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Spectrophotometric Determination of Chloramphenicol in Pharmaceutical Formulations by Diazotization and Coupling Reaction

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Abstract

Chloramphenicol (CAP), a broad-spectrum antibiotic, was quantitatively analyzed in various pharmaceutical formulations using a rapid and sensitive spectrophotometric method, with the characterization via Fourier-transform infrared (FTIR) spectroscopy. The reduction of CAP was achieved using zinc powder in the presence of concentrated hydrochloric acid, followed by diazotization with sodium nitrite ($NaNO_2$). The resulting diazonium salt was subsequently coupled with three distinct chromogenic reagents cresol, resorcinol, and 2-naphthol—yielding yellow, and red azo dyes, respectively. Maximum absorbance for the dye complexes was observed at wavelengths of 420 nm (cresol), 440 nm (resorcinol), and 540 nm (2-naphthol). The method exhibited linearity within the concentration ranges of 3.8–30 μgmL^{-1} for cresol, 0.9–10 μgmL^{-1} for resorcinol, and 3.7–25 μgmL^{-1} for 2-naphthol, in accordance with Beer's law. Molar absorptivity and Sandell's sensitivity were recorded as follows: 2.9 × 10^3 L.mol^{-1}·cm^{-1} and 0.112 μg.cm^{-2} (cresol), 3.4×10^4 L.mol^{-1}·cm^{-1} and 0.0094 μg.cm^{-2} (resorcinol), and 1.4×10^3 L.mol^{-1}·cm^{-1} and 0.23 μg.cm^{-2} (2-naphthol), indicating high sensitivity of the developed method. Precision analysis revealed that the 2-naphthol reagent provided the most accurate results with a coefficient of variation ranging from 0.81% to 2.59%, followed by resorcinol, while the cresol-based method demonstrated comparatively lower precision. This spectrophotometric technique was successfully applied for the determination of CAP in commercial pharmaceutical formulations, including 0.5% eye drops and 1% eye ointments.

1. Introduction:

Antibiotics are used by both humans and animals to treat and prevent bacterial diseases [1]. Improper use and disposal of antibiotics have a detrimental effect on the environment and ecology [2, 3]. The broad-spectrum antibiotic chloramphenicol (CAP) was first produced in 1947 from Streptomyces venezuelae cultures. It quickly gained popularity and is mostly

used as a bacteriostatic agent and a potent inhibitor of bacterial protein synthesis [4, 5]. It is effective against both anaerobic and aerobic bacteria as well as Gram-negative and Gram-positive types [5, 6, 7, 8].

Treatment with CAP remains the best option for severe systemic Arizona infections, as well as salmonella-caused typhoid and paratyphoid fever. Infections of the eyes caused by bacteria, like Bacillus cereus panophthalmitis, are similarly treated with CAP [2, 4, 5, 6, 8].

Because it is affordable and easily accessible, CAP is frequently used and can be given parenterally or orally. Apart from its broad application in managing diverse illnesses, it is also employed in preventing infections in small cuts and in treating a few communicable diseases in birds, bees, and aquaculture [4, 5, 9]. But among the most serious side effects of CAP are bone marrow suppression, aplastic anemia, neurotoxic reactions, leukemia, and gray baby syndrome [2, 4, 5, 7, 9].

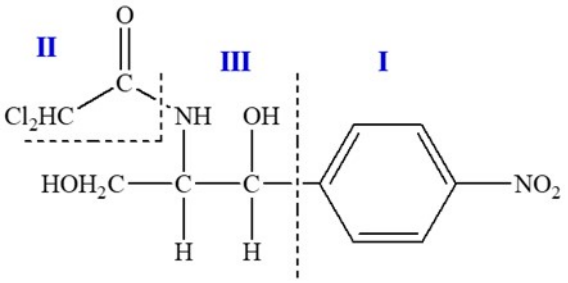
Consequently, the use of CAP for the care of animals used in the production of food products such as eggs, honey, milk, and shrimp has been outlawed by the "European Union" (EU) and numerous other countries [4, 5, 7, 9]. However, CAP is still broadly used in underdeveloped countries because of its great utility, relative affordability, and accessibility [5, 6, 8, 9].

This antibiotic is special since it was the first naturally occurring substance to be discovered with a nitro group and as a derivative of dichloroacetic acid. CAP has gone by a number of generic names. "D-threo-2,2-dichloro-N-[β-hydroxy-α-(hydroxymethyl)-p-nitrophenethyl]-acetamide" is the chosen Chemical Abstracts name, while D(-)threo-2-dichloroacetamido-1-p-nitrophenyl-1,3-propanediol is the term that is used in the literature most frequently [4, 10]. CAP is a finely crystalline powder, needles, or elongated plates that range in hue from white to greyish-white to yellowish-white.

Its chemical structure is $C_{11}H_{12}Cl_2N_2O_5$ (molar mass 323.13 g mol^{-1}), and the range of its melting point (sublimes in high vacuum) is 150.5–151.5 °C. Mildly soluble in water and ether, insoluble in petroleum ether and benzene, and easily soluble in methanol, butanol, ethyl acetate, ethanol, propylene glycol, and acetone [4, 5, 6], and independent of pH between 4 and 6.2 [4, 6, 11]. Scheme 1 shows the chemical structure of CAP.

The significance of this drug has led to the growth of several analytical techniques to determine CAP to date. These techniques include "high-performance liquid chromatography" (HPLC) [12, 13], "liquid chromatography-mass spectrometry" (LC-MS) [9, 14], "gas chromatography- mass spectrometry" (GC-MS) [15], colorimetry [16], electrochemical techniques [17, 18], flow injection chemiluminescence [7], and spectrophotometry [6, 19, 20, 21, 22, 23, 24].

Due to the significance of the drug CAP, which is used more often for medicinal purposes, an effort has been made to verify the spectrophotometric method's accuracy, precision, repeatability, and reproducibility by developing and validating it. The process involves the coupling of the reduced diazotized CAP with one of three coupling reagents (cresol, resorcinol, or 2-naphthol), with the maximum absorbance of each reagent occurring in the visible region. Analytical factors for optimizing azo-dyes have been studied.



Scheme 1. Chemical structure of chloramphenicol (CAP). The p-nitrobenzene moiety (I), the dichloracetyl moiety (II), and the 2-amino-propanediol moiety (III)[6].

2. Material and Methods:

2.1 Apparatus:

Using an ultraviolet-visible (UV-VIS) spectrophotometer (UV1200, Shanghai Yoke Instrument) with a wavelength range of (200–1020) nm and 1 cm matched quartz cells, as well as infrared spectroscopy (IR Spectroscopy NICOLET iS10), all of the spectral measurements were performed. A DENVER instrument model electronic balance and micropipettes measuring 1-10 μl (United), 20-200μl, and 200-1000μl (LAMTEK).

2.2 Chemicals:

Distilled water was used to prepare the solutions, and all of the compounds were of the analytical reagent quality. CAP standard material was provided by "Hawler Medical University - College Pharmacy, Department of Pharmaceutical Chemistry and Pharmacognosy" in Erbil, Iraq. The pharmaceutical preparations of different brands and state forms such as API/phenicol eye drop (Amman Pharmaceutical Industries Co., Jordan 0.5%), RIACHOL, eye drop (Riyadh Pharma Medical and Cosmetic Products Co, Saudi Arabia 0.5%), Aquachlor (eye drop), Allphenicol (eye ointment 1%), Hichlor (eye ointment 1%), and chlorprim (eye ointment 1%) were obtained from different drug industries.

Zinc (metal) dust 99.0% (Thomas Baker, India), Sodium carbonate (Roth, Germany), Sodium nitrite (Roth, Germany), Ortho-Phosphoric acid 85% (Roth, Germany), Urea Extra pure (Sdfcl, India), Ethanol absolute anhydrous (Carlo Erba, France), 2-Naphthol LR (Sdfcl, India), Resorcinol, Cresol, Sulfamic acid, and Ammonia.

2.3 Preparation of the Standard Solutions:

2.3.1 Chloramphenicol Solution (10^4 μgmL^{-1}):

A stock solution was prepared by dissolving 0.5 g of pure CAP in 20 mL of absolute ethanol, and then distilled water was added to complete the volume to 50 mL.

2.3.2 Reduced Chloramphenicol RCAP (500 μgmL^{-1}):

Within a reaction vessel, combine 5 mL of the CAP stock solution (10^4 μgmL^{-1}), 10 mL of distilled water, 20 mL of concentrated hydrochloric acid (37% and 1.18 gmL^{-1}), and 4 g of zinc powder. Following mixing and an hour of standing, the liquid was filtered, and distilled water was added to get the final volume of 100 mL. [6, 20].

2.3.3 RCAP Working Solution (100 μgmL^{-1}):

From the 500 μgmL^{-1} RCAP solution, 20 mL has been taken and placed in a standard volumetric flask. 1.75 g of sodium carbonate was added to the mixture to make the pH 7.0. The filtrate solution was then diluted to 100 mL [20].

2.3.4 2-Naphthol Solution (0.1% w/v):

A solution was prepared by dissolving 0.1 g of 2-naphthol in 100 mL of distilled water and diluted to the mark in a 100 ml volumetric flask.

2.3.5 Resorcinol Solution (0.1% w/v):

A solution was prepared by dissolving 0.1 g of resorcinol in distilled water and diluted to the mark in a 100 ml volumetric flask.

2.3.6 Cresol Solution (0.1% w/v):

A solution was prepared by dissolving 0.1 g of cresol in distilled water and diluting to the mark in a 100 ml volumetric flask.

2.3.7 Sodium Nitrite Solution (0.1% w/v):

A solution was prepared by dissolving 0.1 g of sodium nitrite in distilled water and diluted to the mark in a 100 ml volumetric flask.

2.3.8 Urea Solution (3% w/v):

A solution was prepared by dissolving 3 g of urea in distilled water and diluted to the mark in a 100 ml volumetric flask.

2.3.9 Sulfamic Acid Solution (3% w/v):

A solution was made by dissolving 3 g of sulfamic acid in distilled water and diluted to the mark in a 100 ml volumetric flask.

2.3.10 Phosphoric Acid Solution (1N):

To prepare this solution, 2.27 mL of phosphoric acid (85% and 1.69 gmL^{-1}) was added to a 100 mL volumetric flask containing 20 mL of distilled water, and the volume was then completed to the mark with distilled water.

2.3.11 Ammonia Solution (4M):

To prepare this solution, 30.2 mL of ammonia (25% and 0.90 gmL^{-1}) was added to a 100 mL volumetric flask, and the volume was then completed to the mark with distilled water.

2.3.12 Sodium Carbonate Solution (4M):

This solution was prepared by dissolving 42.4 g of sodium carbonate in distilled water and diluted to the mark in a 100 ml volumetric flask.

2.4 Procedure for Calibration Curve:

2.4.1 Using 2-Naphthol Reagent:

To prepare 100 μgmL^{-1} diazonium salt, take 10 mL of RCAP solution (500 μgmL^{-1}) and combine it with 5 mL of $NaNO_2$ in a 50 mL volumetric flask. Finally, fill a volumetric flask to the mark with distilled water.

A various concentration of 1, 3, 18, 23, and 25 μgmL^{-1} from RCAP (100 μgmL^{-1}) was added to five 25 mL volumetric flasks. Following this, 1 mL of urea (3%), 2 mL of 2-naphthol (0.1% w/v), and 5 mL of phosphoric acid (1N) were added, and the volume was adjusted with distilled water to the desired amount. Following 10 minutes in an ice bath, the absorbance was measured at 540 nm using a "UV-VIS spectrophotometer" against a blank solution that was made similarly but did not contain RCAP.

2.4.2 Using Resorcinol Reagent:

To prepare 100 μgmL^{-1} diazonium salt, take 10 mL of RCAP solution (500 μgmL^{-1}) and combine it with 10 mL of $NaNO_2$ in a 50 mL volumetric flask. Finally, fill a volumetric flask to the mark with distilled water. The concentration range for the diazotized RCAP (100 μg mL) in six 25 mL volumetric flasks is added (0.3, 0.5, 1, 3, 8, 10) μgmL^{-1}. Next, add 1 mL of sulfamic acid, 2 mL of resorcinol (0.1% w/v), and 1.5 mL of ammonia.

The concentration mixture was standing for 10 minutes in an ice bath. Finally, complete the volume to 25 mL with distilled water. Each result solution absorbance was measured at 440 nm using a "UV-VIS spectrophotometer". Similar procedures were used to prepare a blank solution; however, RCAP solution was not added

2.4.3 Using Cresol Reagent:

Take 10 mL of RCAP solution (500 μgmL^{-1}) and mix it with 10 mL of $NaNO_2$ to prepare 100 μgmL^{-1} diazonium salt. Finally, complete the volume to 50 mL with distilled water into a volumetric flask. A concentration range of 2, 5, 8, 10, 20, 30 μgmL^{-1} of diazotized RCAP was used for six 25 ml volumetric flasks. Next, add 1 mL of urea, 2 mL of cresol, and 2 mL of sodium carbonate, let them stand for two minutes in an ice bath, and then fill the flasks to the mark 25 mL with distilled water. Similar procedures were used to prepare a blank solution, however RCAP solution was not added. Each result solution absorbance was measured at 420 nm using a "UV-VIS spectrophotometer".

2.5 Preparation of Pharmaceutical Formulations:

2.5.1 CAP Eye Drop Solutions (500 μgmL^{-1}):

Various brands of eye drop formulations from various drug firms have been utilized; each brand has five eye drop bottles with a 0.5% CAP quantity. The contents of five containers were mixed for each of API/phenicol, RIACHOL, and Aquachlor. A 50 ml volumetric flask was filled with an aliquot equal to 100 mg of CAP (20 mL), which was combined with 20 ml of ethanol and further diluted with distilled water to the required level.

To get a final solution of 500 μgmL^{-1} of RCAP, this solution was put into a 250 mL beaker, reduced as previously mentioned, and diluted to a 100 mL volume with distilled water. The remaining stages of coupling and diazotization were carried out following the usual CAP procedure.

2.5.2 CAP Eye Ointment Solutions (500 μgmL^{-1}):

The eye ointment formulas from several drug companies have been used; each brand comprises five tubes of eye ointment with a quantity of 1% CAP. For every tube of chlorprim, hichlor, and allphenicol, the contents of five tubes were combined. Three times, using 10 mL of ethanol each time, a precisely weighed quantity of ointment equal to 50 mg of CAP was extracted [6] The ethanol fraction should be taken, filtered, and then diluted to 50 mL. 500 μgmL^{-1} of RCAP was obtained by transferring this solution into a 250 mL beaker, diluting it with distilled water to a 100 mL volume, and reducing it as previously mentioned. Following the standard CAP protocol, the final process of diazotization and coupling was completed.

2.6 Statistical Analysis:

The experiments were performed in triplicate. The results are expressed as mean values ± standard deviation (SD). The Excel software version (2021) was used to perform statistical analysis.

3. Results and Discussion:

Based on the diazotization process, CAP was determined in this work using a "UV-VIS spectrophotometric" technique. The Griess reaction has been extensively utilized to quantify nitrate in a variety of samples, including water, soil, vegetables, meat products, and other materials [25, 26]. It is based on the diazotization of aromatic amine and coupling the result with phenols or aromatic amines. In this work, it was applied to estimate CAP in different samples. Since CAP contains -NO_2 group, reducing CAP (RCAP) is the initial step Scheme 2.

The procedure consists of the following three steps for each coupling reagent: cresol, resorcinol, and 2-naphthol:

Step 1: Utilizing zinc powder and concentrated HCl solution, reduce the nitro group on the CAP structure to an amine group (RCAP).

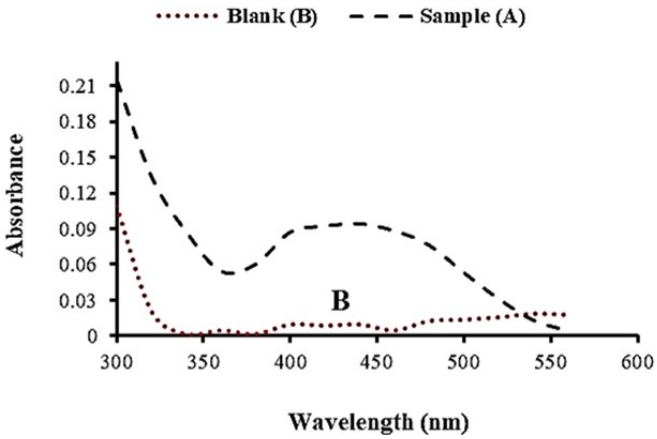


Figure 1. Absorption spectra of the solution prepared by coupling 8 μgmL^{-1} diazotized RCAP with cresol (A) against blank solution (B).

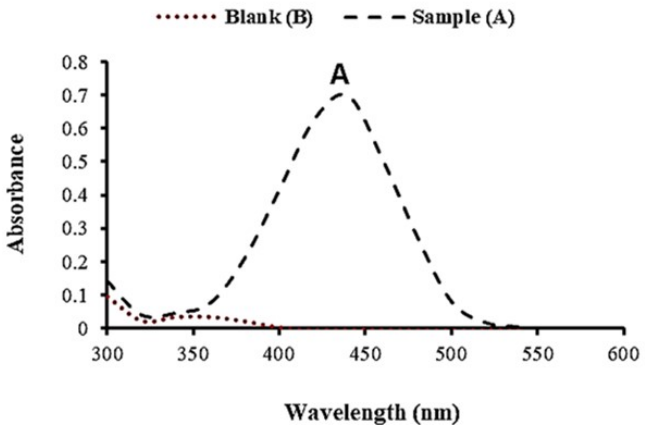
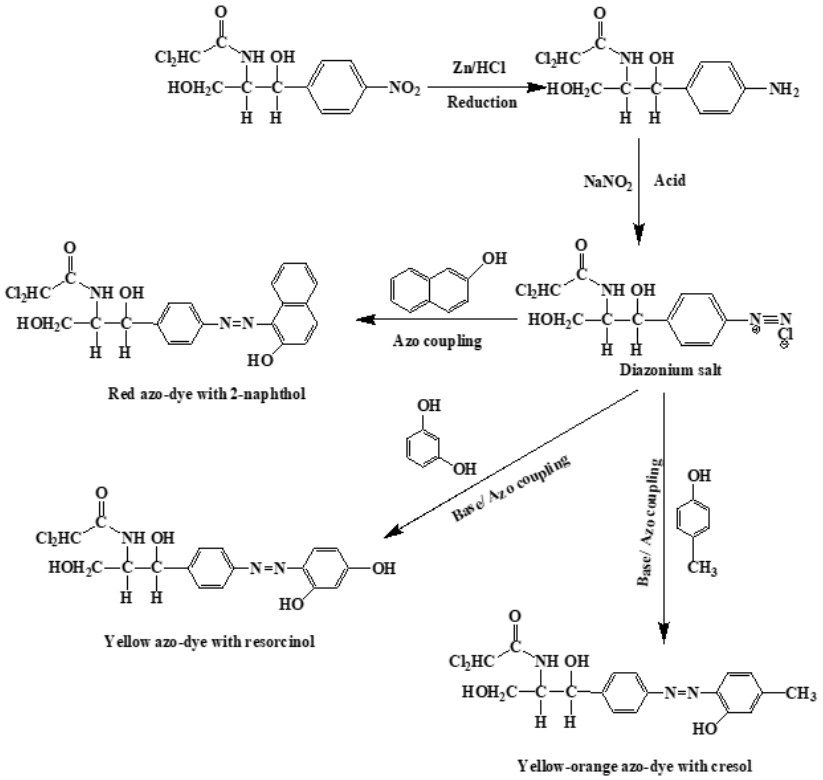


Figure 2. spectra of the solution prepared by coupling 8 μgmL^{-1} diazotized RCAP with resorcinol (A) against blank solution (B).

Step 2: In the presence of mineral acid, the RCAP reacts with $NaNO_2$ to generate a diazonium salt.

Step 3: The following requirements were followed for coupling the diazo compound with the reagents of cresol, resorcinol, and 2-naphthol:

a- An azo-dye that is colored and formed at room temperature between cresol and diazonium salt is produced when sodium carbonate is utilized as the base medium. A yellow azo-dye is created when the product stands for two minutes in an ice bath (-2 °C). This dye absorbs light with a wavelength of 420 nm, as shown in Figure 1.



Scheme 2. Possible reaction pathway for the azo-coupling reaction between RCAP and three different coupling agents [6]

b- After 10 minutes of standing at low-temperature (-2 °C), resorcinol and the diazonium salt react to form a yellow azo-dye that at room temperature, in the presence of ammonia, absorbs light at a maximum wavelength of 440 nm see Figure 2.

c- After standing in a cold bath for 10 minutes (-2 °C), the diazo compound coupled with the 2-naphthol reagent at room temperature to generate a colored product of red azo-dye, which absorbs maximum absorbance at wavelength 540 nm see Figure 3.

3.1 Fourier Transform Infrared (FTIR) Spectral Analysis:

Given its characteristics as an analytical technique for fingerprints, FTIR spectroscopy may be regarded as a selective method. Since it contains several crucial regions for differentiation, the wave number between 4000 and 650 cm^{-1} is frequently employed for sample measurement. There are certain similar peaks in the FTIR spectra of CAP and RCAP, which are displayed in Figures 4 and 5. O-H and N-H stretching corresponds to the peak at wave number 3400-3250 cm^{-1}.

For the aromatic C-H stretching, a peak of around 2990 cm^{-1} is assigned. The carbonyl group (C=O) stretching vibration is linked to the strong peak at 1650/1640 cm^{-1}. The C-O stretching of alcohol group is linked to the strong peak at 1046/1050 cm^{-1}. Some of the peaks, meanwhile, were only identified for CAP and were not found in RCAP. The functional group of -CH2 was visible at wave number 1460 cm^{-1}, however the presence of a functional group of N=O from (R-NO2) was assigned form peak at 1390 cm^{-1}.

3.2 Reaction Condition Optimization for UV-VIS Spectrophotometry:

3.2.1 Study the Effect of the Amount of Coupling Reagent:

This study was looked into several variables that affect the rate at which reactions proceed. One factor is to look at how the amount of reagent affects the approach's sensitivity. To do this, several solutions ranging in volume from 1 to 5 mL of each reagent cresol, resorcinol, and 2-naphthol, with a (0.1% w/v) concentration were investigated. Based on the highest reading of the standard's absorbance and maximum sensitivity, 2 mL of each reagent is found to be the ideal volume Figure 6.

3.2.2 Study the Effect of the Type and the Amount of Removal Agent:

A common procedure for creating azo dyes involves first diazotizing the aromatic amine that will be the diazo component, and then, in the second step, reacting the diazotized amine

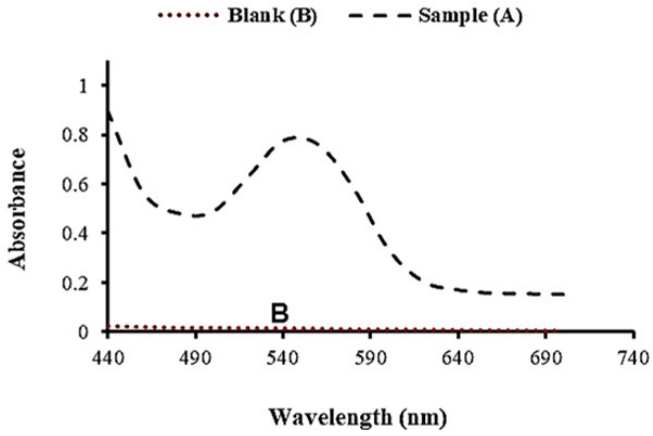


Figure 3. Absorption spectra of solutions prepared by coupling 8 μgmL^{-1} diazotized RCAP with 2-naphthol (A) against blank solution (B).

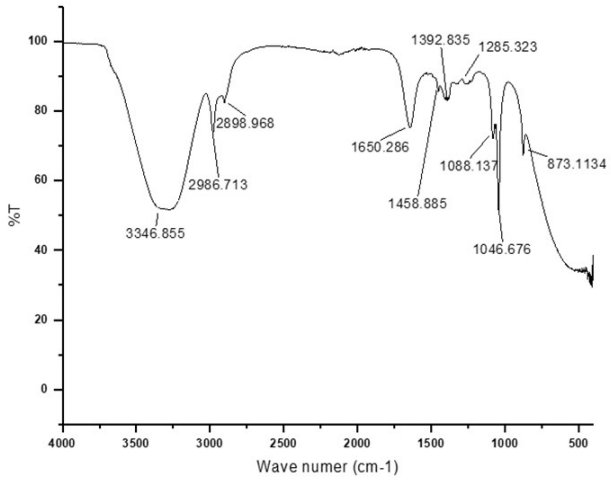


Figure 4. FTIR spectrum of CAP solution.

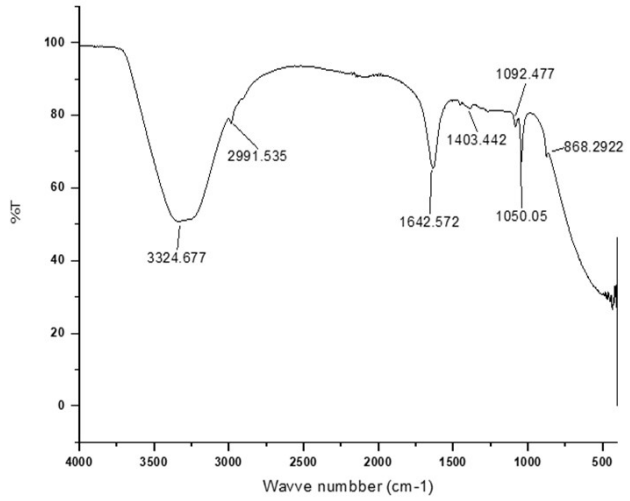


Figure 5. FTIR spectrum of RCAP solution.

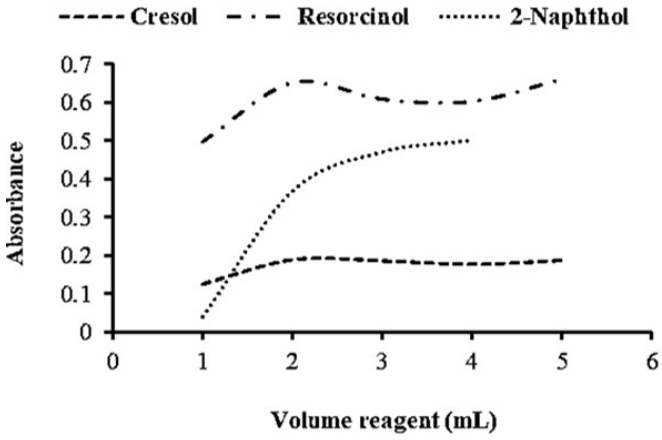


Figure 6. Optimization of the quantity of coupling reagents with diazotized RCAP at the concentration (0.1% w/v) of cresol, resorcinol, and 2-naphthol

with the suitable coupling component. Typically, diazotization is accomplished by adding an excessive amount of nitrite, such as sodium nitrite, to a mineral acid solution. Before azo coupling may happen, excess nitrite needs to be eliminated once diazotization is finished. Usually, urea, amido sulfonic acid (sulfamic acid), or non-diazotized amine in small amounts are added to achieve this [26].

The effects of two distinct nitrite-excess removal agents have been investigated using sulfamic acid and urea at equal quantities (3% w/v). According to the findings, 1 mL of sulfamic acid is the maximum amount that may be used to eliminate excess sodium nitrite when resorcinol is used as a coupling agent. However, when using 1 mL of urea instead of sulfamic acid, cresol, and 2-naphthol work better. As seen in Figures 7 and 8, 1 mL is sufficient to eliminate the excess of sodium nitrite.

3.2.3 Study the Effect of the Type and the Amount of Base: Three distinct bases were used in this investigation. Based on the findings of employing 2-naphthol and cresol, sodium carbonate was found to be the most effective alkaline medium for maximum absorption; this strategy was adopted in all subsequent testing, as shown in Table 1. Using different amounts of sodium carbonate reveals that 2 mL is adequate to be the best environment for cresol because it has the highest absorbance values see Figure 9. On the other hand, ammonia was determined to be the best alkaline medium for utilizing resorcinol Table 1, with 1.5 mL being the perfect volume for this procedure Figure 9.

3.2.4 Study the Effect of the Amount of Sodium Nitrite: The quantity of sodium nitrite reagents is essential for producing a diazonium product. The absorbance of a solution

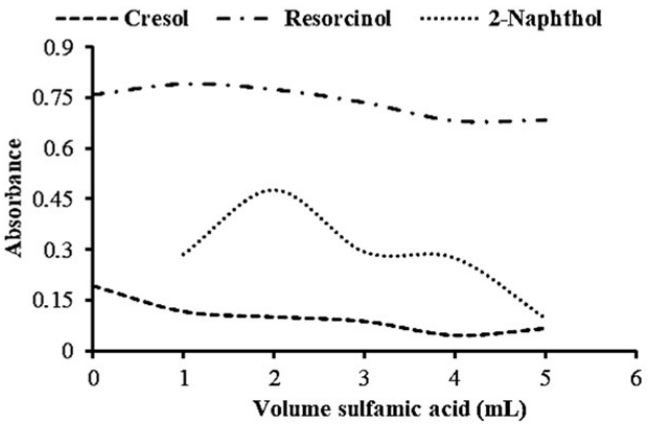


Figure 7. Optimizing the sulfamic acid quantity when diazotized RCAP is combined with cresol, resorcinol, and 2-naphthol.

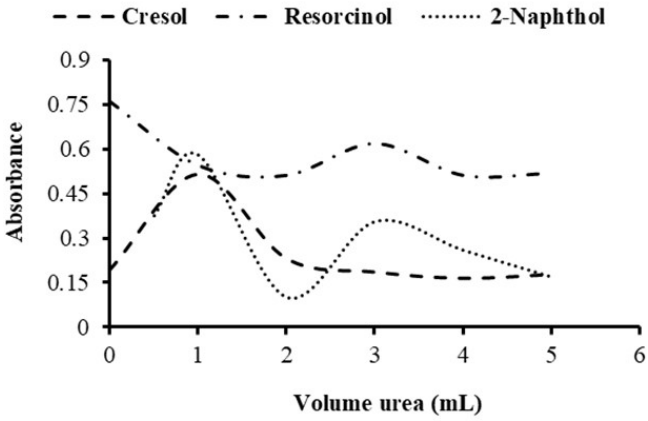


Figure 8. Optimizing the urea quantity when diazotized RCAP is combined with cresol, resorcinol, and 2-naphthol.

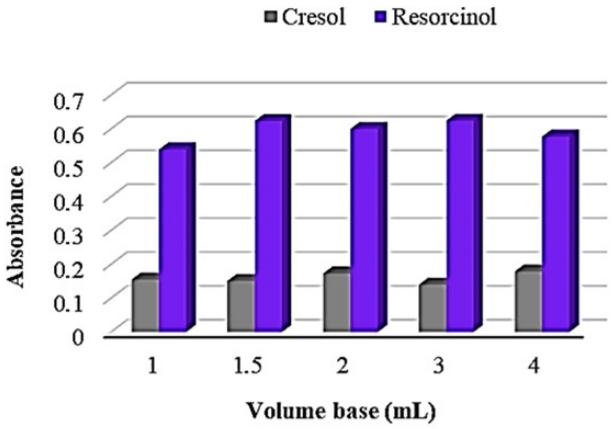


Figure 9. Optimizing the ammonia quantity for resorcinol and sodium carbonate quantity for cresol.

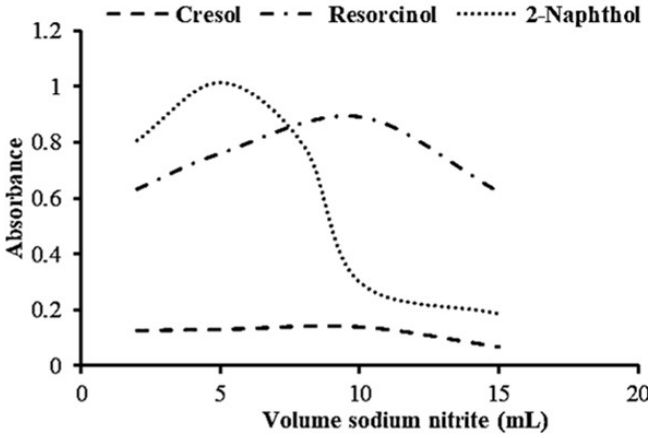


Figure 10. Optimizing the quantity of sodium nitrite when diazotized RCAP is combined with cresol, resorcinol, and 2-naphthol.

Table 1. Study the effect of the type of base used for the reaction progress.

Type of base	Absorbance		
	Cresol	Resorcinol	2-Naphthol
Diethyl amine	0.021	0.364	0.358
Ammonia	0.163	0.512	—
Sodium carbonate	0.173	0.498	0.582

containing diazotized CAP has been examined with varying amounts of sodium nitrite (0.1% w/v). Figure 10 illustrates the results of this experiment, which indicated that 5 ml was the ideal volume for employing 2-naphthol and 10 mL for both cresol and resorcinol.

3.2.5 Study the Effect of the Type and the Amount of Acid:

The kind and concentration of the mineral acid influence the use of 2-naphthol as a coupling agent. Using three acid solutions at the same concentration (1N), the acidity of the medium was investigated see Table 2. Using both phosphoric acid and formic acid, the reaction's progress was shown to produce equivalent results. But in this experiment, 5 mL of phosphoric acid (1N) was selected since, in this case, the reaction is less susceptible to the media environment than in other cases, and the synthesis of the red dye produced the greatest absorbance at this concentration.

3.3 Method Validation:

Method validation is necessary for both the creation of reference techniques and the assessment of a laboratory's ability to generate accurate analysis records. The framework of the process that produced the chemical data includes val-

Table 2. Study the effect of the type and the amount of acids used for the reaction progress.

Volume (mL)	Absorbance		
	Formic acid	Acetic acid	Phosphoric acid
2	0.437	0.019	0.657
5	0.582	0.006	0.789
8	0.465	0.014	0.73
10	0.37	0.009	0.687
Product color	**Red**	**Yellow**	**Red**

idation. To avoid their improper use and ensure scientific accuracy and consistency, analytical method validation, taking into account the most appropriate practices for examining the best parameters of analytical methods, and using a variety of pertinent overall performance indicators, including linearity, selectivity, specificity, precision, accuracy, range, "limit of detection" (LOD), "limit of quantification" (LOQ), robustness and ruggedness, heavily discussed[27].

i Linearity of the Method:
The capacity of an analytical method to produce test results that are proportionate to the analyte concentration in a certain range, either directly or through well-specified mathematical modifications, is known as linearity. Linearity can be thought of as a type of internal or relative precision for a particular focal point. It shows how well a system responds to a series of dilutions in the right matrix. When the correlation coefficient (R2), which was derived from the calibration curve (Y= a + mX), is larger than 0.9, the linear relationship between absorbance and concentration is evaluated [27]. Here, X is the CAP concentration in μgmL^{-1}, Y is the absorbance, m is the slope, and a is the y-intercept.

After RCAP was diazotized and coupled with the three coupling reagents under ideal reaction conditions, a standard calibration curve was created by combining CAP at various concentrations. Plotting absorbance against CAP concentration reveals that, employing cresol, resorcinol, and 2-naphthol, respectively, the generated dye follows Lambert Beer's law from (3.8-30) μgmL^{-1}, (0.9-10) μgmL^{-1}, and (3.7-25) μgmL^{-1} of CAP Figure **??**. The statistical results are shown in Table3.

ii Precision of the Method:
Tables 4, 5, 6 present the calculated results for the suggested approach's linearity and accuracy. It is commonly described as a measure of consistency using the average, "standard deviation" (SD), and "coefficient

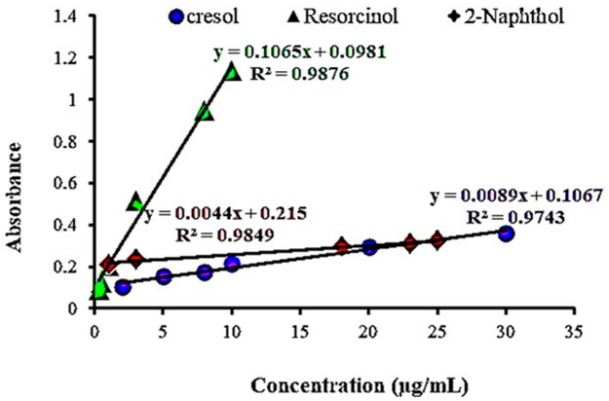


Figure 11. With ideal experimental conditions, the Lambert-Beer calibration curves for RCAP couples with cresol, resorcinol, and 2-naphthol, with max values of 420 nm, 440 nm, and 540 nm, respectively.

of variation" (CV). Comparing the diazotized CAP solution measurements taken in three replicates allowed for the evaluation of the experiment's reproducibility. Table 4 displays the moderate range of CV, whereas Table 5 displays the low range of 0.5-5.1% for resorcinol, and Table 6 displays the best range of 0.8-2.6% for 2-naphthol. These findings are noteworthy. It was predicted that lower CV ranges would follow from higher CAP concentrations. In general, results with coefficients of variation up to 5% can be accepted for analytical techniques. If they go below 1%, it would be fantastic.

iii Sensitivity of the Method:
The sensitivity of the method can be assessed using a number of factors, including molar absorptivity, Sandell's sensitivity, "LOD", and "LOQ" [27]. The molar absorptivity and Sandell's sensitivity values shown in Table 4 suggest that resorcinol is the most sensitive technique. Table 7 presents a comparison of the recommended method's sensitivity with other spectrophotometric methods that have been proposed in the literature.

3.4 Application of the Proposed Methods to Pharmaceutical Formulations:

Many brands of eye drop formulations from different pharmaceutical companies were used; these included eye drops and eye ointments. The formulations were taken in the linearity range and processed under the preparation protocols, following the generally advised procedure. Additionally, a "UV-VIS spectrophotometer" was used to assess the absorbance of the resulting solution mixture in the presence of cresol, resorcinol, or 2-naphthol at 420 nm, 440

Table 3. Study the effect of the type and the amount of acids used for the reaction progress.

Parameters	Results with cresol	Results with resorcinol	Results with 2-naphthol
Color of the product	Yellow	Yellow	Red
Wavelength at maximum absorption (λmax, nm)	420	440	540
Regression equation	y = 0.0089x + 0.1067	y = 0.1065x + 0.0981	y = 0.0044x + 0.215
Linear range (μgmL^{-1})	3.8-30	0.9-10	3.7-25
Intercept (a)	0.1067	0.0981	0.215
Slope (m)	0.0089	0.1065	0.0044
Standard error of slope (Sm)	0.00073	0.0059	0.0003
Standard error of intercept (Sa)	0.0114	0.0322	0.0054
Determination coefficient (R2)	0.9743	0.9876	0.9849
Molar absorptivity (ε, L/mol.cm)	2.9×10^3	3.4×10^4	1.4×10^3
Sandell's sensitivity (μg/cm2)	0.112	0.009	0.227

Table 4. The experimental findings for measuring the amount of diazotized RCAP at various concentrations (from 2 to 30μgmL^{-1}) at 420 nm using cresol.

concentration (μgmL^{-1})	Abs.1	Abs.2	Abs.3	Abs.(average)	SD	CV
2	0.078	0.105	0.124	0.102	0.0231	22.6471
5	0.146	0.154	0.172	0.157	0.0133	8.4733
8	0.182	0.175	0.175	0.177	0.0040	2.2599
10	0.21	0.218	0.218	0.215	0.0046	2.1395
20	0.281	0.297	0.309	0.296	0.0140	4.7297
30	0.372	0.363	0.351	0.362	0.0105	2.9006

Table 5. The experimental findings for measuring the amount of diazotized RCAP at various concentrations (from 0.3 to 10 μgmL^{-1}) at 440 nm using resorcinol.

concentration (μgmL^{-1})	Abs.1	Abs.2	Abs.3	Abs.(average)	SD	CV
0.3	0.086	0.087	0.089	0.087	0.0015	1.7241
0.5	0.125	0.126	0.125	0.125	0.0006	0.48
1	0.201	0.205	0.204	0.203	0.0021	1.0345
3	0.528	0.492	0.525	0.515	0.0200	3.8834
8	0.977	0.890	0.970	0.946	0.0483	5.1057
10	1.133	1.123	1.165	1.140	0.0219	1.9211

Table 6. The experimental findings for measuring the amount of diazotized RCAP at various concentrations (from 1 to 25 μ g/mL) at 540 nm using 2-naphthol.

Concentration (μgmL^{-1})	Abs.1	Abs.2	Abs.3	Abs.(average)	SD	CV
1	0.206	0.215	0.216	0.2123	0.0055	2.5907
3	0.238	0.237	0.232	0.2357	0.0032	1.3577
18	0.293	0.297	0.298	0.2960	0.0026	0.8784
23	0.308	0.313	0.31	0.3103	0.0025	0.8057
25	0.323	0.334	0.326	0.3277	0.0057	1.7394

Table 7. The analytical data generated by the suggested procedure is contrasted with that of other procedures used in the literature.

Parameters	Proposed method			Method (I) [20]	Method (II)[6]	Method (III) [6]
Reagent	Cresol	Resorcinol	2-Naphthol	Promethaizne.HCl	Chromatropic acid	Phenol
Medium	Alkaline	Alkaline	Acid	Acid	Alkaline	Alkaline
Color of the Product	Yellow	Yellow	Red	Blue-green	Red-violet	Yellow
Wavelength at maximum absorption (λmax, nm)	420	440	540	606	515	432
Linear range (μgmL^{-1})	3.8-30	0.9-10	3.7-25	0.4-12	0.5-12	0.4-18
Determination coefficient (R2)	0.9743	0.9876	0.9849	0.9943	0.9979	0.9994
Molar absorptivity (ε, L/mol.cm)	4.6×10^3	3.85×10^4	4.7×10^3	12.9×10^3	$12.4\text{x}10^3$	14.9×10^3
Sandell's sensitivity (μg.cm^{-2})	0.112	0.0094	0.23	——	——	——

Table 8. CAP content measured in pharmaceutical samples with the cresol reagent.

Formulation name	Amount of CAP (μgmL^{-1})		Amount of CAP (%)		Erel.%	Recovery%
	Labelled commercially	Determined ± SD*	Labelled commercially	Determined		
Aquachlor (eye drop)	13	13.15±0.17	0.00130	0.00131	1.15	101.15
	25	26.24±0.31	0.00250	0.00262	4.96	104.96
Chlorprim (eye ointment)	13	12.97±0.70	0.00130	0.00129	-0.23	99.77
	25	26.13±0.63	0.00250	0.00261	4.52	104.52

*SD, standard deviation for triplicate determinations

Table 9. CAP content measured in pharmaceutical samples with the resorcinol reagent.

Formulation name	Amount of CAP (μgmL^{-1})		Amount of CAP (%)		Erel.%	Recovery%
	Labelled commercially	Determined ± SD*	Labelled commercially	Determined		
Aquachlor (eye drop)	4	3.92±0.28	0.00040	0.00039	-2.00	98.00
	6	5.67±0.03	0.00060	0.00057	-5.50	94.50
Chlorprim (eye ointment)	5	4.94±0.05	0.00050	0.00049	-1.20	98.80
	6	5.67±0.04	0.00060	0.00057	-5.50	94.50
[a]API, phenicol (eye drop)	3	3.05±0.03	0.00030	0.00031	1.67	101.67
	6	5.88±0.03	0.00060	0.00059	-2.0	98.00
[b]RIACHOL (eye drop)	3	3.11±0.08	0.00030	0.00031	5.33	105.33
	6	6.23±0.27	0.00060	0.00062	3.83	103.83

*SD, standard deviation for triplicate determinations
[a] Amman Pharmaceutical Industries Co., Jordan
[b] Riyadh Pharma Medical and Cosmetic Products Co, Saudi Arabia

Table 10. CAP content measured in pharmaceutical samples with the 2-naphthol reagent.

Formulation name	Amount of CAP (μgmL^{-1})		Amount of CAP (%)		Erel.%	Recovery%
	Labelled commercially	Determined ± SD*	Labelled commercially	Determined		
[a]API, phenicol (eye drop)	3	3.13±0.22	0.00030	0.00031	4.33	104.33
	22	21.77±1.84	0.00220	0.00218	-1.05	98.95
[b]RIACHOL (eye drop)	3	3.06±1.24	0.00030	0.00031	2.00	102.00
	22	21.39±1.80	0.00220	0.00214	-2.77	97.23
Allphenicol (eye ointment)	5	4.86±0.65	0.00050	0.00049	-2.80	97.20
	23	22.07±1.32	0.00230	0.00221	-4.04	95.96
Hichlor (eye ointment)	9	8.90±0.56	0.00090	0.00089	-1.11	98.89
	23	22.22±1.25	0.00230	0.00222	-3.39	96.61

*SD, standard deviation for triplicate determinations [a] Amman Pharmaceutical Industries Co., Jordan [b] Riyadh Pharma Medical and Cosmetic Products Co, Saudi Arabia

4. Conclusions:

The amount of CAP in pharmaceutical formulations has been ascertained by employing a technique that relies on the drug's diazotization coupling reaction with three unique coupling agents. To control the amount of colored azo-dye produced, the drug dosage is utilized. The approach has been evaluated for accuracy using the assay of CAP in pharmaceutical formulations (eye drops and ointment), and the outcomes have shown promise. Because the current method is sufficiently straightforward and accurate, CAP can be routinely evaluated in both pure form and in various dosage forms. The suggested approach eliminates the need to describe steps that are typically connected to other processes, like chromatographic techniques.

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Data Availability Statement: All of the data supporting the findings of the presented study are available from corresponding author on request.

Declarations:

Conflict of interest: We hereby confirm that the document contains all of our own illustrations, tables, and diagrams.

Ethical approval: Ethical approval is not applicable for this article.

Author contributions: All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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التقدير الطيفي للكلورامفينيكول في المستحضرات الصيدلانية باستخدام تفاعل الازوتة والاقتران

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الخلاصة
الكلورامفينيكول (*CAP*) ، وهو مضادّ حيوي واسع الطيف، جرى تحليله كمّيًا في صيغ دوائية مختلفة باستخدام طريقة قياس طيفيّة سريعة وحسّاسة في المجال المرئي البنفسجي، مع توصيف المركّب بواسطة مطيافية الأشعّة تحت الحمراء بتحويل فورييه (*FTIR*) أُنجز اختزال *CAP* بمسحوق الزنك بوجود حمض الهيدروكلوريك المركّز، تلاه إجراء الديازوتة باستعمال نتريت الصوديوم ($NaNO_2$) .ثمّ أُقْرِن ملح الديازونيوم الناتج بثلاثة كواشف مُولِّدة للّون ـ الكريزول، والريسورسينول، و −2 نفثول مكوّنًا أصباغ أزو صفراء وحمراء تبعًا للكاشف. سُجِّلت قيم الامتصاص العظمى عند أطوال موجية بلغت 420 نانومتر (الكريزول)، 440 نانومتر (الريسورسينول)، و 450 نانومتر (2− نفثول). وأظهرت الطريقة خطيّة ضمن مجالات التركيز: 30 − 3.8 ميكروغرام / مل للكريزول، و 10 − 0.9 ميكروغرام / مل للريسورسينول، و 25 − 3.7 ميكروغرام / مل لـ −2 نفثول، وذلك وفق قانون بير. سُجِّلت الامتصاصية المولارية وحساسية ساندل كما يأتي: 2.9×10^{3} لتر مول 1− سم 1− و 0.112 ميكروغرام سم 2− (الكريزول)، و 3.4×10^{4} لتر مول $^{-1}$ سم $^{-1}$ و 0.0094 ميكروغرامسم $^{-2}$ (الريسورسينول)، و 1.4×10^{3} لترمول $^{-1}$ سم $^{-1}$ و 0.23 ميكروغرام سم $^{-2}$ (2 ـ نفثول)، بما يدلّ على الحساسية العالية للطريقة المطوَّرة. وأظهرت دراسة الدقّة أنّ كاشف −2 نفثول أتاح أدقّ النتائج بمعامل تغاير تراوح بين 0.81% و 2.59% ، تلاه الريسورسينول، في حين أبدت طريقة الكريزول دقّة أدنى نسبيًّا. وقد طُبِّقت هذه التقنية الطيفيّة بنجاح لتعيين تركيز الكلورامفينيكول في مستحضرات دوائية تجارية، منها قطرات عينية بتركيز 0.5% ومراهم عينية بتركيز 1% .

الكلمات الدالة : الاصباغ الازوية، الكلورامفينيكول، تفاعل الاقتران، الكريزول، 2− نفثول

التمويل: لايوجد

بيان توفر البيانات: جميع البيانات الداعمة لنتائج الدراسة المقدمة يمكن طلبها من المؤلف المسؤول.

اقرارات: تضارب المصالح: يقر المؤلف انه ليس لديهم تضارب في المصالح.

الموافقة الأخلاقية: لم يتضمن هذا البحث اي تجارب على البشراوالحيوانات بالتالي لم يكن من الضروري الحصول على الموافقة الاخلاقية.

مساهمات المؤلفين: قام جميع المؤلفين بمراجعة النسخة النهائية المراد نشرها ووافقوا على تحمل المسؤولية عن جميع جوانب العمل.