RESEARCH ARTICLE

# Development of a Visible Spectrophotometric Analysis for Hydrochlorothiazide in Pure and Pharmaceutical Dosage Forms

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***Received: 10th March, 2021; Revised: 16th April, 2021; Accepted: 20th May, 2021; Available Online: 25th June, 2021***

**ABSTRACT**

An accurate, simple, sensitive, and low-cost approach was developed to determine hydrochlorothiazide (HCTH) in pure and pharmaceutical preparations. This method is based on hydrochlorothiazide's acidic hydrolysis, then treated with sodium nitrite($NaNO_2$) to form diazonium salt that couples with resorcinol reagent in the basic medium at pH=13 to form a stable dye with a light orange color that soluble in water. The colored compound has the greatest absorption at 426 nm wavelength. The variables that affect the completion of the reaction were optimized. Beer's law is obeyed over the concentration range (0.25-13 μg/mL) with a molar absorption factor of (14140.75 L/mol.cm). The detection limit (0.1174 $\mu g.mL^{-1}$), limit of quantitative (0.3557μg/mL), and Sandell's sensitivity (0.0210 $\mu g.cm^{-2}$) have been calculated. The suggested methodology has been applied with success to the determination of hydrochlorothiazide in pharmaceutical preparations.

**Keywords:** Azotization, Hydrochlorothiazide, Resorcinol, Spectrophotometric

International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.2.46

**How to cite this article:** Noor HK, Abass SM. Development of a Visible Spectrophotometric Analysis for Hydrochlorothiazide in Pure and Pharmaceutical Dosage Forms. International Journal of Drug Delivery Technology. 2021;11(2):496-500.

**Source of support:** Nil.

**Conflict of interest:** None

## INTRODUCTION

Hydrochlorothiazide is a type of thiazide diuretics in medical care Figure 1. It is used more commonly due to better bioavailability. It is more potent, so the required dose is considerably lower than that of Chlorothiazide.[1] Hydrochlorothiazide operates upon distal convoluted tubules and inhibits the sodium chloride co-transporter system, which results in a diuretic action and potassium loss in urine.[2] Food and Drug Administration (FDA) approved the thiazide diuretics for treating high blood pressure, the adjunctive edema treatment related to cirrhosis and congestive heart failure, for treating the edema related to renal impairments.[3] The Hydrochlorothiazide has been related to higher risks of negative cardiovascular consequences for any specific systolic blood pressure lowering.[4] By comparing HCTH to the rest of the thiazide diuretics, it is safe to treat high blood pressure. Its dose is much lower than Chlorothiazide, as Chlorthalidone associated with an increased risk of kidney and electrolyte abnormalities.[1-5] Hydrochlorothiazide has bioavailability ~70%, while Chorothiazide was weak, hydrochlorothiazide has 6 to 12 hours as half-life.[2] Adverse effects: weakness, nausea, exfoliative dermatitis/TAN.[6] But the most dangerous can cause fluid imbalance, including hypokalemia, hyponatremia, hypercalcemia,[7] and an increased risk of developing skin cancer.[8,9] Results in increasing triglyceride and cholesterol.[10] Contra-indications for hydrochlorothiazide in hypersensitivity to sulfonamide-derived drugs,[11] and during pregnancy.[12,13] A variety of analytical approaches were reported to determine the HCTH, which includes the high-performance liquid chromatography,[14] electrochemical sensors' approach,[15] chemometric assisted fourier transform infrared spectroscopy (FTIR),[16] UV-spectrophotometric.[17,18] The present research aims to develop a simple, sensitive, and selective acidic hydrolysis[19] approach for determining the HCTH in a variety of pharmaceutical forms and samples.

## EXPERIMENTS

### Instrument

The present study has been carried out using the Shimadzu 800, Japan UV-Vis. double beam spectrophotometer, supplied with

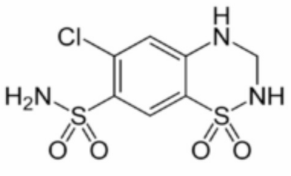


**Figure 1:** Hydrochlorothiazide's chemical structure

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one 10 mm quartz cell, Sartorius BL 210 S Scientific balance (± 0.0001 g), Gottingen–Germany was used for weighting the samples**.**

**Reagent and Materials**

*Standard Drug Solution ($8.397x10^{-4}M$)*

The HCTH drug's standard solution has been produced from the pure substances of the HCTH, which General Company provides for the Pharmaceutical Industry - Samara, Iraq, through the dissolution of 0.1g in a solution mix of 15 mL of the methanol and 25.0mL concentrated HCl acid. This solution has been heated at 75° C, left to cool, and has been transferred afterward to a 100 mL flask, and volume has been completed to the mark of 100 mL volumetric flask, 25 mL of the solution that was prepared has been transferred to a 100 mL volumetric flask, and the volume has been completed with distilled water to the mark to obtain a solution at a 250 µg/mL concentration.

*Reagent Solution of the Resorcinol ($2.72x10^{-3}M$)*

Resorcinol reagent was produced through the dissolution of 0.03gm in a 100mL flask with 5mL of methanol, and after that, the volume has been completed by distilled water to the mark.

*$NaNO_2$ Solution ($1.45x10^{-3}M$)*

The nitrate solution was prepared by the dissolution of 0.01gm of NaNO2 in a small volume of distilled water and transferred into a 100 mL flask. After that, the volume is completed to mark with that same solvent.

*Solution of Sodium Hydroxide ≈ 1M*

A total of 4 gm sodium hydroxide has been dissolved in a little of the distilled water; after that, the solution has been transferred into a 100 mL flask, and the distilled water has completed its volume to the mark.

*Buffer Solution*

A buffer solution has been produced through the mixing of 50 mL of 0.2 M of the potassium chloride solution (produced through a dissolution of 1.49 g potassium chloride in 50 mL distilled water) and 172 mL of 0.20 M sodium hydroxide (which has been produced through dissolution of 2g of the sodium hydroxide with the distilled water in 250 mL volumetric flask) to obtain a solution with pH that equals 13.

**Recommended Process**

Different aliquant 0.01–0.52 mL of 250 µg/mL of hydrolyzed drug solution have been prepared into a 10 mL flask, and 0.40 mL of 1.45 x $10^{-3}$M of the $NaNO_2$ have been added and put in an ice bath for a 5 min period, after which 0.50 mL of the resorcinol reagent ($2.72x10^{-3}$M) has been added and 1.5 mL of the pH 13 buffer solution, absorbance has been evaluated at a 426 nm wavelength against the blank solution.

*Process for the Tablet 50 mg*

Ten HCTH tablets have been precisely weighed and well grinded. A 0.1819g weight Diuzid (safa/ Iraqi), 0.1918g Hydrochlorothiazide, Actavis (Iran) has been taken similar to the 0.10 g weight of the HCTH to make a sample solution, the other solution types may be made with the serial dilution for the preparation of 250 µg/mL, after that, the suggested approach has been applied for quantitatively determining (2.50, 5.0, 7.50) µg/mL.

*Procedure for the Tablet 25 mg*

Ten HCTH tablets have been precisely weighed, well grinded, 0.1379g weight Esidrex 25 (French) has been taken similar to 0.10 g weight of the HCTH to make a sample solution. The other solution types may be produced with the serial dilution for the preparation of 250 µg/mL; after that, the suggested approach has been applied for quantitatively determining 2.5, 5, and 7.5 $\mu g.mL^{-1}$.

## RESULTS AND DISCUSSIONS

The optimal conditions for the development of the color have been carried out through the variation of parameters one by one and fixed the rest for the observation of the obtained effect on the colored product's signals of absorption. The preliminary analyses have discovered that following the diazotization in the alkaline medium, a substance with the light orange color yielded in treating the HCTH solution with resorcinol.

**Spectra of the Absorption**

The main test of this approach included the hydrolyzed HCTH diazotization with the $NaNO_2$, after that, reacted with the resorcinol in the primary mediums to form the colored azo dyes. The absorbance and λ max of the azo dye have been assessed against reagent blank Figure 2. This complex had shown a maximal absorbance at 426 nm.

**Reaction Chemistry**

Hydrochlorothiazide acidic de-composition breaks the bond of the secondary amine with the group of the methylation. At the same time, converting secondary into primary amine that may be azotized easily with the nitrous acid to form diazonium salt and combined with the resorcinol in the alkaline mediums to form a light orange colored compound, which can be seen from Scheme 1.

**Effects of the concentration of the $NaNO_2$**

Effects of the concentration of $NaNO_2$ on the colored complex's absorbance has been studied in a range between (0.1 mL and 0.6 mL) from 1.45 x $10^{-3}$M of the $NaNO_2$, the maximal intensity

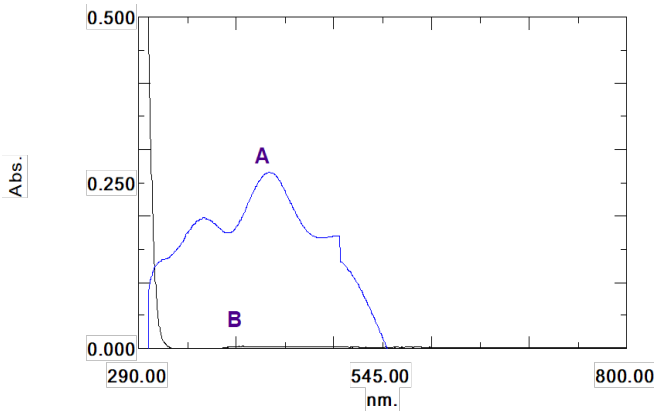
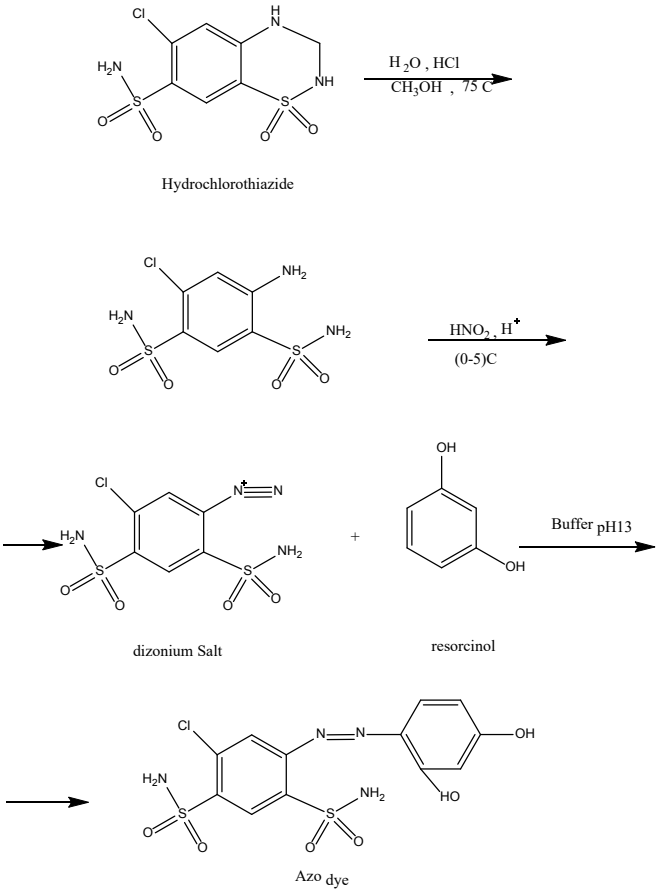



**Figure 2:** Colored complex's absorption spectra for 5 µg/mL of the HCTH (A), against the blank solution (B)




**Scheme 1:** The reaction's suggested in the chemical mechanism

of the color is accomplished with 0.3 mL, high concentration results in the decrease of intensity.

### Effects of the concentration of the reagents

Effects of the addition of various resorcinol reagent concentrations for the determination of 5 $\mu g.mL^{-1}$ of the HCTH have been researched by selecting the optimal concentration that maximizes the absorption. This work has shown that the 0.6mL concentration from 2.72 x $10^{-3}$M resorcinol has shown the optimal increment of the absorption, which is why it has been preferable for the following steps.

### Effect of the Buffer

The colored complex has been formed in the basic conditions, which is why they have been researched through the basic buffer solution, identified by the fact that colored compound had higher stability with the use of the buffer instead of the sodium hydroxide base only. The effects of the buffer solution are researched following the determination of optimal pH value for the optimal value of the absorption. Various prepared buffer solution volumes have been studied for selecting the most suitable volume, this study has shown that the 1.5 mL volume of the buffer solution has been most suitable for giving the maximum intensity of absorption and this, has been fixed for the following works. The colored compound has been produced in a basic environment, so experiments have been done using buffer solution.

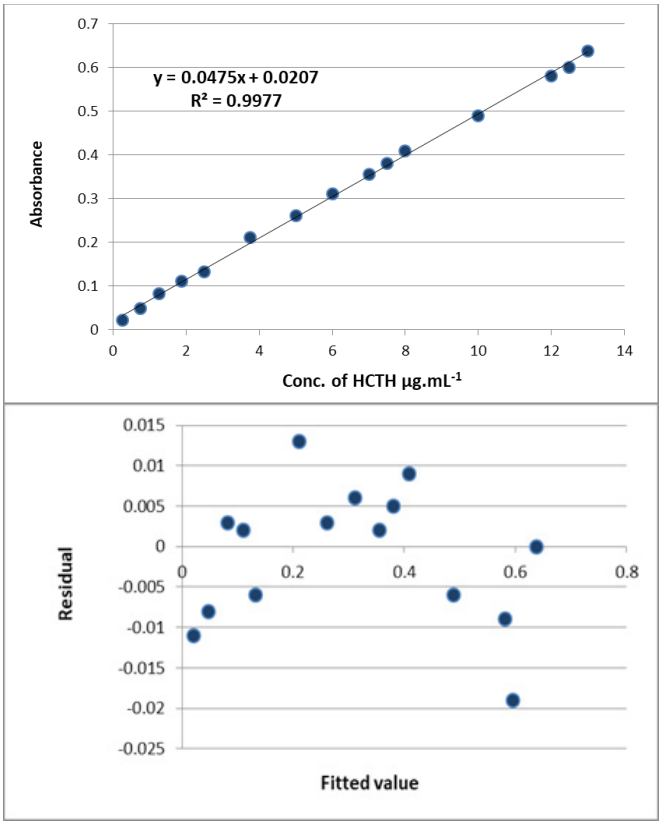



**Figure 3:** (a) Calibration curve (b) and residual chart

**Table 1:** Optical characterization and quantitative parameters of the proposed work.

| *Parameters* | *Values* |
|---|---|
| λ max (nm) | 426 |
| Color | Light orange |
| Linear ranges (µg/mL) | 0.25-13 |
| Slop | 0.0475 |
| Intercept | 0.0207 |
| Regression equation | y = 0.0475x +0.0207 |
| Molar absorptivity ($L.mol^{-1}.cm^{-1}$) | 14140.75 |
| Coefficient of Correlation | 0.9977 |
| Quantitative limit ($\mu g.mL^{-1}$) | 0.3557 |
| Limit of Detection ($\mu g.mL^{-1}$) | 0.1174 |
| Sandell's sensitivity ($\mu g.cm^{-2}$) | 0.0210 |

### Effects of the Various Solvents

The impacts of the variety of the solvents upon the signal of the absorption of color product formed have been investigated with the use of the following solvents: water (426 nm), ethanol (422 nm), methanol (422 nm), 2-propanol (423 nm), and acetone (421 nm). This study has shown that the optimal solvent is water, giving the highest value of absorption.

### Linearity and Calibration Curve

The optimum experimental settings for evaluating the drug compound applied and the linear curve of calibration have been obtained in a range of concentration between

0.25 and 13 µg/mL. Some of the optical characteristics of the formed colored compound have been measured, as can be seen from Table 1. By plotting residual of standard concentration values with the value of the absorption, Figure 4b shows that residual has been evenly distributed around the mean of its values and = 0 and random distribution around mean of values, which indicates that there aren't any systematic errors in suggested working approach.

## Precision and Accuracy

The proposed approach's compatibility and accuracy have been researched by measuring the relative standard deviation (RSD) values and the relative error (RE). With five replicates for three different drug concentrations have been chosen, which are in a linear range. The relative error didn't exceed 5%, whereas the RSD has been within (≤4%) limits, as shown in Table 2, indicating the likelihood for the assessment procedure with good compatibility and accuracy.

**Table 2:** Accuracy and precision data

| *Hydrochlorothiazide. Conc. (µg.mL$^{-1}$)* | | | |
|---|---|---|---|
| *Taken* | *Found** | *Relative Error %* | *RSD%* |
| 2.5 | 2.494 | -0.240 | 3.376 |
| 5 | 5.117 | 2.340 | 1.630 |
| 7.5 | 7.581 | 1.080 | 0.603 |

* *Five replicate*

**Table 3:** Quantitative determination of 2.5µg/mL of HCTH from a Variety of the Origins

| *Drugs* | *Companies* | *Rec.%* |
|---|---|---|
| Diuzid, tab | S.P.I / Iraq | 100.4 |
| Hydrochlorothiazide, tab | Iran | 101.16 |
| Esidrex 25, tab | JUVISE /France | 101.16 |

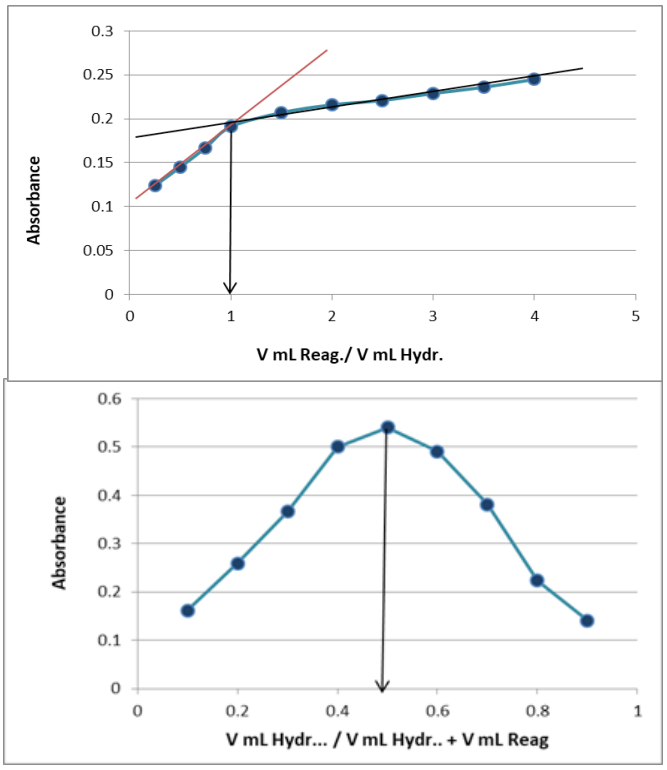



**Figure 4:** Stoichiometric research with (a) Mole ratio (b) Continuous variation

**Table 4**: Quantitative determination of a variety of the HCTH concentrations from a variety of the origins

| *Pharmaceutical preparation* | *Labeled amount (mg)* | *Conc. µg.mL$^{-1}$* | | | |
|---|---|---|---|---|---|
| | | *Taken* | **Found* | *Recovery%* | *RSD%* |
| Diuzid | 50 | 2.5 | 2.153 | 86.12 | 0.826 |
| | | 5 | 4.848 | 96.96 | 0.398 |
| | | 7.5 | 7.374 | 98.32 | 0.268 |
| Hydrochlorothiazide | 50 | 2.5 | 2.111 | 84.44 | 0.398 |
| | | 5 | 4.827 | 96.54 | 0.400 |
| | | 7.5 | 7.332 | 97.76 | 0.405 |
| Esidrex 25 | 25 | 2.5 | 2.174 | 86.96 | 0.806 |
| | | 5 | 4.869 | 97.38 | 0.793 |
| | | 7.5 | 7.395 | 98.60 | 0.536 |

* *Three replicate*

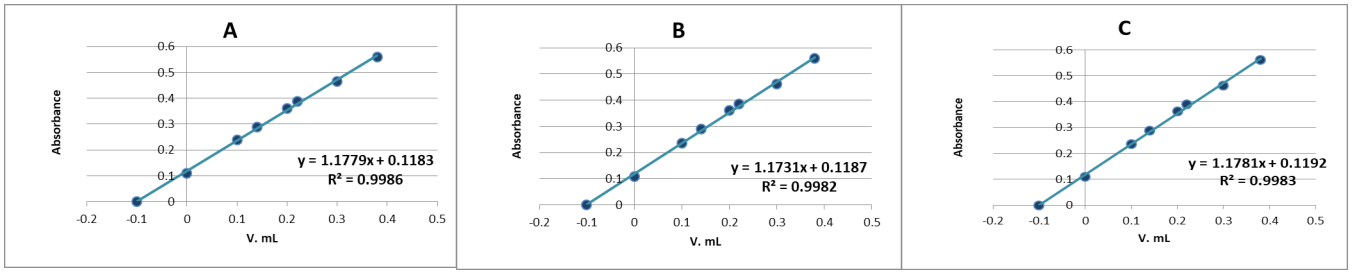



**Figure 5:** Determinations of the HCTH in the pharmaceutical preparations with the use of the approach of the standard addition (A) Diuzid (SPI) Iraqi, (B) Hydrochlorothiazide (Iran), (C) Esidrex 25 (French).

### Study of the Stoichiometry

Equal concentrations of the resorcinol and HCTH ($8.397 \times 10^{-4}$M) have been prepared to study the stoichiometric ratio of azotized drug complex and the resorcinol reagent at a pH value of 13 Figure 4, continuous variation approaches and molar ratios have been applied for that purpose. The study has shown that the drug compound ratio to coupling reagent has been 1:1.

### Application in the Pharmaceutical Formulations

*Tablets 25 and 50 mg*

As can be seen from Figure 5, the proposed was implemented successfully on the quantitative HCTH generated by various companies using the standard addition approach. Table 3 shows the value of recovery for determining 2.5 µg/mL HCTH from various pharmaceutical formulation sources.

## CONCLUSIONS

The suggested approach has been identified by the speed, accuracy, and ease in estimating the medication in the aqueous mediums. The drug assessment was possible in the case of the conversion of the secondary group of the amines to the primary amine with the use of acid hydrolysis procedure with the concentrated HCl acid. Outcomes have shown good compatibility and accuracy in applying to the estimate HCTH in the pharmaceutical formulations.

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