Journal of Pharmaceutical and Applied Chemistry
An International Journal

10.18576//JPAC.2023.305119

Spectrophotometric Assay of Cefadroxil via Oxidation and Bleaching Color of Leishman Dye

*Amina D. Sulaman[1] and Nabeel S. Othman[1]**

[1] Department of Chemistry, College of Science, University of Mosul, Mosul, Iraq.
[1] Department of Chemistry, College of Science, University of Mosul, Mosul, Iraq.

Received: 10 May. 2023, Revised: 11 June. 2023, Accepted: 20 June. 2023.
Published online: 1 September 2023.

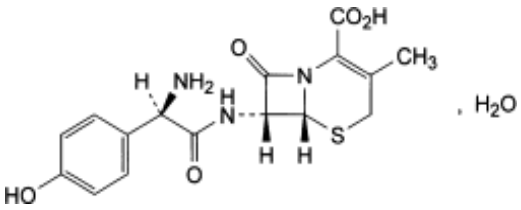
Abstract: A simple and sensitive spectrophotometric method has been developed for the estimate of cefadroxil in the pure form and in the dosage form(capsule). The suggested method involves the oxidation of cefadroxil with a known excess amount of N-bromosuccinimide (NBS) in an acidic medium (hydrochloric acid, 1M). The residual amount of oxidant (NBS) is determined by decolorization of the Leishman color dye. The absorbance of excess dye was measured at 651 nm. The method follows Beer's law in the concentration range of 0.5-7.0 µg/ml. The linearity with determination coefficient 0.9941, molar absorptivity 3.5813 x 10^4 L.mol^{-1}.cm^{-1} and Sandell′s sensitivity index value 0.0106 µg/cm^2. The LOD and LOQ values were 0.004 µg/ml and 0.015 µg/ml respectively. The relative standard deviation value was not more than 4.88% and the relative error was from -2.0 to 1.3%. The method was applied successfully to the pharmaceutical preparations as a capsule.

Keywords: Leishman dye, Cefadroxil, N-bromosuccinimide, Spectrophotometry.

Introduction

Cefadroxil is a drug used in many treatments of infections resulting mainly from Gram-positive bacteria [١,2].

The chemical structure of Cefadroxil is mentioned below in Fig. 1.



$C16H_{17}N_3O_5S,H_2O$ M.wt=381.4 g /mol.

Fig. 1: The chemical structure of cefadroxil.

Various techniques in the literature for the microassay of cefadroxil in it is formulations and also in some biological fluids. These techniques included high-performance liquid chromatography [4-7], voltammetric and spectrophotometric methods [8], modified electrode [9], fluorescence [10], electrochemical [11], flow injection analysis (FIA)[12], or using potassium permanganate and formaldehyde system [13].

There is no doubt that some of the overhead methods mainly the chromatographic and the electro methods are sensitive and extra selective for the determination of the analyte in the presence of more than one component in sample, but expensive instruments are needed.

The main spectrophotometric methods remain prevailing in most laboratories due to the cheapness of the devices and the variety of reactions that can be used in the different estimations [14-19].

The indirect spectrophotometric methods included measuring the absorbance of colored product that the analyte is not a part of the final complex such as measuring the absorbance of the residual color of Fe +2 - 4,7- diphenyl 1,10phenanthroline complex in the determination of trifluoperazine [20], and the color of residual dye(Leishman) in the determination of chloramphenicol[21].

Leishman pigment was discovered by the Scottish General William Leishman in 1923-1926, it is used in blood staining to detect malaria infection. Leishman stain is violet in color and stable [22]. Through the literary survey about its use as a reagent for the determination of pharmaceutical compounds, there is only its use in the estimation of chloramphenicol [21]. Therefore, we used it to estimate the drug compound under study (cefadroxil).

The present work included an indirect method for the assay of cefadroxil via oxidation of cefadroxil and the excess of oxidized reagent bleaching the color of Leishman dye, and the absorbance of a bleached Leishman dye is directly proportional to the amount of cefadroxil in solution.

EXPERIMENTAL

Instrumental

A JASCO-360 (Japan) spectrophotometer with 1 cm light path glass cells were used for all the absorbance and spectral measurements. A BEL-sensitive balance was used for weighing all solid materials.

Reagents and solutions

* Corresponding author E-mail: nsn20002004@uomosul.edu.iq

All chemicals used were of an analytical reagent grade and solutions were prepared in distilled water.

The cefadroxil solution, 100 µg/ml of pure form was prepared by dissolving 0.0100 g of cefadroxil powder in 100 ml of warm distilled water.

Leishman solution, 1.8 x 10^{-4} M was prepared by dissolving 0.2000 g of pure dye in 50 ml methanol with stirring for 24 minutes then was filtrated and diluted 1 ml of filtrate with 25 ml distilled water.

N-bromosuccinimde solution, 1x10^{-3} M was prepared by dissolving 0.0177 g of oxidant reagent in 100 ml distilled water.

Hydrochloric acid approximately, 1M was prepared by diluting 8.4 ml from hydrochloric acid 11.8 M with 100 ml of distilled water.

Preparation of pharmaceutical form by weighting and mixing 3 capsules contents of cefadroxil 500 µg/ capsule (Tabuk Company), then weighted amount equivalent to 0.0100 g of pure cefadroxil and dissolving in 100 ml warm distilled water in 100 ml a volumetric flask to prepare 100 µg/ml.

Genera method and calibration curve

Different volumes of cefadroxil solution 0.05-0.7 ml with a concentration of 10 µg/ml were added to a series of 10- mL volumetric flasks then followed by adding 0.75 ml of hydrochloric acid (1 M), then 1.25 ml of the oxidizing agent NBS was added and waited for 5 minutes, then followed by adding 2 mL of Leishman dye and wait for 10 minutes, then the absorbance was measured at 651 nm. Fig. 2 shows that the calibration curve follows Beer's law within the range from 0.05 to 0.7 µg/mL, and the value of the molar absorptivity was 3.5813x 10^4 l/mol. cm and Sandell's index was 0.0106 µg/cm^2.

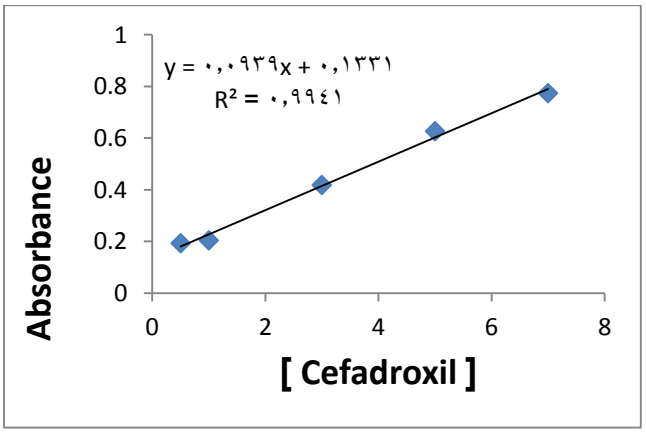


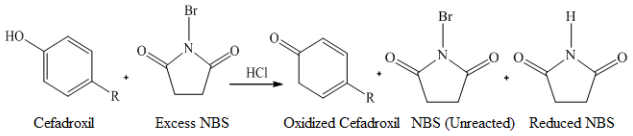
Fig. 2: Calibration curve for cefadroxil determination**.**

Both the limit of detection (LOD) and the limit of quantification (LOQ) were calculated, and values 0.004 and 0.015 µg/mL respectively.

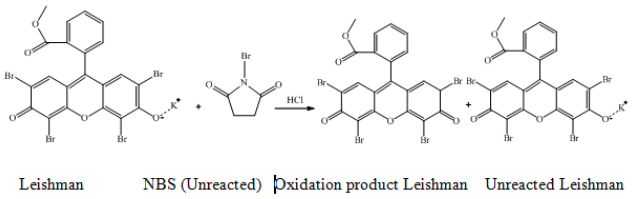
Results and Discussion

The principle of the method

The method depends on the oxidation of cefadroxil by adding an excess of N-bromosuccinimide in an acidic medium in the presence of hydrochloric acid (1M) as following equations:



Then the color of Leishman dye was bleached by unreacted NBS.



Then the absorbance of unreacted Leishman dye was measured at 651 nm. The values of absorbance are proportional to the amounts of cefadroxil.

The optimum conditions

All parameters that affected the intensity of unreacted Leishman dye were studied and the optimal results were chosen.

Amount of Leishman dye

Different volumes of 0.1-2.5 ml of Leishman dye solution were added to a series of volumetric flasks and the volume was diluted to 10 ml with distilled water(Fig. 3).

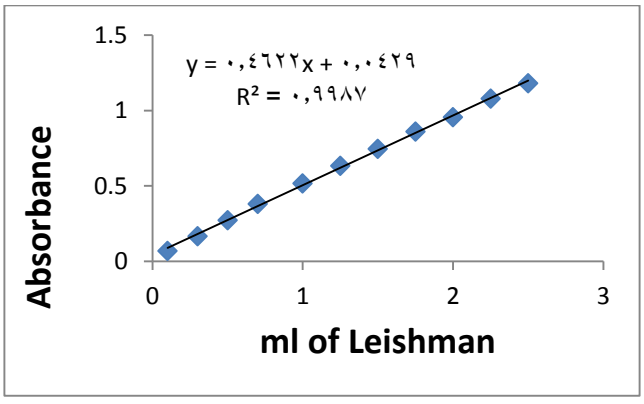


Fig. 3: The effect of Leishman dye amount on absorbance.

The volume of 2 ml has been selected as a useful amount for the reaction. It gave good absorbance and is in the linear relationship of amount Leishman with absorbance.

Effect of acid types and their amounts

The effect of acid types and amounts used for the oxidation of cefadroxil was studied by adding different types and

volumes of acids HCl, CH_3COOH, HNO_3, and H_2SO_4 (Fig.4).

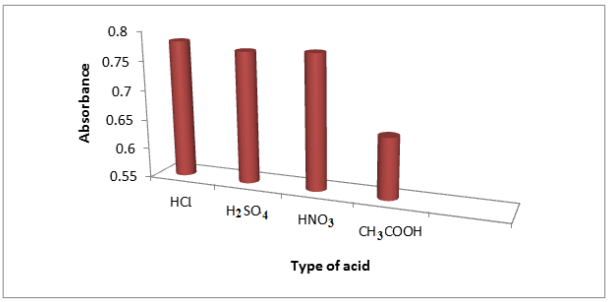


Fig.4: Effect of acid type.

The results as shown in Fig. 4 and Table 1 show that 0.75 mL of 1M HCl gave high absorbance, So it has been used in the next experiments.

Table 1: Effect of acid amount.

HCl (ml)	Absorbance
0.5	0.7867
0.75	0.7936
1.0	0.7887
1.25	0.7818
1.5	0.7741
2.0	0.7714

The volume of 0.75 ml of HCl gave high absorbance, this result indicated that a large amount of cefadroxil oxidized and needs a large amount of NBS and a low amount of unreacted NBS so less bleaching in the color of Leishman and high absorbance.

Effect of oxidant reagents types

Different types of oxidant reagents N-bromosuccinimide, N-chlorosuccinimide, potassium periodate, and sodium periodate had been tested(show Fig.5).

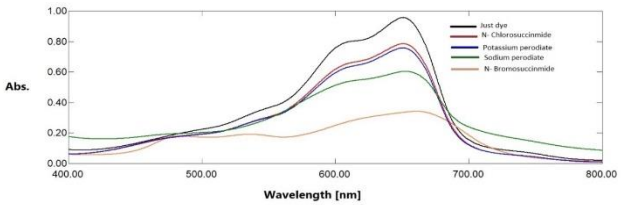


Fig. 5: Effect type of oxidant on bleaching the color of the dye.

The results in Fig.5 indicated that N-bromosuccinimide was a useful oxidizing agent because it gave the highest bleaching of Leishman dye.

Effect of the oxidant amount

0.1-2.0 mL of 10^{-3} M of NBS has been added to 2mL amount of Leishman dye without cefadroxil as shown in Fig. 6.

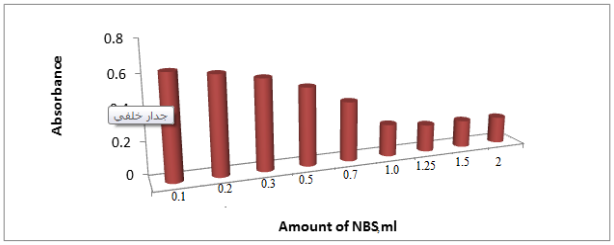


Fig. 6: Effect of the oxidant amount.

1.25 mL of NBS solution was a sufficient amount (volume) to obtain a maximum bleaching of the dye color, So 1.25 mL of NBS was used in the next experiments.

Effect of oxidation time

The results in Table 2 were obtained from the study of the effect of optimum time on both oxidation of cefadroxil by NBS in an acidic medium and for bleaching of Leishman dye.

Table 2: Effect of time on oxidation of cefadroxil and bleaching color of dye.

Time(min.) after adding NBS	Absorbance /minute standing time before dilution				
	Immediately	5	10	20	30
Immediately	0.6323	0.6483	0.6507	0.6492	0.6512
5	0.7564	0.7578	0.7771	0.7618	0.7632
10	0.7523	0.7133	0.7034	0.7155	0.7194
20	0.7552	0.7070	0.7042	0.7511	0.7447

The results in Table above 5 minutes were selected as a suitable time for oxidizing the drug and 10 minutes for color bleaching Leishman dye.

Effect of oxidation order

Different experiments have been done, to know the best order of the addition reaction. The results as shown in Table 3.

Table 3: The order of addition.

Reaction component	Order number	Absorbance
S + H + OX + Dye	I	0.7601
S + Dye + OX + H	II	0.5728
Dye + OX + H + S	III	0.2368
S + OX + H + Dye		0.7593

The order I was selected for the next experiments because it gave the highest absorbance compared with other orders. Order III gave the lowest absorbance because the dye first oxidized by NBS and bleached it is color, also there is a decrease in absorbance**.**

Absorption spectrum

Absorption spectrum at optimum conditions for the unreacted dye in the presence of 0.3 and 0.7 µg/mL of cefadroxil and without cefadroxil (only Leishman. The results are shown in Fig. 7.

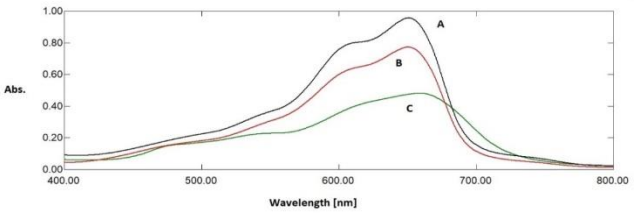


Fig. 7: Absorption spectrum with and without cefadroxil: Absorption spectrum for Leishman dye(A), absorption spectrum for 0.7 µg/mL cefadroxil and Leishman dye. (B) and C absorption spectrum for 0.3 µg/mL cefadroxil and Leishman dye.

The results from Fig. 7 indicated that the oxidant share in the oxidation of the drug compound and Leishman dye and an increase in the amount of cefadroxil caused a lease bleaching of dye color because more amount of NBS used in oxidized cefadroxil.

Analytical application

The suggested method was checked for the determination of cefadroxil(Cef.) in capsule form. Drug concentration was calculated by direct measurements using the relationship in the standard calibration curve. The results are shown in Table 4.

Table 4: Determination of cefadroxil in pharmaceutical form (capsule).

Drug	Cef. taken µg/ml	Cef. found µg/ml	Rec. %	Er %	RSD %	Drug content measured (mg)
Cefadroxil /capsules 500 mg (Tabuk)	3	3.01	100.33	0.33	0.002	501.65
	7	6.94	99.14	-0.86	0.042	495.70

Standard addition method

The standard addition method was used in the estimation of 2 and 3 µg/ml of cefadroxil in capsule solution (Tabuk company-Kingdom of Saudi Arabia), The obtained results were shown in Fig. 8 and illustrated in Table 5.

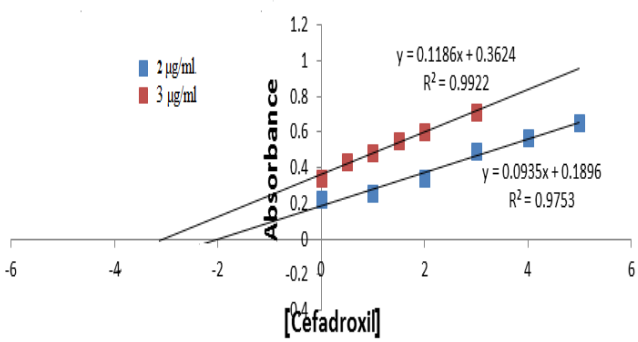


Fig. 8: Determination of cefadroxil in capsule (Tabuk company-Kingdom of Saudi Arabia) by standard addition plot.

Table 5: Standard addition results.

Pharmaceutical preparation	Amount taken µg/ml	Amount measured µg/ml	Recovery %	Drug contain mg
Cefadroxil / (500mg/ Capsule) Tabuk Company	2	2.02	101.00	505.00
	3	3.05	101.66	508.30

Similar results in Table 5 compared with results in Table4(with accepted analytical error) were obtained by applying the suggested method at the same concentration of pharmaceutical form (capsule) and indicated that there are no interferences of additive used in drugs manufacturing via direct determination using the relationship of linearity and standard addition method

Comparison of methods

A comparison has been made of the most important analytical variables of the proposed method with their counterparts in other spectrophotometric methods(Table 6).

Table 6: Comparing some of the important analytical variables of the method with other methods.

Method	Reagent	λ_{max} (nm)	Beer's law (µg/ml)	ε l/mol.cm.	Ref.
Oxidation bleaching color	Leishman dye	651	0.5-7	$3.58x10^4$	Present method
Oxidation bleaching color	Methyl orange dye	508	2-6	$4.77x10^4$	[23]
Azo coupling	2,4-Dinitrop-henylhydrazine	515	7.5-75	$7.00x10^3$	[24]
Oxidation reduction reaction	1,10-Phenathr-oline/ Fe^{+2} 2,2`-bipyridyl/ Fe^{+2}	510 521	0.5-5 0.5-6	$1.14x10^5$ 6.30×10^4	[17]
Oxidation reaction	Cerium (IV) ammonium sulphate Iron (III) ammonium sulphate	397	5-30	$0.82x10^4$ $1.37x10^4$	[25]

From the results of the comparison, we conclude that the proposed method is no less important than the other methods used in the comparison.

CONCLUSIONS

The suggested method showed a simple, accurate, and sensitive spectrophotometric method for the determination of cefadroxil using NBS as an oxidant agent. The unreacted NBS bleached the Leishman dye. The linearity of the method from 0.5 to7 µg/ml and with molar absorptivity equal to $3.5813x\ 10^4$ l/mol. cm that indicated a sensitive method. The method has been applied successfully to determine cefadroxil in capsule form.

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