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Using of 2-Aminothiazole Diazotised in Spectrophotometric Estimation of Cefadroxil in Dosage

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Abstract
A sensitive and simple spectrophotometric method was proposed to estimate cefadroxil in pure and capsule form. The colored product was achieved in two steps, the first step included diazotization of 2-aminothiazole with acidic NaNO2 at 0-5°C and the second step included coupling of diazotization 2-aminothiazole with cefadroxil, the orange-colored azo dye has a maximum absorption at a wavelength of 503 nm. Beer's law was obeyed at ranging of concentrations from 1 to 12.5 µg/ml with a determination coefficient of 0.9945. A stoichiometric ratio of the reaction of diazotized 2-aminothiazole with cefadroxil was 1:1. The application for determination of cefadroxil in dosage form (capsule) via the suggested method gave accepted analytical results.

Keywords: Cefadroxil, Diazotization, Coupling, 2-Aminothiazole, Spectrophotometric

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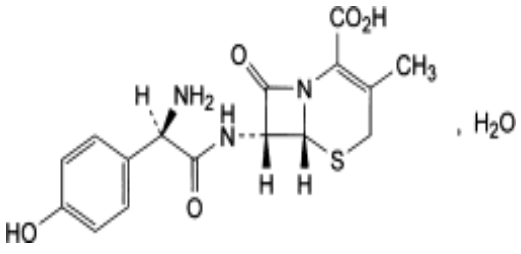
Introduction

Cefadroxil is one of cephalosporin drugs group. It has many uses one of them the being treatment of contagions which resulted largely from Gram-positive bacteria. It has the following structure as shown in figure 1 (Marco. and Salgado,2017; British Pharmacopeia, (2013) [1,2].



$C_{16}H_{17}N_3O_5S, H_2O$ M.Wt=381.4 g /mol

Figure 1: The chemical formula and chemical structure of cefadroxil.

Many different techniques used in the assay of cefadroxil were published in the literature. One of these techniques included voltammetric, using square wave adsorptive stripping [3], glassy carbon as a modified electrode [4], normal phase and reverse phase liquid chromatography [5-7], also fluorescence [8], Electrochemical [9], and flow injection [10] techniques have been used in the assay of cefadroxil, although the above techniques are accurate

and sensitive, the devices are expensive and the possibility of their presence in all laboratories are difficult. Therefore, researchers tended to use the spectroscopic technique, as the spectrometer is cheap and accessible to most researchers, and also the availability of many organic and inorganic reagents. The method below included using various reagents in spectrophotometric determination of cefadroxil, 2, 4-dinitrophenylhydrazine [11], Lawsonia inermis (Henna) as a natural reagent [12], 1,10-phenathroline (method I) or 2,2`-bipyridyl (method II) [13], and sodium hydroxide [14], and also a green analytical method has been used [15].

This present work included a suggestion for a spectrophotometric procedure for assay Cefadroxil in its pharmaceutical dosage(capsule) via a diazo-coupling reaction using the diazotized 2-thiazole.

Experiment

Apparatus

Spectral measurements and readings of absorbance were achieved by using a JASCO-360 (Japan) spectrophotometer, with two glass cells (1 cm light path), BEL-Sensitive balance, and BP3001 pH-meter were used in the present investigation.

Chemical and Solutions

All chemicals used in the present work were of analytical grade, The drug in pure form was given by General Establishment for Medical Appliance and Drugs / SDI, and other reagents from Fluka company.

Cefadroxil solution, 100 µg/ml

The cefadroxil solution was prepared by dissolving 0.0100 g of cefadroxil in warm distilled water and the volume was completed to100 ml in a calibrated flask with the same solvent.

2-Aminothiazole solution, 2.5 x 10-3 M

This solution was prepared by dissolving 0.0250 g of 2-aminothiazole in 10 ml distilled water and 5 ml of concentrated HCl, then the mixture was transferred to a 100 mL volumetric flask, and another 70 ml distilled water was added before cooling in an ice bath to 0-5°C, After that 0.0170 g of sodium nitrite (dissolved in 10 ml distilled water) gradually with stirring was added and then the volume was completed to 100 ml with cold distilled water and cooled the solution for five minutes at 0-5°C, transfer the diazotized reagent to a darkened container and used after leaving it at room temperature for 1.5-2 hours, this solution was prepared daily.
Sodium hydroxide (Approximately 2M) was prepared by dissolving 40 g of NaOH in 500 ml of distilled water.

Pharmaceutical preparation (Capsules form), 100µg/mL

The contents of 3 capsules of cefadroxil (500 mg/capsule) (Tabuk company-Kingdom of Saudi Arabia), were weighed and mixed well, and a weight of 0.0116 g of the capsule powder equivalent to 0.0100 g of pure cefadroxil was taken, and dissolved in 100 ml of warm distilled water in a calibrated flask.

General procedure and calibration curve

Added 0.1-1.25 ml of 100 µg/ml cefadroxil solution to a series of 10 ml volumetric flasks, then 2 ml of the diazotized reagent 2-aminothiazole solution and 3 ml of 2M NaOH were added. The volume was completed to the mark with distilled water and the absorbance of the colored azo dye solutions was measured at 503 nm against the blank. A linear calibration graph was gained over a concentration of 1-12.5 µg cefadroxil /ml, as shown in Figure (2) with molar absorptivity 5.1 x 103 $L.mol^{-1}.cm^{-1}$

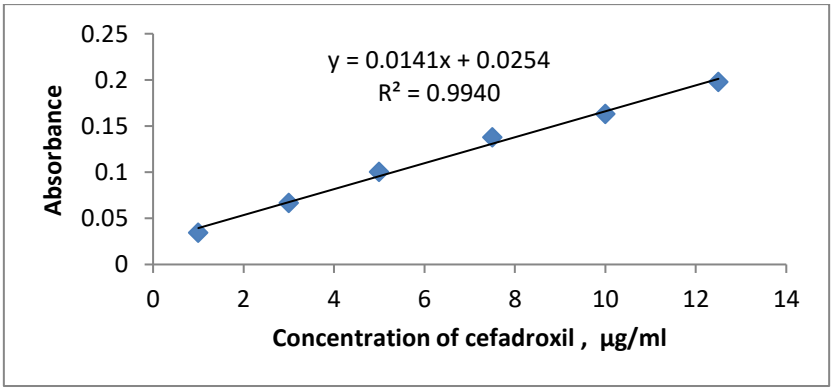


Figure 2: Calibration graph for cefadroxil determination via the suggested method.

Results and Discussion

All factors which affected the intensity of the formed colored azo dye were studied by varying the factor under study and fixing the other factors and the optimal conditions were selected.

Effect of diazotized 2-aminothiazole amount:

Different volumes from 1.5 to 2.5 ml of diazotized 2-aminothiazole 2 x 10-3M were added to a series of 10 ml - volumetric flasks which contained different volumes of cefadroxil 0.25-2.0 ml of 100 µg cefadroxil /ml standard solution i.e., 2.5-20 µg/ml, The results as shown in Table 1.

Table 1: Effect of diazotized 2-aminothiazole amount on the colored azo dye.

2-Amino-thiazole (2.5x10-3 M)	Absorbance / µg of cefadroxil / ml						
	2.5	5	10	12.5	15	17.5	20
1.5	0.0176	0.0371	0.0580	0.0592	0.066	0.0751	0.0863
2.0	0.0495	0.0962	0.1518	0.1715	0.1894	0.2053	0.2275
2.5	0.0463	0.0828	0.1278	0.1570	0.1747	0.2047	0.2157

From the results in Table 1, 2 ml gave the highest absorbance for the formed azo dye, so 2 ml had been selected in the next experiments.

Effect the type and amount of base:

Different types of bases have been used as a medium of reaction, sodium hydroxide gave the highest absorbance, Therefore NaOH was fixed in the next experiments, The results as shown in Figure 3.

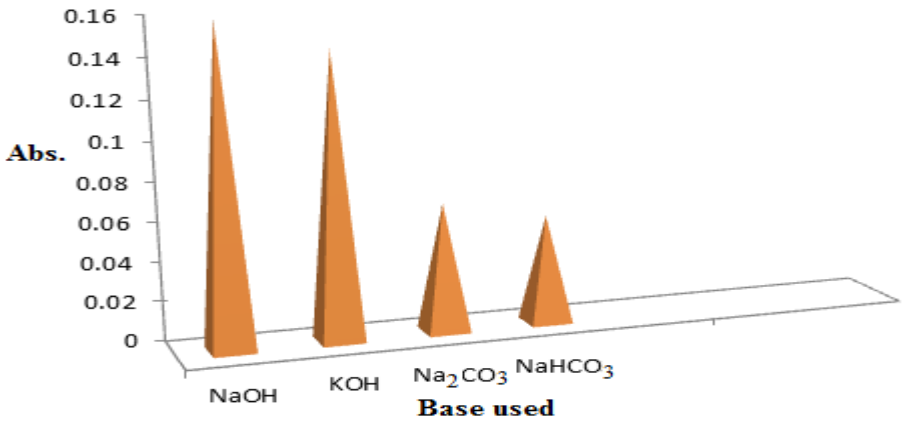


Figure 3: The effect of the base types.

The results in Figure 3 show that sodium hydroxide was the optimal base according to the highest absorbance. Also, the optimal volume of NaOH has been studied by adding volumes from 2 to 4 ml of sodium hydroxide (2M) to various volumes of 0.25-2.0 ml of cefadroxil and 2 ml of diazotized 2-aminothiazole (2 x 10-3 M). The results were fixed in Table 2.

Table 2: Effect of the amount of sodium hydroxide on absorbance.

NaOH (2M)	Absorbance / µg of cefadroxil / ml						
	2.5	5	10	12.5	15	17.5	20
2	0.0377	0.0640	0.0894	0.1310	0.1477	0.1546	0.1620
3	0.0441	0.0964	0.1598	0.1799	0.2000	0.2179	0.2164
4	0.0371	0.0596	0.0875	0.1060	0.1198	0.1511	0.1852

The results in Table 2 show that 3ml of sodium hydroxide (2M) was the optimal volume, It gave the highest intensity of the colored azo dye, so it was recommended to use it in the next experiments.

The order of addition

To know the best sequence of the addition for the components of the reaction, different experiments have been done, The results showed that Cefadroxil + diazotized 2-aminothiazole (R)+ base(NaOH) gave the highest absorbance (as shown in Table 3).

Table 3: The sequence of addition.

Component of reaction	Number of orders	Absorbance
Cefadroxil + R + NaOH	1	0.1560
R + NaOH + Cefadroxil	2	0.0284
NaOH + Cefadroxil+ R	3	0.0421

Effect of solvents

The effect of various solvents used in dilution such as ethanol, methanol, acetone, acetic acid, and distilled water has been studied. The results indicated that in using methanol and acetic acid turbid solutions have appeared. Ethanol gave the highest absorbance as illustrated in figure 4 and table 4.

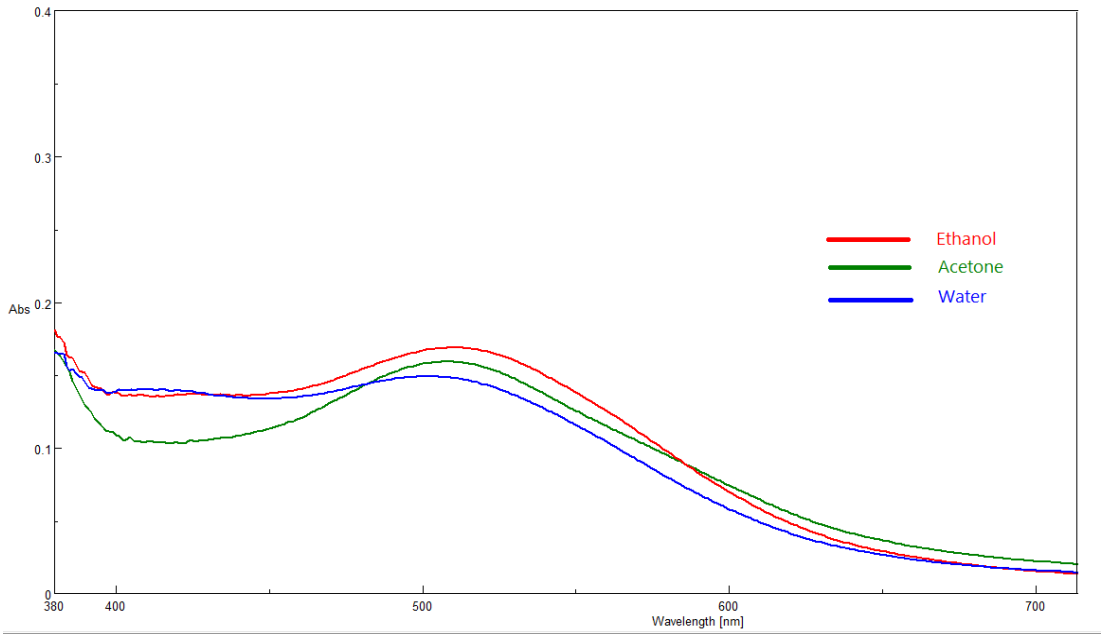


Figure 4: Absorption spectra of azo dye by using different solvents.

Table 4: The effect of solvents.

Solvents		Absorbance	max (nm) λ	ε l/mol.cm
A	Ethanol	0.1620	511	6.1x103
B	Acetone	0.1529	507	5.7x103
C	Water	0.1467	503	5.3x103

According to its availability, ease of use, and safety of distilled water, based on the advantages of water that I mentioned above, use of water was kept in dilution.

Study of the time affecting the stability of the orang azo dye

The effect of time on the formed dye was studied by taking a fixed amount of cefadroxil and it was modified according to the proposed method. Absorbance was measured at different time periods. The results obtained indicate that the dye is stable for a period of not less than 60 minutes.

The final absorption spectrum of the orange azo dye

Using the optimal conditions previously proven (Table 5), 2 ml of the diazotized reagent 2-aminothiazole was added to volumetric flasks of 10 ml containing 1 ml of the prepared drug solution (cefadroxil), then 3 ml of the base (NaOH) was added, the volume was completed to the mark with distilled water, where a spectrum of the azo dye was taken against the blank solution, and also the absorption spectrum of the blank solution against distilled water was done, as shown in Figure 5.

Table 5: Ideal conditions for the proposed method.

Optimization condition	The values or selected materials
Reagent used	2-Aminothiazole
Amount of reagent(mL), Molarity	2, 2.5x10-3
Base used, Molarity, mL	NaOH,2,3
Solvent used	Distilled water
λmax (nm)	503

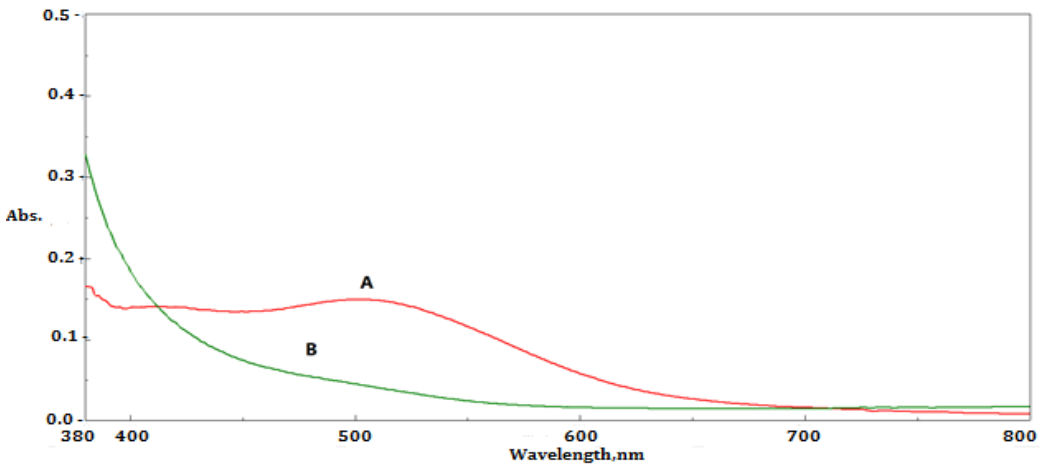


Figure 5: The final absorption spectrum of the formed azo dye vs blank solution(A) and the blank solution vs. distilled water(B).

Stoichiometric ratio

Job's (continuous variation) method was used via using equimolar solutions of diazotized 2-aminothiazole(D-2ATE) and cefadroxil (CEF) solution (2.5 x 10-3 M). To a series of 10-ml volumetric flasks, different volumes of the CEF were added from 0.5 to 2 mL. Different volumes of D-2ATE were added so that the final volume of the two components in all volumetric flasks is equal (the same), then 3 ml of NaOH (2M) was added, the volume was completed with distilled water, and the absorbance had been measured at 503 nm. As shown in figure 6 the ratio of the formed azo dye is 1:1 of the CEF: D-2ATE. The mole ratio method is also done by adding various volumes of 0.25- 2.0 ml of the D-2ATE to 1 ml of the CEF solution in the same concentrations (2.5 x 10-3M). The results in Figure 7 prove the ratio resulted from the application of Job's method.

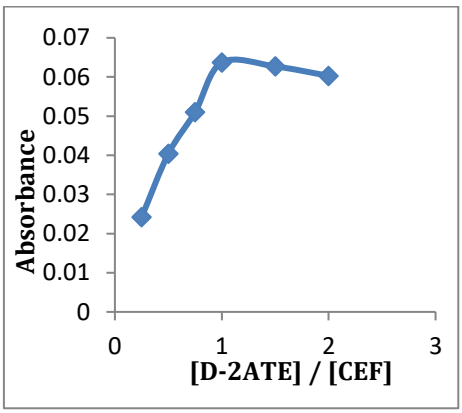


Figure 6: Plot according to Job's method

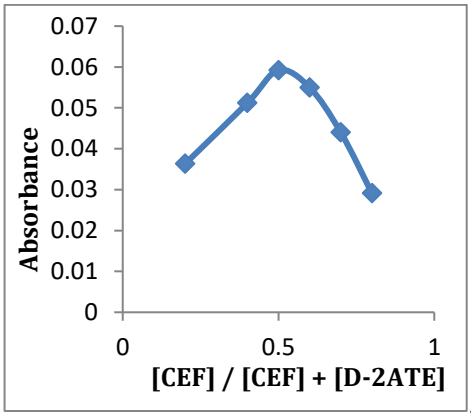


Figure 7: Plot according to mole ratio method.

Therefore, according to the obtained results fixed in figures 6 and 7 the suggested structure of the orange azo dye is as in Figure 8.

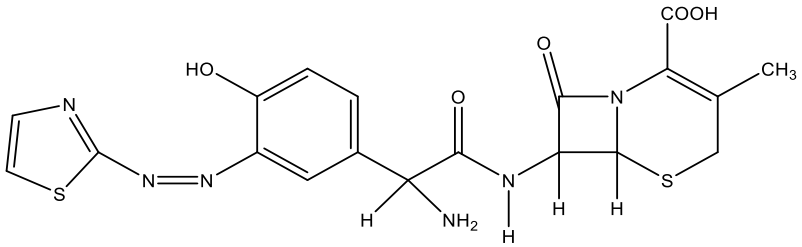


Figure 8: Structure of orange azo dye.

Analytical application:

The proposed method is applied to estimate cefadroxil in capsule form. On applying the proposed procedure good results of recoveries, accuracy (expressed by Er%), and precision (expressed by Relative Standard Deviation, RSD%) are obtained, as shown in Table 6.

Table 6: Analytical application for pharmaceutical in dosage form (capsule).

Pharmaceutical preparation	Amount taken µg/ml	Amount measured µg/ml	Recovery* %	Error %	RSD %	Drug contains (mg)
Cefadroxil Capsule (5mg, Tabuk)	5	5.13	102.60	2.60	2.6405	5.13
	10	10.09	100.90	0.90	0.5104	5.04

Conclusion

A sensitive, simple, and economical proposed method based on the coupling of cefadroxil with the diazotized 2-aminothiazole reagent in an alkaline medium was suggested. The method is successfully used for the assay of cefadroxil in it is formulation (capsule), and the results indicated that there is no interference that is present in the commercial dosage form.

References

[1] Marco, D. B. & Salgado, H. R. (2017). Characteristics, properties and analytical methods of cefadroxil: a review, Critical Reviews in Analytical Chemistry, 47(2):93-98.

[2] British Pharmacopeia on CD-ROM, 7th Edn., System Simulation Ltd the Stationary Office, London, 2013.

[3] Atif, S.; Baig, J. A., Afridi, H. I., Waris, M., Asif, W., & Naeem, A. (2022). Analytical comparison of cefadroxil determination by square wave adsorptive stripping voltammetric and spectrophotometric methods, Austin J Anal Pharm Chem, 9(1): 1138.

[4] Kassa, A.; Amare, M.; Benor, A.; Tigineh, G. T.; Beyene, Y., Tefera, M., & Abebe, A. (2022). Potentiodynamic poly (resorcinol)-modified glassy carbon electrode as a voltammetric sensor for determining cephalexin and cefadroxil simultaneously in pharmaceutical formulation and biological fluid samples. ACS Omega, 7(38):34599-34607.

[5] Kano, E. K.; Serra, C. H. D. R.; Koono, E. E. M.; Fukuda, K., & Porta, V. (2012). An efficient HPLC-UV method for the quantitative determination of cefadroxil in human plasma and its application in pharmacokinetic studies. Journal of liquid Chromatography & Related Technologies, 35(13): 1871-1881.

[6] Bosch, M. E.; Sanchez, A. R., Rojas, F. S., & Ojeda, C. B. (2008). Recent developments in the analytical determination of cefadroxil, Asian Journal of Pharmaceutical Sciences, 3(3): 217-232.

[7] Ting, S. (1988). Reverse-phase liquid chromatographic analysis of cephalosporins". Journal of the Association of Official Analytical Chemists, 71(6): 1123-1130.

[8] Elbalkiny, H. T.; Yehia, A. M.; Safa'a, M. R., & Elsaharty, Y. S. (2020). Derivative constant wavelength synchronous fluorescence spectrometry for the simultaneous detection of cefadrine and cefadroxil in water samples. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 229:117903.

[9] Sanz, C. G.; Serrano, S., & Brett, C. M. (2019). "Electrochemical characterization of cefadroxil β-lactam antibiotic and Cu (II) complex formation". Journal of Electroanalytical Chemistry. 844: 124-131.

[10] Metwally, F. H.; Alwarthan, A. A., & Al-Tamimi, S. A. (2001). Flow-injection spectrophotometric determination of certain cephalosporins based on the formation of dyes. Il Farmaco, 56(8): 601-607.

[11] Zakaria, S. A.; Talal, Z., & Othman, N. S. (2022). Using 2, 4-dinitrophenylhydrazine in spectrophotometric determination". Samarra Journal of pure and Applied Science, 4(2): 107-117.

[12] Hassan, M.; Abeed, F. A., & Saif, B. (2014). A new kinetic spectrophotometric method for determination of cefadroxil in pharmaceutical formulations using Lawsonia inermis (Henna) as natural reagent, Advances in Biological Chemistry, 2014(4):116-128.

[13] Omer, A.; Sehree, M., & Othman, N. (2017). Indirect spectrophotometric methods for the determination of cefadroxil in its pure and pharmaceutical preparations. Rafidain Journal of Science, 26(1): 56-65.

[14] Shantier, S. W.; Gadkariem, E. A.; Ibrahim, K. E., & El-Obeid, H. A. (2011). Spectrophotmetric determination of cefadroxil in bulk and dosage form using sodium hydroxide". E-Journal of Chemistry, 8(3): 1314-1322.

[15] Marco, B.; Kogawa, A., & Salgado, H. (2019). New, green and miniaturized analytical method for determination of cefadroxil monohydrate in capsules. Drug Analytical Research, 3(1):23-28.