M. Schachter, P. Smits, and A. D. Hughes. Inhibition of carbonic anhydrase accounts for the direct vascular effects of hydrochlorothiazide. Hypertension. 1999; 33(4):1043-1048.
3. Naguib IA, Abdelaleem EA, Abdallah FF, Emam AA. Development and Validation of Two Chromatographic Methods for Simultaneous Determination and Quantification of Amiloride Hydrochloride, Hydrochlorothiazide, and Their Related Substances, in Pure and Tablet Forms. J AOAC Int. 2020; 103(3):747-754.
4. S. A. Hapse, V. S. Wagh, P. T. Kadaskar, M. D. Dokhe, and A. S. Shirsath. Spectrophotometric estimation and validation of hydrochlorothiazide in tablet dosage forms by using different solvents. Der Pharma Chemica. 2012; 4(1):10-14.
5. Naguib IA, Abdelaleem EA, Zaazaa HE, Draz ME. Simultaneous determination of hydrochlorothiazide and benazepril hydrochloride or amiloride hydrochloride in presence of hydrochlorothiazide impurities: chlorothiazide and salamide by HPTLC method. J Chromatogr Sci. 2015; 53(1):183-188.
6. Boggula N, Bhadru B, More K. RP-HPLC Method Validation for Levomilnacipran Estimation in Bulk and Formulation. International Journal of Pharmaceutical Quality Assurance. 2023; 14(4):900-903.
7. Sharma M, Kothari C, Sherikar O, Mehta P. Concurrent estimation of amlodipine besylate, hydrochlorothiazide and valsartan by RP-HPLC, HPTLC and UV-spectrophotometry. J Chromatogr Sci. 2014; 52(1):27-35.
8. Kiran Rapolu, Raja Reddy Aleti, Rama Rao Tadikonda, Pravalika Patlolla, Narender Boggula. Method Development and Validation of Pregabalin in Bulk and Tablet Dosage Forms by UV Spectroscopy. International Journal of Advanced Chemistry Research. 2024; 6(1):53-57.
9. Boyka G Tsvetkova, Lily P Peikova. Development and validation of RP-HPLC method for simultaneous determination of Amlodipine Besylate and Hydrochlorothiazide in the pharmaceutical dosage form. J Chem Pharm Res. 2013; 5(1):271-275.
10. Joshi SJ, Karbhari PA, Bhoir SI, Bindu KS, Das C. RP-HPLC method for simultaneous estimation of bisoprolol fumarate and hydrochlorothiazide in tablet formulation. J Pharm Biomed Anal. 2010; 52(3):362-371.
11. Kartal M, Erk N. Simultaneous determination of hydrochlorothiazide and amiloride hydrochloride by ratio spectra derivative spectrophotometry and high-performance liquid chromatography. J Pharm Biomed Anal. 1999; 19(3-4):477-85.
12. Thaidala Sriveni, Vanamala Naveen, Vemula Sai Rupa, Aeruva Renuka, Sunil Porika, M Akiful Haque, Vasudha Bakshi, Narender Boggula. Development and Validation of Dolutegravir in Bulk and Formulation: An Anti-Retroviral Drug Using UV-Spectroscopy. International Journal of Pharmaceutical Quality Assurance. 2021; 12(1):57-60.
13. Sachin Bhagwate and N. J. Gaikwad. Stability Indicating HPLC Method for the Determination of Hydrochlorothiazide in Pharmaceutical Dosage form. J App Pharm Sci. 2013; 3(02):088- 092.