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Spectrophotometric determination of Hydrochlorothiazide by diazotization- coupling using a meta-aminophenol reagent and application to pharmaceutical formulations.

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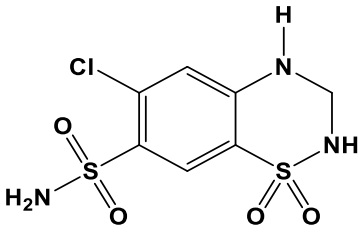
A B S T R A C T

A Spectrophotometry method was developed to be faster and more sensitive to determine the purity of Hydrochlorothiazide compound. This method is relying on the reaction of diazotization-coupling using a meta-aminophenol reagent medium and factor to give a yellow product at the wavelength 443 nm. This is will be within the range of Beer's law (0.4-20 ppm), molar absorbance (3.7843×10^4 $L.mol^{-1}.cm^{-1}$), correlation coefficient (0.9998), and Sandell′s sensitivity (0.00786 $\mu g/cm^2$) Limit of detection (LOD) (0.0226 ppm) and LOQ (0.0754 ppm). It has been successfully applied to pharmaceutical preparations...

Introduction

Scientific name of a chemical compound 6-chloro-3,4 dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1- dioxide[1] Its chemical composition is as shown in Figure (1-1).



(Figure1: Structure of HCTZ)

$C_7H_8ClN_3O_4S_2$

M.Wt:297.74 gm/mol[2]

Hydrochlorothiazide is a white powder that dissolves easily in sodium hydroxide solution and moderately in ethanol as well as ammonia and is slightly soluble in water, its melting point is 266-269 C°[3] It is used in the treatment of high blood pressure[4] It inhibits the reabsorption of sodium and chloride in the beginning of the distal convoluted tubule (causes a natriuretic effect) mainly by reducing the reabsorption of sodium and chloride in the cortical part of the ascending end of the Loop of Henley by inhibiting Na^+, Cl^-[5] The gastrointestinal absorption of hydrochlorothiazide is 70%[6].

Apparatus

The absorption and spectroscopic reading were achieved by using a Shimadzu UV/Vis 1800 spectrophotometer (Japan), and quartz cells with a light path of 1 cm. The weight of chemical compounds was measured using the sensitive scale (S 201 BL Sartorius). Heating process was carried out using the water bath (Karl Kolb-Germany).

Materials and chemical solutions

All the chemicals and analytical reagents were used in this study have the highest purity. As described below :

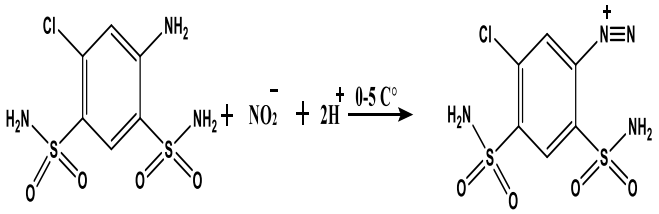
1. **Hydrochlorothiazide solution, hydrolyses in acidic medium (100 µg/mL)**

The solution was prepared by dissolving 0.01 g of pure hydrochlorothiazide in .05 ml of ethanol, then adding 20 ml of concentrated hydrochloric acid, and then clean 50 ml of distilled water, then we perform an escalation process for half an hour at a temperature (70-75 ° C) until the completion of the acid decomposition process.

2. **Meta-aminophenol reagent solution (9.16×10^{-4}M)**
   Prepare this solution by dissolving 0.01 g of the reagent in distilled water and then bring the volume up to the mark in a 100 mL vial.
3. **sodium nitrite solution (0.1450 M)**
   Prepare this solution by dissolving 1g of sodium nitrite with distilled water and bring the volume up to the mark in a 100ml vial.
4. **urea solution (0.1 M)**
   Prepare by dissolving 0.6g of urea in a given amount of distilled water and bring the volume up to the mark in a 100ml vial.
5. **potassium hydroxide solution (25 M) (approximate concentration)**
   Dissolve 1.403 g of potassium hydroxide in distilled water and bring the volume up to the mark in a 100 mL vial.
6. **hydrochloric acid solution (1M) (approximate concentration)**
   Prepare the solution by diluting 8.4 ml of concentrated hydrochloric acid with distilled water to 100 ml using a volumetric vial.
7. **Pharmaceutical solution (100 µg/mL)**
   The pharmaceutical preparation containing hydrochlorothiazide is available in the form of tablets produced by Al-Safa Company for Pharmaceuticals and Medical Supplies, Diyala-Iraq. (10 tablets weighing 1.7961 gm). Equivalent to 100 µg/mL dissolved in 5.0 mL of ethanol then add hydrochloric acid and make up to mark with distilled water in a 100 mL vial and filter the solution using filter paper.

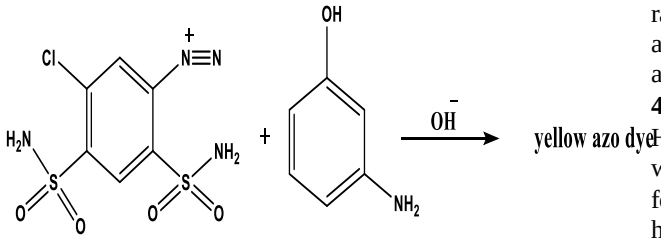
The general principle of the method

This method relied on reacting hydrochlorothiazide hydrolyzed with sodium nitrite in an acidic medium to obtain the disonium salt according to the following equation:



4-amino -6- chloro -1,3 bezo disolfnamide — Dizonium Salt

Then reacting the nitrogenous hydrochlorothiazide with a meta-aminophenol reagent in an alkaline medium to form a yellow dye as shown in the following equations:



Dizonium Salt Meta amino phenol

Preliminary study

1.0 ml of 0.1450 M sodium nitrite solution was added to 2.0 ml of 100 µg/ml hydrochlorothiazide solution, and the resulting solution was placed in an ice bath (0-5 °C) for 5 minutes, then .01 ml of urea solution was added. A concentration of 0.1 M and the addition of 1.0 milliliters of a meta-aminophenol reagent solution at a concentration of 9.16×10^{-4} M, and the medium was made alkaline by adding 0.01 ml of potassium hydroxide solution at a concentration of 0.25 M in a volumetric vial of 25 ml. absorption at a wavelength of 443 nm.

Study optimal conditions

Different optimal conditions were studied for the purpose of obtaining high absorption and stability. To complete the measurements and obtain high sensitivity for the determination of hydrochlorothiazide, use 2.0 ml of hydrochlorothiazide hydrochloride solution at a concentration of 100 µg/ ml in a final volume of 25 ml, then the absorbance of the stained solution was measured at a wavelength of 443 nm.

1. A study of the effect of the amount of sodium nitrite with time

Different volumes of sodium nitrite solution at a concentration of 0.1450 M were taken with a hydrochlorothiazide solution of a concentration of 100 µg/mL and the resulting solution was placed in an ice bath for 5 minutes, then 1.0 ml of a meta-aminophenol reagent solution at a concentration of 9.16×10^{-4} M was added and the medium was made. Alkaline by adding a 0.25 M potassium hydroxide solution in a 25 ml volumetric vial. The prepared solutions were measured at a wavelength of 443 nm, and it was found that 0.5 ml of sodium nitrite is the best at a time of 5 minutes.

2. Study the effect of the amount of urea

This effect was studied by taking different volumes of 0.1 M urea to a solution of nitrogenous hydrochlorothiazide 100 µg/ml, in order to get rid of the remaining excess of sodium nitrite because the increase in nitrite causes side reactions that affect the absorption values of the solution, the results shown indicate that the addition of 0.25 ml of urea gave the highest absorption, so it was adopted in subsequent experiments.

3. Study the effect of detector size

A study was conducted on the effect of different amounts of meta-aminophenol reagent solution on the absorption of the resulting product. Volumes ranging from (0.1 – 2 ml) of the reagent were used at a concentration of 9.16×10^{-4} M. It gave the best absorption at a volume of 0.01 ml.

4. Study the type and size of the base

He studied the effect of different types and sizes of weak and strong bases on the absorption of the formed solution. It was found that potassium hydroxide with a concentration of 0.25 M gave the best absorption at a volume of 1.5 ml, so it was adopted in subsequent experiments.

5. Study the effect of surface active substances

This study included the effect of surfactants on the intensity of adsorption by adding different volumes of surfactants to an atomized hydrochlorothiazide solution, then adding 1.5 milliliters of 0.25 M potassium hydroxide solution. The following studies.

6. Study the effect of time on the stability of the reaction product

The effect of time was studied on the stability of the product formed by adding 1.0 ml of sodium nitrite solution with a concentration of 0.1450 M to 2.0 ml of hydrochlorothiazide solution with a concentration of 100 µg/ml and placing the resulting solution in an ice bath at a temperature of (0-5 °C) for 5 minutes, then add .01 ml of 0.1 M urea solution and add 1.0 ml of meta-aminophenol reagent solution with a concentration of 9.16×10^{-4} M, and the medium was made alkaline by adding .51 ml of a 0.25 M potassium hydroxide solution in a 25volume vial Then the absorption of the dye intensity formed during different periods of time was tracked against the mock solution at room temperature. It was found that the resulting dye solution has high stability.

Table 1: Summary of optimal conditions for estimation of HCTZ

Material solution	Concentration (M)	Optimum amount (ml)
m-amino phenol	9.1×10^{-4}	1.0
$NaNO_2$	0.1450	0.5
Urea	0.1	0.25
KOH	0.25	1.5
λ_{max} (nm)	443	
Color	Yellow	
Temp (C°)	Room temp	Temp (C°)
Stability Time	More than four days	

7. final absorption spectrum

Based on the pre-established optimal conditions, the final absorption spectrum of the nitrogenized hydrochlorothiazide solution was measured, as 1.0 ml of a meta-aminophenol reagent solution was added at a concentration of 9.16×10^{-4} M, then 1.5 ml of a potassium hydroxide solution was added with a concentration of 0.25 M, to form a yellow solution. The highest absorption at a wavelength of 443 nm against the mock solution reagent in Fig2.

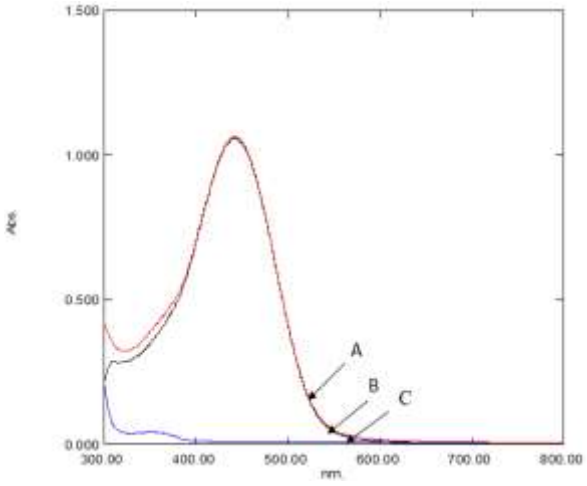


Figure. 2: Final absorption spectrum of 8 µg/25 mL of a nitrogenized HCTZ solution. (A): vs. mock solution, (B): vs. distilled water, (C): mock solution versus distilled water.

Method of work and preparation of the standard curve

The standard curve for the determination of hydrochlorothiazide was prepared using proven optimal conditions (Table 1). Increasing volumes (0.1 – 6.0 ml) were taken at concentrations (0.4-24 µg/ml) of a 100 µg/ml hydrochlorothiazide solution in 25 ml bottles and 0.5 ml of sodium nitrite solution was added at a concentration of 0.1450 M. The solution was left for 5 minutes on ice to complete the nitro enation process. Then add 0.25 milliliters of urea with a concentration of 0.1 M to get rid of excess nitrite, then add 1.0 milliliters of a meta-aminophenol reagent solution with a concentration of 9.16×10^{-4}M and add 1.5 milliliters of a solution of potassium hydroxide with a concentration of 0.25 M, complete the volume to the mark with water Then the absorbance was measured against its mock solution at a wavelength of 443 nm, The standard curve of the working method is shown in Figure (1), which follows Beer's law in the concentration range 0.4-20 µg/mL. It was shown through the value of the correlation coefficient that the linear specifications of the curve are good.

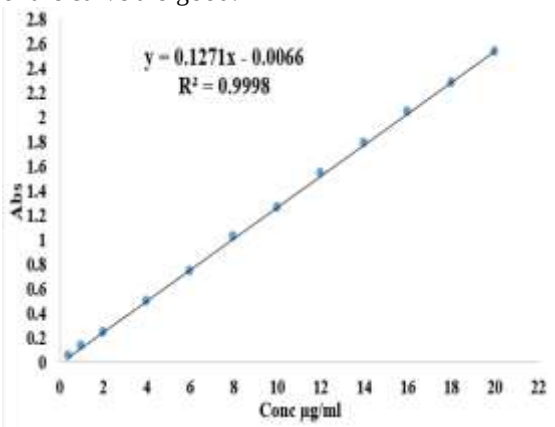


Figure. 3: Standard curve for the determination of HCTZ using nitrogenase and conjugation.

Accuracy and compatibility

Accuracy and compatibility were studied by measuring five replicates of three different concentrations (0.4, 2, 4 µg/mL) of a hydrochlorothiazide drug solution that fall within the limits of Beer's law for the standard calibration curves. The method was carried out using the approved method. The results shown in Table (2) show that The method has good accuracy and compatibility.

Table2: Accuracy and compatibility

C. Taken HCTZ µg/ml	C.Foud HCZ µg/ml	Recovery %*	RSD %*
0.4	0.401	100.2	1.395
2	1.996	99.8	0.448
4	4.007	100.175	1.562

*Average of five determinations

Study the nature of the resulting dye

1. method of continuous changes

The Job method[7] was applied to find out the interaction ratio between hydrochlorothiazide and a meta-aminophenol reagent by preparing the drug and the reagent at the same concentration (3.35×10^{-4} M), and from which hydrochlorothiazide solutions were prepared in increasing volumes of(0.25-1.5 ml), and in contrast volumes of reagent (0.25-1.5 ml), so that the total final volume is equal to 1.5 ml, the previously suggested optimal conditions were applied, then the solution was diluted with distilled water to the mark in bottles of 25 ml, then the absorbance was measured against the mock solution at a wavelength of 443 nm. Figure (4) shows the coupling ratio between hydrochlorothiazide and the detector is (1:1) (medicine: detector).

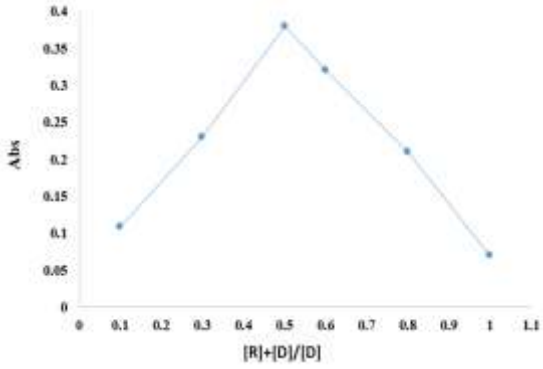


Figure. 4: Continuous changes curve of the resulting solution between HCTZ and a meta-aminophenol reagent.

2. Molarity method

This method was applied to confirm the actual ratio of the nature of the product formed[7] between hydrochlorothiazide and the reagent, as a number of volumetric vials containing 0.5 ml of a hydrochlorothiazide solution with a $^{-4}$ M) were prepared, ×10concentration (3.35 and at the same concentration a meta-aminophenol reagent solution was added to it

in volumes Increasing from (0.1 -1.0 ml), taking into account the addition of the rest of the solutions at the previously proven optimal conditions, then the absorbance was measured at a wavelength of 443 nm against the mock solution. It was found that the correlation ratio is (1:1) (drug: reagent) as shown in Figure (5).

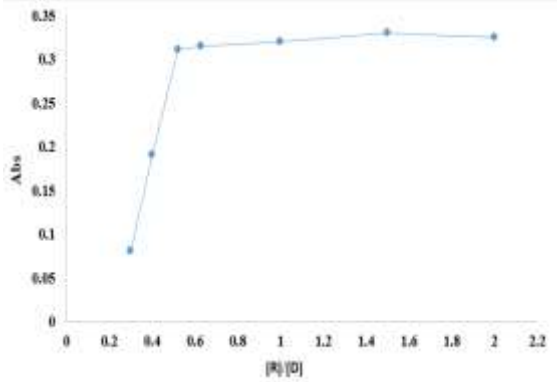


Figure. 5: Molar ratio curve of the resulting solution between HCTZ and meta-aminophenol reagent.

Application in pharmaceutical preparations

1. Direct method

The proposed method was applied to the pharmaceutical preparation hydrochlorothiazide, which is available in the form of tablets, by taking three different concentrations of the pharmaceutical solution. The method was applied depending on the previously proven optimal conditions. The average of five readings for each concentration was calculated against the mock solution, then the recovery ratio and the relative error were found. And the relative standard deviation as shown in Table (3).

Table3: Estimation of HCTZ in pharmaceuticals

Amount Taken HCTZ µg/ml	Amount Foud HCZ µg/ml	Recovery % *	RSD % *
0.4	0.398	99.65	1.077
2	1.950	97.51	0.465
4	3.993	99.82	0.571

*Average of five determinations

2. Standard addition method

For the purpose of evaluating the efficiency of the developed method for the determination of hydrochlorothiazide in its pharmaceutical preparations, the standard addition method was applied by taking constant volumes (0.25, 0.5 ml) of pharmaceutical solutions with a concentration of 100 µg/mL and taking increasing volumes (0.25-4.5 ml) of hydrochlorothiazide standard solution in vials. Volumetric capacity of 25 ml. The above solutions were treated according to the pre-established optimal conditions, and the results in Figure (6) show that the standard addition method is well compatible with the proposed method.

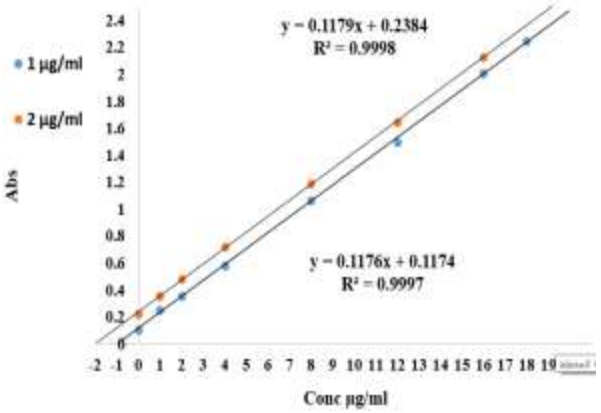


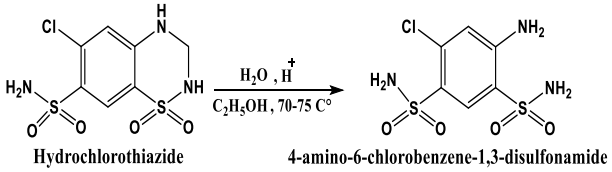
Figure. 6: Standard addition curve for the determination of HCTZ in pharmaceutical formulations

Table4: Standard addition to the proposed working method

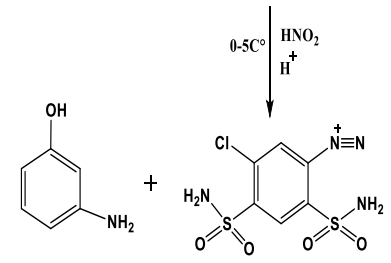
Amount Taken HCTZ µg/ml	Amount Foud HCZ µg/ml	Recovery % *
1	0.998	99.8
2	2.022	101.1

proposed reaction mechanism

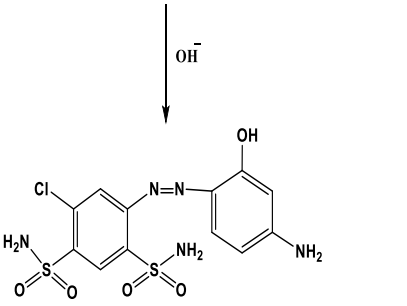
Through this study, we conclude that the proposed chemical formula resulting from the coupling of azotized hydrochlorothiazide with a meta-aminophenol reagent is as follows[8]:



Hydrochlorothiazide — 4-amino-6-chlorobenzene-1,3-disulfonamide



Meta amino phenol — Dizonium Salt



4-((4-amino-2-hydroxyphenyl)diazenyl)-6-chlorobenzene-1,3-disulfonamide

Compare the proposed method

The proposed analytical method for the determination of hydrochlorothiazide was compared with some parameters of another spectrophotometric method, and the results were recorded in Table (5).

Table5: Compare the proposed spectroscopic method with another method

Parameter	Present method	Literature method[9]
Reagent	m-aminophenol	O-phenylene diamine
Color of the product	yellow	Orange
λ_{max}	443	450
Medium of reaction	Acidic	Acidic
Beer's law range (µg/ml)	0.4-20	6-36
Molar absorptivity (L/mol.cm)	3.7843×10^4	6.96045×10^3
Sandell sensitivity (µg/cm^2)	0.00786	0.057
Slope	0.1271	0.0175
Intercept	0.0066	0.3107
Correlation coefficient(R)	0.9998	0.9917
LOD (µg/ml)	0.0226	0.0829
LOQ (µg/ml)	0.0754	————
Ave RSD%	1.135	————
Ave Recovery %	100.058	99.11
Stability of Colour (min)	More than four days	60

Conclusions

A simple spectroscopic method was developed for the determination of hydrochlorothiazide based on the reaction of diazotization- coupling in an acidic medium and its coupling with a meta-aminophenol reagent in a basic medium. A yellow dye was formed that gave the highest absorption at a wavelength of 443 nm and with concentrations ranging from 0.4-20 µg/ml. The molar absorption is 3.7843 x 10^4 L/mol. cm, and the recovery rate was 100.058% with a relative standard deviation of 1.135%. The sensitivity of Sandel was 0.00786 µg/cm2, the detection limit was 0.0226 µg/mL, and the quantitative limit was 0.0754 µg/mL. followed.

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