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Spectrophotometric determination of hydrochlorothiazide using charge transfer interactions with alizarin red s reagent

Ikhlass Thanone Al-Allaf [1], Nabeel Sabeeh Othman[2*], Amer Thanoon Al-Taee[3]
[1,2,3] Department of Chemistry, College of Science, University of Mosul, Mosul, Iraq

[*]*Corresponding author: nsn20002004@uomosul.edu.iq*

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Abstract:
The present work included using transferred charge complex reaction in the assay of Hydrochlorothiazide using Alizarin Red S as an electron acceptor. The method is based on direct spectrophotometric estimation of hydrochlorothiazide by its reaction with Alizarin Red –S in absolute methanol media through forming a red charge transfer complex solution that gives a maximum absorption at 537 nm. The relation between concentration and absorbance was linear within the concentration range of 0.1-2.5 µg / ml with the molar absorption coefficient equal to 1.2774×10^5 l. mol^{-1}. cm^{-1}. The method was successfully applied to estimate hydrochlorothiazide in its pharmaceutical preparations (tablets)**.**

Keywords: Hydrochlorothiazide, Alizarin Red S, Charge transfer, Tablets

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Introduction

Hydrochlorothiazide (HYD) belongs to the class of thiazide diuretics that are used in cases of Edema (people with congestive heart failure) to eliminate an excess of water, HYD relieves fluid retention in the stomach, and continuing kidney failure. The most excellent common use is to treat high blood pressure (Hypertension), and kidney stones can be treated with HTZ [1].

HTZ is a white crystalline powder, odorless, and solubility in water at 25 ° C is very slight. It dissolves in alcohol and acetone and is also dissolved in dilute alkaline medium ammonia, and sodium hydroxide solutions, and it is insoluble in an acidic medium. HTZ has the chemical structure as illustrated in Figure 1[2].

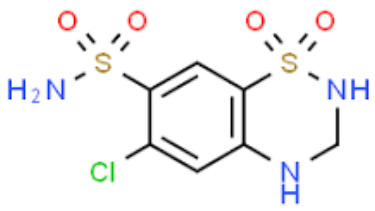

Figure 1: The chemical structure of Hydrochlorothiazide.

The transferred charge complexes are formed as a result of the interaction between an electron donor compound (n-donor) and the other electron acceptor (acceptor) through electrostatic attraction, which leads to the stability

of the complex. The complexes of the transferred charge give strong colors [3,4]. The charge transfer interactions were used in the determination of numerous drug and organic compounds using different reagents.
Alizarin (ALI-R) is an organic compound of anthraquinone compounds. It was used as a natural dye to color fabrics and textiles and has numerous names, the most well-known are Alizarin Brilliant Blue R, Alizarin Cyanine Green G, and Alizarin Cyanine Green and Alizarin Red S it has the chemical formula is C14H7NaO7S and chemical structure as shown in Figure2[5].

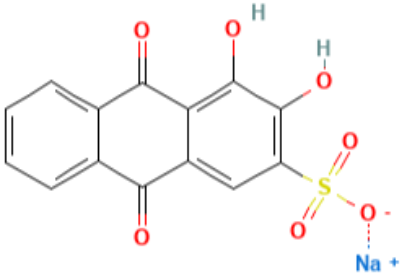

Figure 2: The structure of Alizarin Red S.

Various methods mentioned in the literature survey used in the assay of HYD these methods included spectrophotometric determination using UV and visible regions [6-10], electrochemical method using DPV, SWV [11], DPV [12,13], CV/ DPV[14,15], various types of chromatographic techniques used in the determination of HYD in presence of other drugs, high-performance liquid chromatography [16], reverse phase high-performance liquid chromatography [17-19], LC /Mass/Mass [20], HPTLC[21], TLC[22], UHPLC/MS/MS [23]. Fluorescence technique [24]. Capillary zone electrophoretic [25,26], and flow injection analyses was also used [27].

Experiment

Devices used:
All spectral measurements were carried out using a double beam device of the type Jasco V-630, UV-Vis spectrophotometer (Japanese origin), and glass cells with a thickness of 10 mm were used, and a sensitive balance of type ABS 120-4 by Kern and Sohn, (German manufactured by GmbH), and measurements were made of the pH of the solutions using the device of type BP3001.

Chemicals
The chemicals and the analytical reagents used were of high purity.

Solutions
Hydrochlorothiazide solution 100 µg/ml (HYD).
This solution was prepared by dissolving 0.0100 g of pure hydrochlorothiazide [produced by the Chinese company (SINOPHARM) (China National Pharmaceutical Industries Group (CNPGC) and supplied by Samarra Pharmaceutical Company], in 20 mL of methanol, and then the volume is completed to 100 mL with the same solvent using 100 mL -volumetric flask,
Hydrochlorothiazide solution 10 µg/ml: prepared by diluting 10 mL of a 100 µg/mL solution to the mark of a 100 mL volumetric flask with methanol. Alizarin Red S (ALI-R) solution (0.05 W/V%). This solution was prepared by dissolving of 0.0500 g of pure Alizarin Red-S(BDH) in 100 ml of methanol in a volumetric flask. It was kept in a shaded container and it was stable for a month.
Pharmaceutical preparation solution HCT (100 µg / mL).
Two types of tablets from various sources, the German company T & D pharma GmbH, as each tablet contains 25 mg of HYD, by measuring the average weight of 10 tablets, which is 161.8 mg, crushing and mixing well, and weighing from powder the equivalent amount to 0.0100 g of pure HYD and add 20 ml of methanol and shake the solution well and complete the volume to 100 ml with the same solvent, and in order to obtain the working solution at a concentration of 10 µg / ml take 10 ml of the previous solution and dilute to 100 ml with methanol.
Pharmaceutical solution (Hydrazide Awa) tablets (100 µg / ml). The pharmaceutical preparation is produced by the Iraqi Awa medical company, as each tablet contains 50 mg of HYD. The solution was prepared by taking 10 tablets crushing and mixing them well, the average weight is 161.8 mg, weighing equivalent to 0.0100 g of pure HYD, and completing its preparation as in the previous preparation.

Results and discussion

The general principle of the reaction

The interactions of the formation of transferring charge complexes or the so-called electron-donor-acceptor complexes depend on the presence of electron-donor atoms that have the ability to participate in them and an electron-acceptor system such as the double π bonds in the aromatic compound.

Optimal conditions for the reaction

10 µg of HYD in a final volume of 10 mL was used for subsequent experiments and the absorbance of the solutions against their blank solutions was measured.

Studying the effect of pH

The pH of HYD dissolved in methanol was measured and the reading was 7.30, after adding 2.5 mL of ALI-R reagent, the pH converted to 7.0. For the purpose of knowing the optimal acidity of the reaction, various volume of hydrochloric acid solution (0.001 M) or sodium hydroxide (0.001 M) were added. The results are shown in Table (1) and Figure (2).

Table 1: Effect of the pH value on the formation of the colored product.

pH Value	Absorbance
3	0.021
5	0.141
6	0.347
6.5	0.579
6.8	0.642
Without addition 7.0	**0.667**
7.5	0.657
8.0	0.584
8.5	0.473
9.0	0.253
9.5	No. color contrast

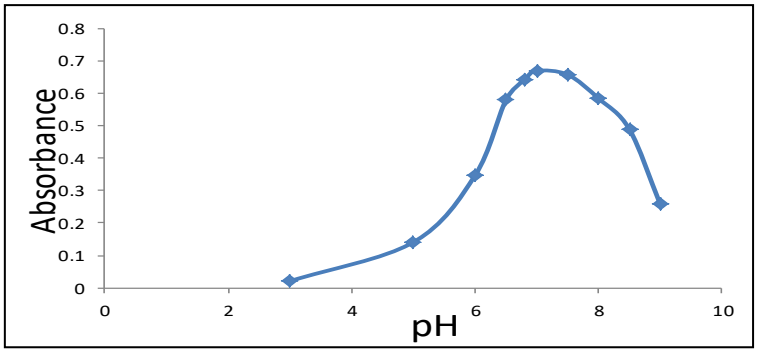

Figure 3: The effect of the pH value on the composition of the colored product.

From the observation of the above results in Table 1 and Figure 3, it is clear to us that the pH value of 7.0 gives the highest absorbance. Therefore, buffer solutions of pH 7.0 were prepared and 1.0 ml of each one was added, and its effect on the formation of the resulting product was studied(Table 2).

Table 2: The effect of the buffer solution on the formation of the colored product.

Buffer Solution pH7	Absorbance
Borate	0.066
Acetate	0.481

Citro	0.501
Phosphate	0.201
Without buffer	**0.658**

From observing the results recorded in Table 2, it is clear that the buffer solutions negatively affect the absorbance of the colored product, therefore, it was not recommended for use in subsequent experiments.

Studying the effect of the amount of reagent

The effect of the amount of ALI-R on the absorbance of the colored product was studied by adding increasing volumes of the reagent solution 0.5-3.0 ml to different concentrations 0.25-2.0 µg of HYD/ mL and waiting for 5 minutes at room temperature and completing the volume with methanol to the mark of 10-ml flask, and the absorbance was measured for these solutions against blank solutions at a wavelength of 537 nm, as shown in Table 3.

Table 3: The effect of the amount of reagent on the absorbance of the colored product.

ml of ALI-R 0.05%	**HYD µg/ml Absorbance**					**R^2**
	0.25	**0.5**	**1.0**	**1.5**	**2**	
0.5	0.039	0.127	0.189	0.258	0.293	0.9451
1	0.169	0.240	0.460	0.577	0.642	0.9806
1.5	0.251	0.399	0.516	0.706	0.838	0.9768
2.0	0.223	0.366	0.586	0.896	1.020	0.9848
2.5	**0.335**	**0.445**	**0.678**	**0.884**	**1.172**	**0.9930**
3.0	0.244	0.394	0.541	0.869	1.110	0.9883

It was found from the results recorded in Table 3 that the highest absorbance was given by the product when using 2.5 mL of ALI-R solution, in addition to the highest value of the determination coefficient and this volume, which was adopted in subsequent experiments.

Studying the effect of time on product formation

To find the required time for the completed reaction, an experiment was done by adding 2.5 ml of ALI-R reagent to 1.0 ml of a 10 µg of HYD solution and following the formation time of the colored product (Table 4).

Table 4: The effect of time on the composition of the colored product.

Time (min)	**Absorbance**
Immed.	0.589
2	0.608
5	0.663
10	**0.668**
12	0.664
15	0.668

The results showed that 10 minutes before filling the volume with methanol was sufficient to complete the reaction, and it gave the highest absorbance, so it was recommended to leave the solutions for 10 minutes in the subsequent experiments.

Study the effect of solvents

The effect of a number of different solvents on the resulting product was studied by taking a series of volumetric flasks, taking 1.0 ml in a 10 µg of HYD, adding 2.5 ml of ALI-R reagent, waiting for 10 minutes to

complete the reaction, then diluting the reaction product for these solutions, and measuring the absorbance of the product, as shown. Figure 4 and Table 5.

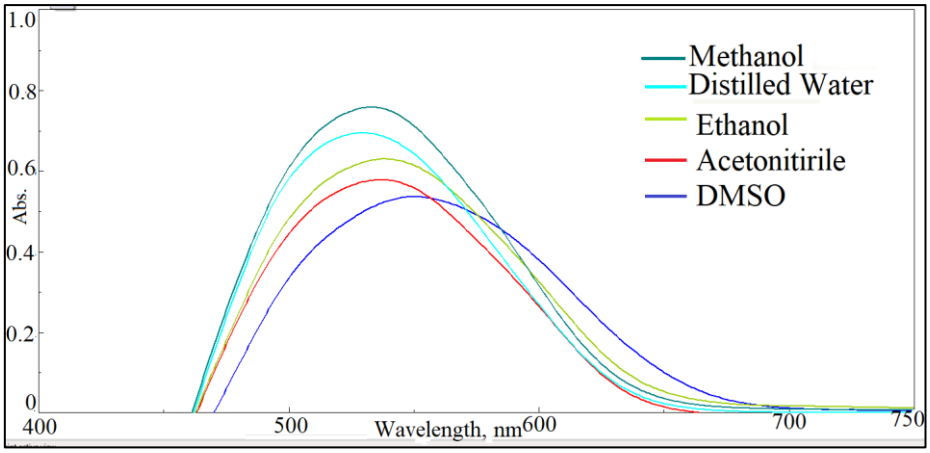

Figure 4: Absorption spectrum of the colored product formed using different solvents.

Table 5: Effect of different solvents.

Solvent	λ_{max}, nm	Absorbance	l.mol^{-1}.cm^{-1}
Water	533	0.693	2.064x10^5
Acetone	Turbid		
Methanol	537	0.757	2.254 x10^5
Acetonitrile	537	0.649	1.932 x10^5
DMSO	550	0.535	1.592 x10^5

From the results shown in Table 5, indicated that methanol gives the highest absorption of the product and water in the second degree, so the stability of the product formed in methanol and water was later studied. Colored product stability

The effect of time on the stability of two different concentrations of HYD in methanol and one concentration in distilled water was studied, and the results are shown in Table 6.

Table 6: The effect of time on the stability of the colored product.

Time, min.	Absorbance/µg of HYD/ml		
	With methanol		With D.W.
	0.5	1.0	1.0
2	0.4370	0.7040	0.6914
5	0.4633	0.7102	0.6874
10	0.4725	0.7492	0.6873
15	0.4797	0.7538	0.6833
20	0.4801	0.7609	0.6772
25	0.4879	0.7627	0.6591
30	0.4880	0.7630	0.6481
35	0.4871	0.7638	0.6473
40	0.4874	0.7643	0.6293
45	0.4869	0.7640	0.6188
5	0.4884	0.7643	0.6014
55	0.4873	0.7641	0.6010
60	0.4875	0.7644	0.5903

The results in Table 6 indicate that the colored product gives an absorbance reading that indicates analytically acceptable stability, two minutes after completing the additions and remains stable for an hour if methanol is used as a solvent to complete the dilution, but if water is used to supplement the volume, the results indicate inconsistent stability.

Final absorption spectrum

When mixing 1.0 ml of a 10 µg HYD solution with 2.5 ml of ALI-R reagent (0.05%), optimal conditions were applied, and the volume was supplemented with methanol to form a red color product that gives the highest absorption at the wavelength of 537 nm compared to the blank solution, which gives little absorbance (0.0905) at the maximum wavelength of absorption. versus methanol at the same wavelength, as in Figure 5.

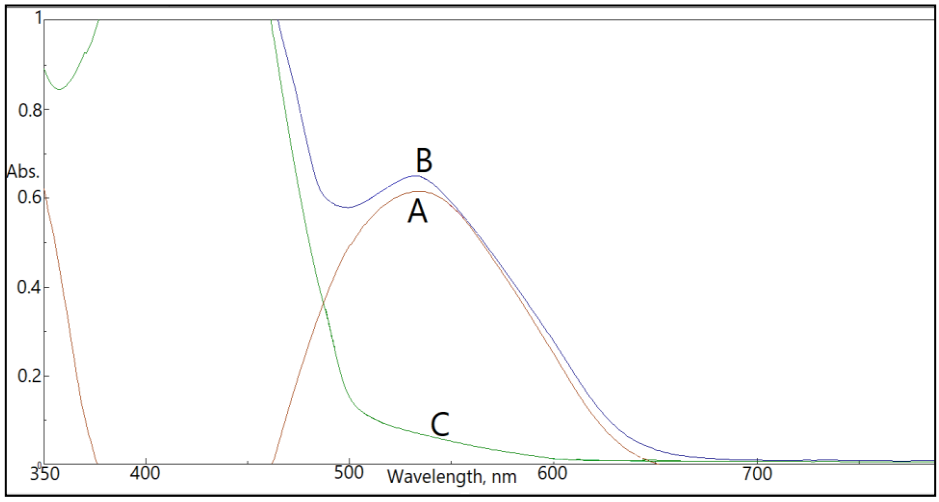

Figure 5: Absorption spectrum of 10 µg/mL of HYD as measured by (A) versus blank solution, (B) versus methanol, and (C) blank versus methanol.

Procedure and standard curve

After establishing the optimal conditions empirically for estimating HYD, the calibration curve for the present method was prepared as follows: Increasing volumes of 0.1-2.5 mL of a 10 µg/mL HYD solution were added to a series of 10 mL volumetric flasks, then 2.5 mL of ALI-R solution was added, waited 10 minutes at room temperature. Then after dilution with methanol, the absorbance of the colored solutions was measured against the blank solution at the wavelength of 537 nm, as in Figure 6. The standard straight curve, which is in accordance with Beer's Law in the range of concentrations 0.1-2.5 µg HYD / ml, the value of the molar absorption coefficient of the product was found to be 1.2776×10^5 l mol^{-1}. cm^{-1} and Sandell's index value was 0.002330 µg. cm^{-2} The values of the limit of detection and limit of quantification (LOD and LOQ) were 0.0112 and 0.0373 µg/mL, respectively.

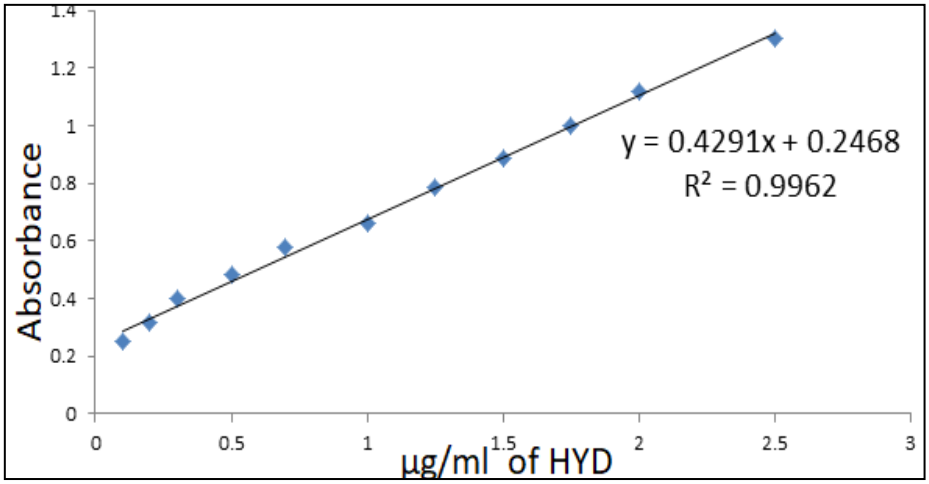

Figure 6: Standard curve for HYD estimation using the charge-transfer interaction with the ALI-R.

The nature of the resulting product

The continuous variation method (Job's method) was used to find out the ratio of the HYD reaction with the ALI-R reagent. As it is clear from Figure 7, the coupling ratio of HYD with RLI-R reagent is 1:1. For verifying this ratio, the mole ratio method was applied, and Figure 8 confirms that the reaction ratio is 1:1

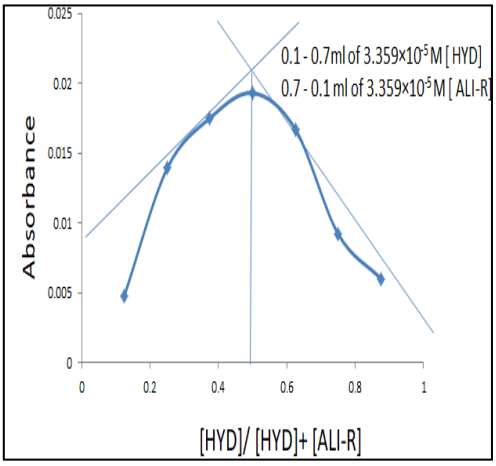

Figure 7: Continuous variation curve.

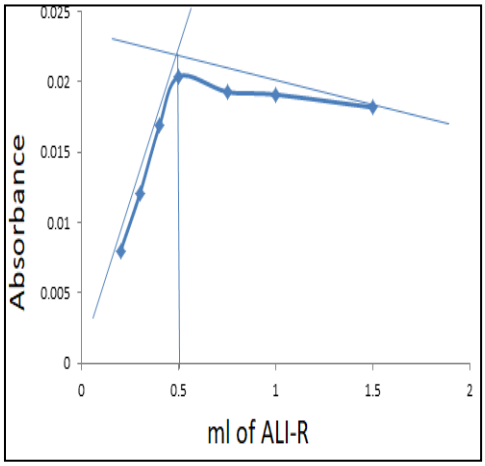

Figure 8: Molar ratio curve.

Accordingly, to the results shown in Figures 7 and 8, the structure of the colored product is as follows, as shown in Figure 9.

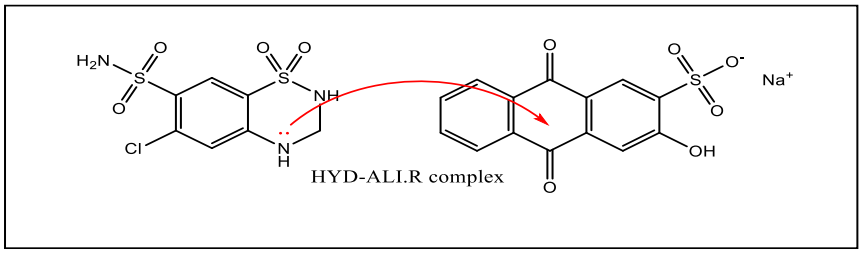

Figure 9: Complex charge transfer diagram between HYD and ALI-R.

Application of method

The proposed method was applied in the estimation of HYD in the pharmaceutical preparations by taking different concentrations of 0.25, 0.5, 1.0, and 1.5 µg/ml of drug solutions and performing the pre-established optimization steps that represent optimal conditions and the recovery percentage is well calculated, which indicates that the method has good selectivity (Table 7).

Table 7: Results of application of the proposed method in pharmaceutical preparations.

Drug	µg HYD taken / ml	µg HYD measured /ml	Recovery* %	Average reading %	Drug contain measured, mg/tab.
CHT T&D pharma 25mg/tab. GmbH/Germen	0.25	0.240	96.00	98.46	24.61
	0.5	0.497	99.40		
	1.0	0.976	97.60		
	1.5	1.504	100.26		
HydraziAwa 50mg/tab. Awamedica, Erbil, Iraq	0.25	0.246	98.40	99.12	49.56
	0.5	0.496	99.20		
	1.0	1.001	100.10		
	1.5	1.479	98.60		

Method comparison

The comparison of the method was based on a t-test to find out the degree of agreement between the proposed method and the standard method adopted in the British Pharmacopoeia by calculating the recovery percentages of five samples of pharmaceutical preparation solutions (HYD) under study (10 µg), using the known relationship [28] to find the experimental t value, as in the results shown in Table 8.

Table 8: Comparison of the proposed method with the standard method.

Drug	Recovery * %		t.exp.
	Present method	Standard method [29]	
CHT T&D pharma, 25mg/ tab. GmbH/Germen	99.98	99.16	0.525
HydraziAwa, 50mg/ tab. Awamedica, Erbil,Iraq	101.2	100.8	0.641

***Average of five determinations.**

The results in Table 8 indicate that the calculated experimental t values are less than the tabular t value established in the statistical tables at a 95% confidence level for eight degrees of freedom. This value indicates that the difference is not significant between the standard method and the proposed method, which indicates that the present method has good applicability to pharmaceutical preparations.

Conclusions

The present method was characterized by simplicity, speed, and good sensitivity. The method depends on reacting hydrochlorothiazide with the reagent Alizarin Red S in a neutral medium to form a complex of the transferred charge, in a methanolic medium, stable, and gives the highest absorption at the wavelength of 537 nm, and follows Beer's law in the concentration range 1.0-2.5 µg / ml. The value of the molar absorptivity is 1.2774×10^5 l. $mol^{-1}.cm^{-1}$ and Sandell's index 0.002330 $\mu g.cm^{-2}$. The method was successfully applied to determine hydrochlorothiazide in tablets of different origins.

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