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Using of 2,7-Dihydroxynaphthalene as a Novel Reagent in Spectrophotometric Assay of Chloramphenicol

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ABSTRACT

An indirect spectrophotometric method for the determination of chloramphenicol (CAP) has been suggested. The method is based on the oxidative coupling reaction of reduced chloramphenicol (R-CAP) with 2,7- dihydroxynaphthalene (2,7-DHN) reagent in the presence of potassium dichromate as oxidizing agent to produce brown colored, stable and soluble product. This product showed maximum absorption at 544 nm. Beer's law is obeyed over the range 0.5-7.5 $\mu g.ml^{-1}$ of R – CAP and molar absorptivity of 1.069×10^{5} $l.mol^{-1}.cm^{-1}$ and Sandell's sensitivity index of 0.0030 $\mu g.cm^{-2}$, limit of detection (LOD), limit of quantitation (LOQ), relative error (RE%) and relative standard deviation (RSD%) have been estimated. The method has been successfully applied to the determination of CAP in drug formulations.

Keywords: chloramphenicol, 2,7- dihydroxynaphthalene, spectrophotometry, oxidative coupling.

استخدام 7،2–ثنائي هيدروكسيل النفثالين بوصفه كاشف جديد للتقدير الطيفي للكلورامفينيكول

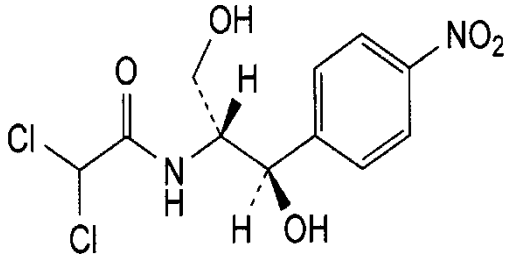
الملخص

أُقترحت طريقة طيفية غير مباشرة لتقدير الكلورامفينيكول. تعتمد الطريقة على تفاعل الاقتران التأكسدي بين الكلورامفينيكول المختزل والكاشف 7،2– ثنائي هيدروكسيل النفثالين بوجود العامل المؤكسد ثنائي كرومات البوتاسيوم لتكوين ناتج ذي لون بني والذي يكون مستقراً وذائباً ويعطي أعلى امتصاص عند الطول الموجي 544 نانوميتر. كانت حدود قانون بير في مدى التركيز من 0.5 الى 7.5 مايكروغرام. مللتر$^{-1}$ من الكلورامفينيكول المختزل وكانت الامتصاصية المولارية 1.069×10^{5} لتر. مول$^{-1}$. سم$^{-1}$، ودلالة ساندل للحساسية 0.0030 مايكروغرام. سم$^{-2}$، وتم حساب كل من حد الكشف (LOD) والتقدير الكمي (LOQ) والخطأ النسبي (RE%) والانحراف القياسي (RSD%). وطُبقت الطريقة المقترحة بنجاح لتقدير الكلورامفينيكول في المستحضرات الدوائية.

الكلمات الدالة: 7،2–ثنائي هيدروكسيل النفثالين، طيفي، الاقتران التأكسدي.

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 (Falagas *et al*., 2008). 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 no 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. 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 (British Pharmacopeia, 2013).



CAP structure, M.wt = 323.1322 g / mol

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 (Hoang *et al*., 2015; Suguna *et al*., 2014; Mallu *et al*., 2011), also LC-MS (Bjorn, 2013), LC-MS-MS (Rocha *et al*., 2015). Other analytical methods have been reviewed in literature these methods included: voltammetry (Yafeng *et al*., 2014), Polarography (Suliman and Razzak, 2000), so that various spectrophotometric methods have been used (Alshirifi and Alhameedi, 2016; Suguna *et al*., 2016; Wafi *et al*., 2015; Al-Abachi *et al*., 2014; Al-Sabha and Al-Hammoshi, 2013; Sinan and Al-Abachi, 2010; Sayhood *et al*., 2013; Al-Sabha and Rasheed, 2010; Al-Ward, 2012).

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

EXPERIMENTAL

Apparatus

A JASCOV - 630 UV / V spectrophotometer (Japan), with 1cm matched quartz cells were used for all measurements. 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

2,7- dihydroxynaphthalene (0.005 M)

This solution was prepared by dissolving 0.0400 g of 2,7- dihydroxynaphthalene (Fluka) in 50 ml distilled water.

Reduced – CAP(R-CAP) Solution (500 µg.ml^{-1}).

This solution was attended by dissolving 0.0500 g of pure 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 (1 M) and 3 g of zinc powder were added, mixed well and allowed to stand for 1hr at the temperature of the laboratory. Then the residue filtered and wash the residue with distilled water into a 100 ml volumetric flask then the volume completed to mark with distilled water to prepare a solution at a concentration of 500 µg.ml^{-1} (1.547×10^{-3}M) of R-CAP. More diluted solutions were prepared daily by appropriate dilution using distilled water (Al-Abachi and Abed, 2014) .

Reduced – CAP(R-CAP) Solution (50 µg.ml^{-1}).

This solution was prepared by taking 5 ml of Reduced – CAP(R-CAP) solution (500 µg.ml^{-1}) and then diluted to 50 ml with distilled water in a volumetric flask.

Potassium Dichromate (0.005 M)

This solution was prepared by dissolving 0.0735 g of potassium dichromate (Fluka) in 50 ml distilled water in a volumetric flask.

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 proceeded as mentioned above in the preparation of R-CAP (50 µg.ml^{-1}) (Al-Abachi and Abed, 2014).

2- Chloramphenicol Capsules

Weight the contents of 5 capsules (each one contain 250 mg of CAP). An accurately weighed amount of powder(0.0603g)equivalent to 50 mg CAP was dissolved in 50 ml ethanol in a volumetric flask, then the solution was transferred into 125 ml beaker and was proceeded as mentioned above in preparation of R-CAP (50 µg.ml^{-1}) (Al-Abachi and Abed, 2014).

3- Injection

The contents of 3 vials were mixed, a 0.073 g equivalent to 50 mg CAP was dissolved in 50 ml ethanol in a volumetric flask then the solution was transferred into 125 ml beaker and was proceeded as mentioned above in preparation of R-CAP (50 µg.ml^{-1}) (Al-Abachi and Abed, 2014).

Procedure and Calibration Graph

To a series of 10.ml calibrated flasks, transferred 0.1 – 1. 5 ml of 50 ppm of R-CAP, then 2.0 ml of 2,7-DHN solution (0.005 M) and 2.5 ml of potassium dichromate solution (0.005 M) were added, after dilution to the mark with ethanol. The absorbance was measured at 544 against the blank. A linear calibration graph was obtained over the concentration range from 0.5 to 7.5µg.ml^{-1} (Fig. 1).

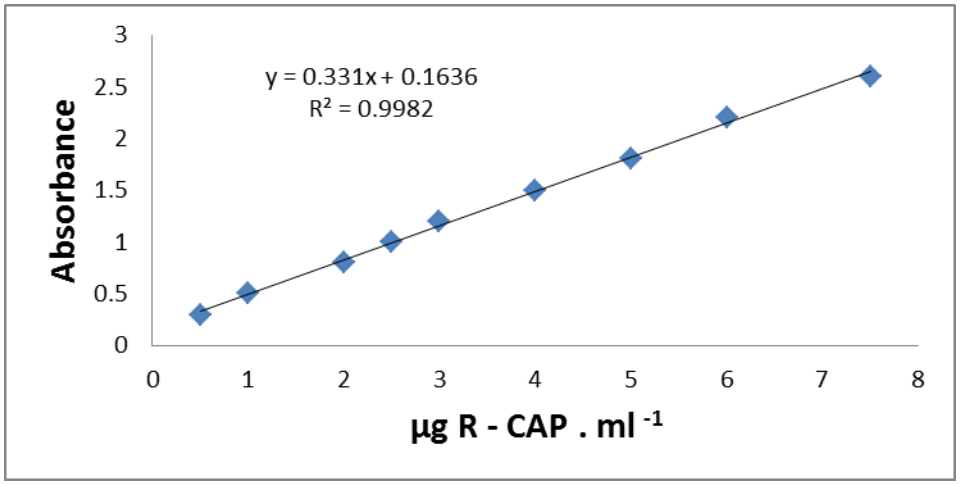


Fig. 1: Calibration graph for R-CAP determination

Optical and Regression Characteristics of the Present Method

The molar absorptivity, Sandell's sensitivity, the limits of detection (LOD) and limit of quantitation (LOQ) were given in (Table 1) which indicated good sensitivity of the suggested method.

Table 1: Optical and regression characteristics of the present method

Parameter	Value
Beer's law(µg.ml^{-1})	0.5 – 7.5
λ_{max},(nm)	544
Molar absorptivity l.mol^{-1}.cm^{-1}	1.069×10^{5}
Linear regression equation Slope = a Intercept = b	Y=ax* + b 0.331 0.1636
Determination coefficient(R^2).	0.9982
Relative standard deviation.	Not more than 0.18%
Limit of detection.(µg.ml^{-1})	0.00238
Limit of quantitation.(µg.ml^{-1})	0.00791

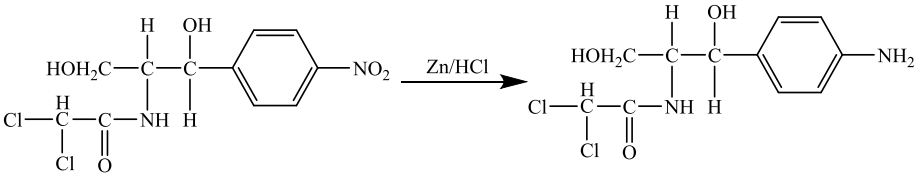
* concentration in µg.ml^{-1}

RESULTS AND DISCUSSION

Principles of the Method

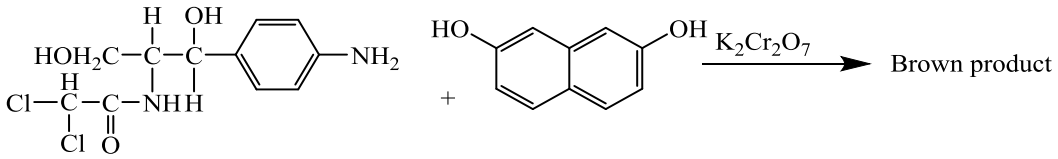
The method included 2 steps of reactions:

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



CAP **R-CAP**

2- Coupling of R–CAP with 2,7-DHN reagent in presence of potassium dichromate.



R-CAP **2,7-Dihydroxynaphthalene**

Optimization of the Experimental Conditions

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

Effect of 2,7-DHN amount:

The effect of different amounts of 2,7-DHN solution (0.005 M) on the intensity of the colored product at different amounts (2.5 – 7.5 µg) of R-CAP has been studied. A 2.0 ml of 2,7-DHN 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 2).

Table 2: Effect of 2,7-DHN amount

Amount of 0.005 M 2,7-DHN (ml)	Absorbance/µg of R–CAP.ml^{-1}				R^2
	2.5	3.75	5	7.5	
1	0.2397	0.2896	0.4935	0.6269	0.9714
1.5	0.2785	0.3190	0.5720	0.6900	0.9528
2.0	0.3119	0.4575	0.6203	0.7595	0.9738
2.5	0.2837	0.4071	0.5285	0.6272	0.9719

Choice the Oxidizing Agent

Several oxidizing agents have been tested (KIO_4, $K_2Cr_2O_7$, N-Bromosuccinimide, N-Chlorosuccinimide), $K_2Cr_2O_7$ gave the most sensitive reaction. (Table 3).

Table 3: Choice the oxidizing agent

Oxidizing agent (0.005M , 1ml)	$\lambda_{max.(nm)}$	Absorbance
N-Chlorosuccinimide	No color contrast	
Potassium periodate	475	0.2277
N-Bromosuccinimide	No color contrast	
Potassium dichromate	526	0.6205

Effect of Potassium Dichromate Amount

The effect of potassium dichromate amount on the absorbance has been investigated. The suggested procedure has been carried out with different amounts of $K_2Cr_2O_7$; the high intensity of the colored product was achieved by using 2.5 ml of $K_2Cr_2O_7$ (0.005 M); therefore; it has been selected for the subsequent experiment. (Table 4).

Table 4: Effect of potassium dichromate amount

Amount of 0.005 M $K_2Cr_2O_7$ (ml)	Absorbance/µg of R – CAP.ml^{-1}			
	2.5	5	7.5	R^2
1	0.3106	0.6208	0.7399	0.9685
2	0.6302	1.2588	1.5092	0.9705
2.5	0.9279	1.4804	1.9596	0.9717
3.0	0.8233	1.4797	1.7499	0.9460

Effect of pH

The effect of pH on the absorbance has been studied. The result for adding acid (HCl, H_2SO_4, CH_3COOH, 1M) r base (NaOH, Na_2CO_3, KOH, $NaHCO_3$, 1M) gave unsatisfactory results.

Effect of Surfactant

The effect of surfactant (SDS, CPC, CETAB, Triton X-100) on absorbance was studied and the results were unsatisfactory. All surfactant used given a turbid solutions. It was therefore not recommended for use in subsequent experiments.

Effect of Temperature

Some of the oxidative-coupling reactions depended heavily on the degree of temperature, so the reaction was conducted at different temperatures and the results showed that the reaction was not adopted on the temperature significantly, so investigation continued at room temperature (Table 5).

Table 5: Effect of temperature

Temperature C°	Absorbance of 50 µg R - CAP in 10 ml/minute standing time			
	15	20	30	40
20	1.0953	1.1685	1.3866	1.3945
RT=25	1.1053	1.2799	1.4785	1.4889
30	1.1024	1.2543	1.4639	1.4745
40	1.1013	1.2015	1.4030	1.4183
50	1.009	1.1903	1.3575	1.3719

Effect of time on the color development

The effect of the time needed to get full color development has been tested , 30 minutes were found to be optimum time (Table 6).

Table 6: Effect of time on oxidation

Time, minutes	5	10	15	20	25	30	40
Absorbance	1.2144	1.2885	1.3261	1.3873	1.4359	1.4793	1.3983

The stability of Colored Product

The effect of time on the stability of the color product was studied (Table 7). It was one of the most important problems that faced the work because it is stability in aqueous medium (**1**) was unsatisfactory, so there have been several attempts to improve the stability, including changing the

order of addition of the reaction components (**2**), adding 5 ml of (0.01 M) EDTA solution (**3**) and using ethanol as a solvent in dilution to the mark (**4**).

Table 7: The stability of colored product

Time/min	Absorbance of 50 µg of R-CAP/ 10 ml			
	$\lambda_{max.(nm)} = 526$			$\lambda_{max.(nm)} = 544$
	1*	**2****	**3**	**4**
After dilution	0.2459	1.4420	0.0763	1.7760
5	0.4693	1.4729	0.0776	1.7784
10	0.7863	1.5084	0.1180	1.7833
15	1.1058	1.5574	0.1566	1.7902
20	1.2873	1.5797	0.2223	1.8002
25	1.3567	1.6044	0.3208	1.8017
30	1.4788	1.6100	0.5477	1.8015
40	1.4893	1.6267	0.8575	1.8014
50	1.5052	1.6453	1.2215	1.8013
60	1.5235	1.6542	1.3013	1.8012
120	1.6011	1.6842	1.6590	1.7707

* R-CAP + 2,7-DHN + $K_2Cr_2O_7$
** 2,7-DHN + $K_2Cr_2O_7$ + R-CAP

The results in (Table 7) showed that the fourth treatment gave a better stability of colored product with red shift. Therefore, ethanol and 544 nm were used in subsequent experiments.

Final Absorption Spectra

Under the above optimized conditions, absorption spectra of the colored product was formed from the reaction of R-CAP with 2,7-DHN in presence of potassium dichromate against its corresponding reagent blank which showed a maximum absorption at 544 nm., and this wavelength was selected on the subsequent experiments (Fig. 2).

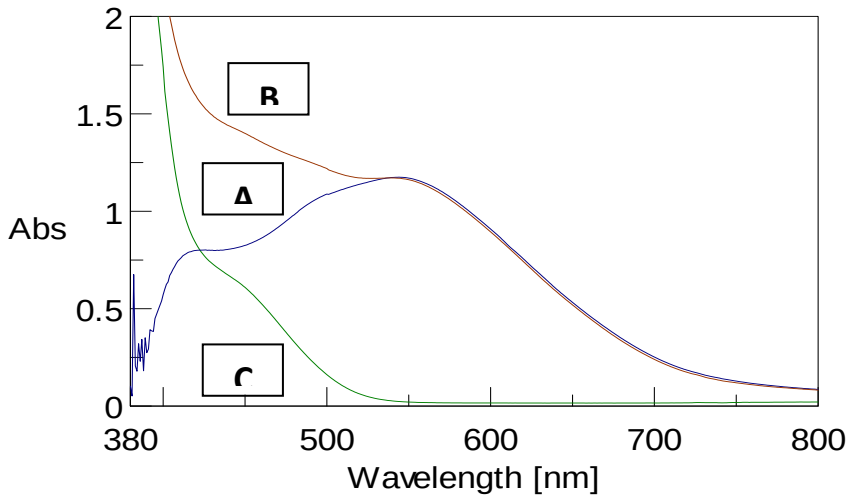


Fig. 2: Absorption spectra of 25 µg CAP / 10 ml treated according to the recommended procedure and measured against (A) reagent blank, (B) ethanol and (C) reagent blank measured against ethanol.

Analytical Application

The proposed method was applied to determine CAP in different drug formulations. On applying proposed procedure, good recovery, accuracy and precision were obtained as shown in (Table 8).

Table 8: Analytical application

Pharmaceutical preparation	µg R - CAP present/10ml	µg R - CAP measured/10ml	%* Recovery	RSD %*	RE %*
PHENICOL Eye Drop (Api , Jordan)	25	24.9	99.6	0.05	-0.4
	50	50.05	100.1	0.11	-0.1
Chloramphenicol sodium succinate equivel 1G base powder vail (Macleods, India)	25	24.9	99.6	0.02	-0.4
	50	49.9	99.8	0.08	-0.2
Chloramphenicol capsules Bp 250 mg (Brawn, India)	25	24.9	99.6	0.18	-0.4
	50	49.8	99.6	0.08	-0.4

*Averge of 5 determination.

CONCLUSION

The suggested procedure for CAP determination was sensitive, accurate and can be used in the determination of CAP in different types of formulations without extraction or separation.

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