Thymol as a Novel Reagent in Spectrophotometric Determination of Chloramphenicol

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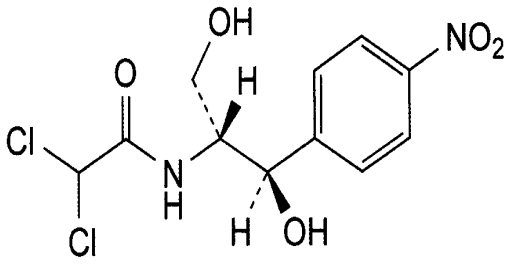
ABSTRACT

A simple, accurate and sensitive indirect spectrophotometric method for the determination of chloramphenicol (CAP) in various formulations has been suggested. The method is based on the coupling of the diazotized of reduced CAP (DR-CAP) with Thymol in alkaline medium to yield a colored product showed maximum absorbance at 459 nm. . Beer's law is obeyed in the concentration range of 1 – 12.5 $\mu g.ml^{-1}$. The molar absorptivity and Sandal's sensitivity of the colored azo dye were 2.28 $x10^4$ $l.mol^{-1}.cm^{-1}$ and 0.0141 $\mu g.cm^{-2}$ respectively. Limit of detection(LOD) 0.104 $\mu g.ml^{-1}$,limit of quantitation (LOQ) 0.348 $\mu g.ml^{-1}$.A relative error ranged from 0.16 to -2.4 % and a relative standard deviation from ±0.03 to ±0.08 % depending on the concentration of the drug. The method was successfully applied to the determination of CAP in its pharmaceutical preparations.

Keywords: Chloramphenicol, thymol, spectrophotometry, azo dye.

INTRODUCTION

Chloramphenicol,a broad spectrum antibiotic, is first isolated from cultures of *Streptomycest*,and is effective against a wide variety of Gram-positive and Gram-negative bacteria . It is widely used because it is inexpensive and readily available [1,2] Chloramphenicol is 2,2-dichloro-N-[(1R,2R)-2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl) ethyl] acetamide, produced by the growth of certain strains of *Streptomyces venezuelae* in a suitable medium. It is normally prepared by synthesis. It contains not less than 98.0 and not more than the equivalent of 102.0 % of $C_{11}H_{12}Cl_2N_2O_5$, calculated with reference to the dried substance.[3]CAP is a white, greyish-white or yellowish-white, fine, crystalline powder or fine crystals, needles or elongated plates, slightly soluble in water, freely soluble in alcohol and in propylene glycol, and has the following structure [3]:



CAP structure, M.wt = 323.1322

For the determination of studied drug various methods have been reported in literature these methods included ;

High performance liquid chromatography (HPLC) which is one of the most powerful and versatile tool for the quantitative determination of CAP[4-7], also LC-MS[8],LC-MS-MS [9], LC-ESI-MS-MS [10]and GS-MS methods[11], Other analytical methods have been reviewed in literature in determination included flow injection[12,13], atomic absorption spectrometer[14],Voltammetry[15],Chem -iluminescence[16] and spectrophotometric methods[17- 25].

The aim of the present work is to provide a sensitive , simple and accurate indirect spectrophotometric method to the determination of CAP in its pharmaceutical preparations.

EXPERIMENTAL

Apparatus

A JASCOV - 630 UV / Vis spectrophotometer (Japan), with 1cm matched quartz cells were used for all measurement . pH measurements have been done by HANNA 211 pH-meter. The balance BEL ENGNEERING was used in the weighing process.

Reagents

All chemicals used in this investigation are of analytical – reagent grade, and CAP standard material was provided from General Establishment for Medical Appliance and Drugs / SDI – Samaraa / Iraq

Solutions:

Thymol,0.5 % .

This solution was prepared daily by dissolving 0.25 g of thymol (Fluka) in 50 ml distilled water

Sodium hydroxide .

This solution was attended by a dilution of the concentrated volumetric solution supplied from Fluka company with distilled water and the solution was saved in a plastic container.

Reduced – CAP(R-CAP) solution (500 $\mu g.ml^{-1}$).

This solution was attended by a dissolving 0.0500 g of CAP in 50 ml ethanol, then transfer the solution to beaker size 125 ml and 20 ml of distilled water ,20 ml of hydrochloric acid and 3 g of zinc powder were added and allowed to stand for 1hr at the temperature of the laboratory. Then filtered and wash the residue with distilled water into a 100 ml volumetric flask then the volume completed to mark with distilled water to prepared a solution at a concentration of 500 $\mu g.ml^{-1}$(1.547×10^{-3}M) of R-CAP. More diluted solutions were prepared daily by appropriate dilution using distilled water[18] .

Sodium nitrite (1.52×10^{-3}M).

This solution was prepared freshly by dissolving 0.0535g of $NaNO_2$ in small amount of distilled water then completed to 500 ml with the same solvent.[18]

DR-CAP ($3.1x10^{-4}$ M) solution.

This solution was prepared by mixing 20 ml of 1.547×10^{-3}M R-CAP with 20 ml of 1.547×10^{-3}M sodium nitrite in 100 ml a volumetric flask ,then the volume was completed to 100 ml with distilled water. More diluted solutions were prepared daily by dilution of the stock solution with distilled water [18].

Solutions of pharmaceutical preparations:

1- Phenicol Eye Drop.

The contents of three bottles of eye drops were mixed. An aliquot corresponding to 50 mg of CAP (10 ml) was diluted to 50 ml with ethanol in a volumetric flask. This solution was

transferred into 125 ml beaker and was proceed as mentioned above preparation R-CAP and DR-CAP of solutions[18].

2-Aphenicol Capsules.

Weight the contents of 5 capsules(each one contain 250 mg of CAP).An accurately weighed amount of powder(0.0603 g)equivalent to 50 mg CAP was dissolved in 50 ml ethanol in a volumetric flask, then transferred the solution into 125 ml beaker and was proceed as mentioned above preparation R-CAP and DR-CAP of solutions[18].

3-Injection.

The contents of 3 injections were mixed, a 0.073 g equivalent to 50 mg CAP was dissolved in 50 ml ethanol in a volumetric flask, then transferred the solution into 125 ml beaker and was proceed as mentioned above preparation R-CAP and DR-CAP of solutions[18].

Procedure and calibration graph

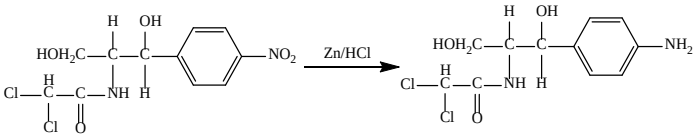
To a series of 10.ml calibrated flasks , transferred 0.1 – 1.25 ml of 100 ppm of DR-CAP , then 1.5 ml of thymol solution (0.5 %) and 2 ml of NaOH solution (1 M) were added , after dilution to the mark with distilled water. The absorbance was measured at 459 against the blank . A linear calibration graph was obtained over the concentration range from 1 to 12.5µg.ml^{-1}(Fig 1).

RESULTS AND DISCUSSION

Principle of reactions :

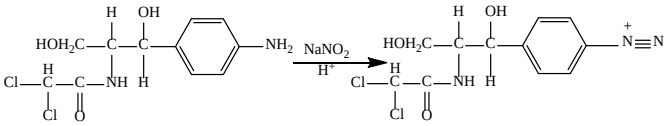
The method include 3 steps :

1-Convention of CAP to reduced - CAP (R-CAP)



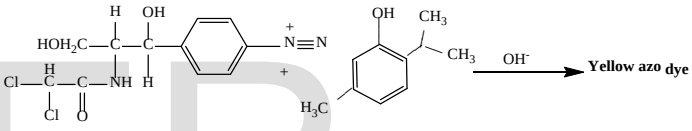
CAP **R-CAP**

2-Prepration of DR -CAP



R-CAP **DR-CAP**

3-Coupling of DR – CAP with thymol in alkaline medium



DR-CAP **Thymol**

Optimization of the experimental Conditions

During the investigation, DR-CAP solution equivalent to 50 µg.ml^{-1} CAP, was taken and the final volume was brought to 10 ml with distilled water.

Effect of thymol amount:

The effect of different amounts of thymol solution (0.5 %) on the intensity of the azo dye at different amounts (25 – 125 µg) of DR-CAP has been studied. A 1.5 ml of thymol solution in a total volume of 10 ml gave the higher sensitivity and higher value of determination coefficient (R^2), therefore it has been selected for subsequent experiments (Table 1).

Effect of base:

Previous experiment has been showed that the colored azo dye formed in alkaline medium, therefore different types of strong and weak bases have been studied , the results in (Table 2) showed that 2 ml of 1M NaOH was the optimum volume .

The stability of azo dye :

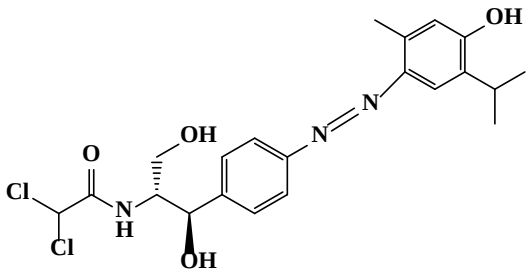
The maximum color of azo dye was reached immediately after mixed the components of the suggested method , and the absorbance remained constant for at least 24 hours (Table 3) .

Final absorption spectrum

All factors affecting on the sensitivity and stability of the colored azo dye resulting from the coupling reaction between DR-CAP and thymol in alkaline medium were carefully studied. A typical spectrum for the formed azo dye was measured versus reagent blank which has negligible absorbance at λmax 459 nm (Fig.2).

The nature of azo dye :

Job`s and mole ratio methods[26] showed that the azo dye formed in composition 1:1 thymol to DR-CAP .The location of the para of thymol is empty, so the DR-CAP is connected to this location[27]. Therefore the suggested structure of azo dye may be written as follows :



Yellow azo dye

Application of method :

The proposed method was successfully applied for the determination of CAP in various formulation forms . The results in (Table 4) showed good recovery with excepted value of recovery and RSD % .

CONCLUSION

The present method is sensitive, accurate and simple for determination of CAP in it`s pharmaceutical preparations without extraction or temperature control.

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Table (1) : Effect of thymol amount.

Thymol (ml)	Absorbance / µg of DR - CAP in 10 ml					R^2
	25	50	75	100	125	
0.5	0.1348	0.3135	0.5009	0.6544	0.8081	0.9959
1	0.1496	0.3248	0.5290	0.6576	0.8160	0.9947
1.5	0.1562	0.3725	0.5160	0.7430	0.9030	0.9960
2.0	0.1526	0.3539	0.5439	0.7067	0.8550	0.9958
2.5	0.1393	0.3416	0.5042	0.7675	0.9265	0.9947

Table (2) : Effect of base on absorbance of azo dye.

Base	NaOH			KOH			Na_2CO_3			NaHCO3		
ml of base	A	pH	λ_{max}	A	pH	λ_{max}	A	pH	λ_{max}	A	pH	λ_{max}
1	0.3554	12.03	459	0.3988	12.80	458	0.5110	10.33	457	0.3682	7.92	381
2	0.3725	12.51	459	0.3662	13.02	459	0.4645	10.69	457	0.6432	8.00	381
3	0.3630	12.83	457	0.3237	13.25	459	0.6875	10.75	455	0.6536	8.33	380

Table (3) : The effect of time on the absorbance of azo dye.

Time/min	Absorbance / µg of DR-CAP	
	50	100
After dilution	0.3723	0.7424
5	0.3723	0.7429
10	0.3722	0.7428
15	0.3723	0.7430
20	0.3725	0.7431
25	0.3722	0.7434
30	0.3721	0.7434
40	0.3722	0.7438
50	0.3722	0.7442
60	0.3723	0.7448

120	0.3725	0.7482
1440	0.3724	0.7261

Table (4) : The result of determination of CAP in its pharmaceutical preparation.

Pharmaceutical preparation	µg CAP present/10ml	µg CAP measured/10ml	Recovery*,%	RSD* %
PHENICOL Eye Drop (Jorden)	50	50.08	100.16	0.08
	100	97.5	97.5	0.04
Chloramphenicol sodium succinate equivel 1G base powder vail (India)	50	49.3	98.6	0.09
	100	96.8	96.8	0.04
Chloramphenicol capsules Bp 250 mg (India)	50	48.8	97.6	0.07
	100	97.7	97.7	0.03

*Average of five determinations.

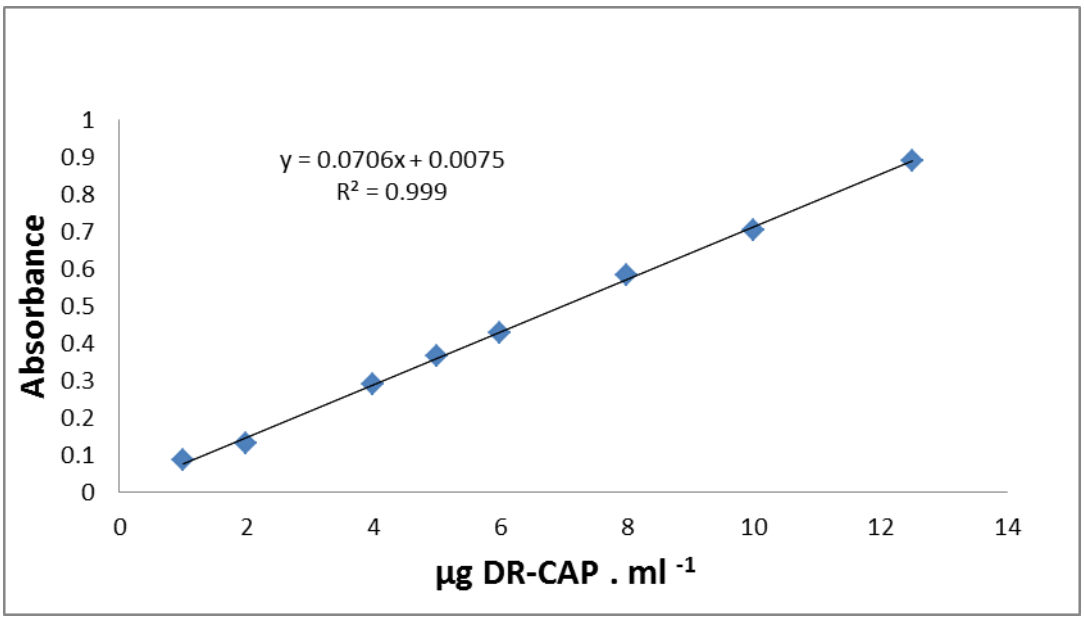
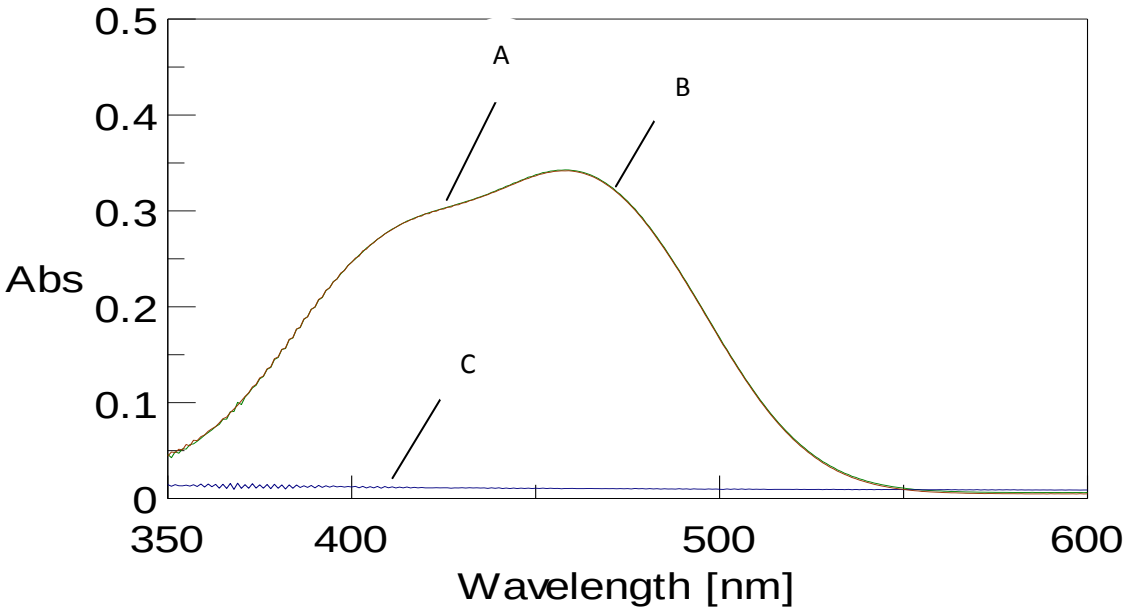


Fig (1) Calibration graph for CAP determination.



Fig(2) : Absorption spectra of 50 μg CAP / 10ml treated according to the recommended procedure and measured against (A) reagent blank, (B) distilled water and (C) reagent blank measured against distilled water.