Spectrophotometric Determination of Chloramphenicol in Bulk and Pharmaceutical Preparation

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Abstract

Chloramphenicol is spectrophotometrically determined by the formation of a colored charge transfer exhibiting λ_{max} at 445 nm after the reaction of the secondary amine with sodium nitroprusside in the presence of NH_2OH in alkaline medium. Classical univariate and chemometric central composite experimental design approaches were used to find the optimum experimental conditions for the parameters affecting the formation of CT-complex. The proposed method is simple and sensitive for the determination of the drug in a concentration rage of 1.0-25.0 µg.mL^{-1} with molar absorptivity8.142 x 10^{3}L.mol^{-1}.cm. and r= 0.9995. The validity of the method was confirmed by finding the regression equation, accuracy, precision, and detection limit. Chloramphenicol was successfully determined in its pharmaceutical preparations by the developed procedure with a reasonable of recovery and precision. Statistical validation of the obtained results was made and the method shows acceptable recovery and repeatability.

Key words: *chloramphenicol determination, spectrophotometry,central composite experimental design.*

Introduction

Chloramphenicol (CAP) is a powerful bacterial protein synthesis inhibitorfirstly isolated from cultures of *Streptomyces venezuelae*[1, 2]. It is used for salmonella infections (i.e. typhoid and paratyphoid fever and in severe systemic Arizona infections) and in various bacterial eye infections which occurs particularly in drug abusers. Chemically, the name of the drug is 2,2-Dichloro-N-[1,3-dihydroxy-1-(4-nitrophenyl)-2-propanyl]acetamide and its chemical formula is and molar mass 323.132 g/mol. The structural formula of CAP is given in Figure 1.

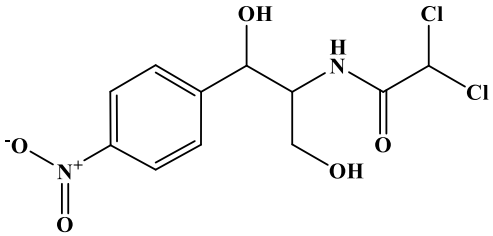


Figure 1: The structural formula of chloramphenicol.

Several analytical methods have been reported for the determination of chloramphenicol CAP in both pure form and as pharmaceutical preparations. Including determination of Simple photometric and colorimetric determinations of chloramphenicol were investigated [3-5]. Determination of chloramphenicol in tablets by electrogenerated chemiluminescence's coupled to flow injection analysis (FIA)(6), high performance liquid chromatography (HPLC) (7), spectrophotometry (8, 9).

This works aims to introduce a new simple and sensitive method for spectrophotometric determination of CPA in its pure form and in pharmaceutical preparations after complexation with sodium nitroprusside. The CT complex is formed in alkaline medium the presence of NH2OH.HCl(10). Univariate and chemometric central composite design (11) were followed for optimization experimental variables.

Experimental

Instrument

CECLL CE 7200. UK (7000 series) Double beam UV- Visible spectrophotometer with 10 mm quartz cell.

Reagents:

All reagents and chemicals used were of Analar grade. Chloramphenicol powder was obtained from the State Company for Drug Industries and Medical Appliances Samara- Iraq (SDI)in pure form (99.99%).

- Sodium carbonate -1- hydrate solution(6% w/v): six grams of the reagent was dissolved in distilled water and the volume of the solution was made to the mark with the same solvent in a 100 mL-volumetric flask.
- Chromogenic reagent (SNP) solution: 100mL of [0.8% (w/v) sodium nitroprusside and 0.186% (w/v) Hydroxylamine hydrochloride]. Prepared by dissolving 0.8 g of sodium nitroprusside and 0.186 g of hydroxylamine hydrochloride in distilled water. The mixed solution was then diluted to 100 mL in a volumetric flask.

Preparation of standard stock solution (200 µg.mL^{-1})

0.02 gm of pure chloramphenicol powder was dissolved in 3 mL of 6% Na_2CO_3 solution. The resulted solution was diluted to 100 mL with distilled water. Working standard solutions were prepared daily by serial dilutions of the stock solution as required with distilled water.

Preparations of sample solutions

1- In Capsule

The contents of 10 capsules were individually weighted out to get the average weight of the capsules. An amount of the powder equivalent to 0.02 gm. of CAP was weighted, then about 5 mL of 6% Na_2CO_3 was added, the mixture was transferred into 100 mL volumetric flask, and the flask contained was swirled. Distilled water was then added to make the volume 100 mL to get 200 µg.mL^{-1} solution of chloramphenicol. The undissolved materials were filtered-out by passing

them through a filter paper (Whatman No.41) before using distilled water to prepare more dilute working solution by subsequent dilutions and applying the recommended procedure.

2- In Eye Drop

4 mL of the sample solution was transferred into a 100 mL volumetric flask, 5 mL of 6% Na_2CO_3 was the added with continuous swirling. The mixture was left to stand for 5 min. before dilution to the mark with distilled water to get 200 $\mu g.mL^{-1}$ of chloramphenicol solution. More dilute solutions were freshly prepared as required via dilutions with distilled water, and the recommended procedure was applied for their analysis.

Recommended Procedures

i- Under conditions established via univariate method

Into a series of 10mL-calibrated flasks containing 2.5 mL of chromogenic reagent (SNP) solution, different volumes of the standard (200 µg/mL) solution containing (10-250) µg of chloramphenicol were transferred. The formed mixtures were shaken for 3 minutes at 25 °C followed by the addition of 0.5 mL of 6%Na_2CO_3 to each solution and the flasks were kept aside for 10 minutes in dark. Thereafter, the volumes of the mixtures were made with distilled water to the mark. The absorbance values were measured at 451.0 nm, after mixing and homogenizing the solutions, against the reagent blank.

ii- Under conditions established via chemometric multivariate

aliquots of the standard solution 200 µg/mL containing (5- 260) µg of chloramphenicol were added to a set of 10 mL-calibrated flasks that contain 2.04 mL of chromogenic reagent (SNP) solution were shaken for 3 minutes at 25 °C. Then 0.85 mL of 6%Na_2CO_3 was added to each mixture and the solutions were kept for two and half minutes in dark. The total volume in each flask was brought to the mark with distilled water and mixed well before measuring the value of absorbance at 451.0 nm against blank solution.

Results and discussion

Absorption spectra

Absorption spectrum for the reaction of chloramphenicol with SNP in presence of hydroxylamine under alkaline conditions was recorded under the optimum conditions and showed the maximum absorption at 451.0 nm for the red color against the reagent blank Fig [2].

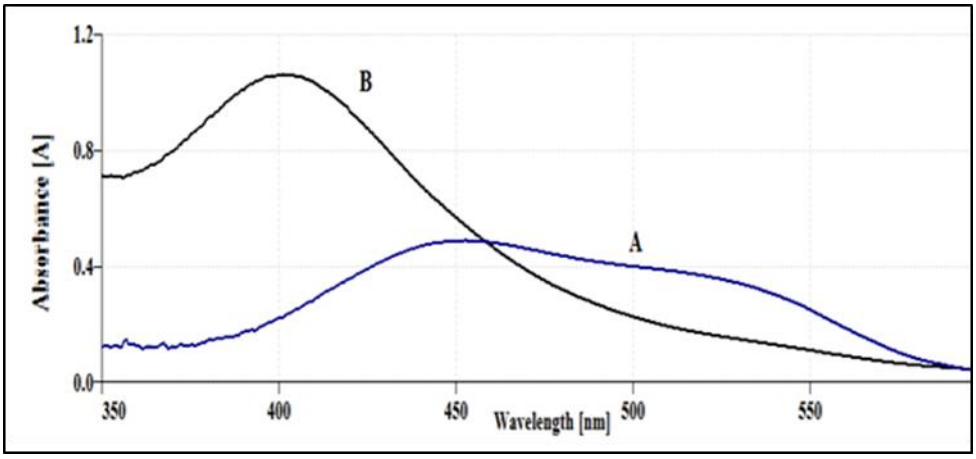


Figure (2): Absorption spectra of (20.0 µg.mL^{-1}) chloramphenicol against reagent blank(A), and of reagent blank against D.W. under the optimum conditions.

Optimization of reaction variables

I-Univariate method

Optimum conditions were established by classical univariate one factor a time strategy and following the absorbance of the colored product.

Figures 3, 4 and Table 1 show the results obtained for the study of the effects of volume of chromogenic reagent, volume of Na_2CO_3 solution, reaction time and different diluting solvents on the measured signal .

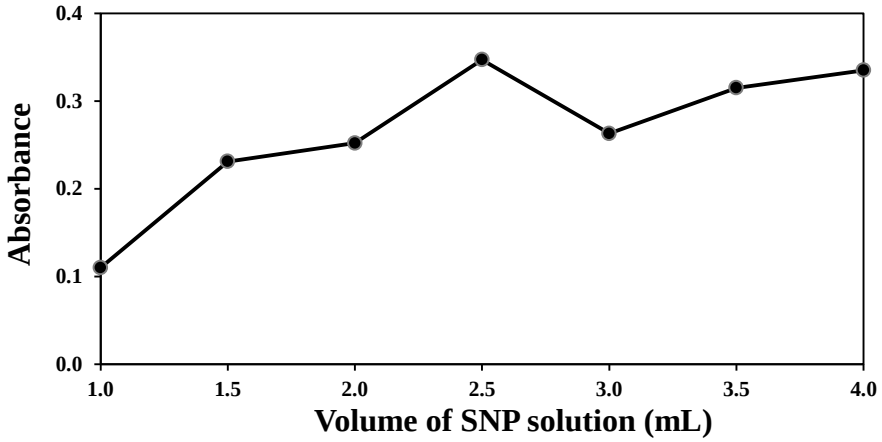


Figure (3): Effect of volume of chromogenic reagent on the color development in the determination of (20.0 µg.mL^{-1})chloramphenicol.

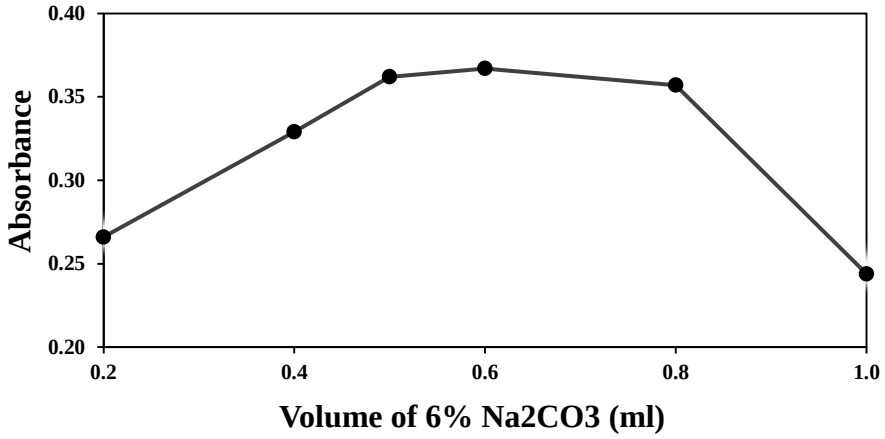


Figure (4): Effect of vol. of 6% Na_2CO_3solution on the color development in the determination of (20.0 µg.mL^{-1})chloramphenicol.

Table (1): Effects of reaction time and different diluting solvents on the color development of (20.0 µg.mL^{-1})chloramphenicol.

Time (min.)	*Absorbance*	*Solvents*	*Absorbance*
0	0.122	Distilled Water	0.437
5	0.325	Methanol	0.525
10	0.367	Ethanol	Turbid
15	0.367	Acetone	Turbid
20	0.276	Acetonitrile	0.364

II- Design of experiment method

A chemometric multivariate optimization approach was used to obtain the optimum values of three of variables having the greatest importance on the reaction yield (viz. the volume of SNP solution, the volume of 6% Na_2CO_3 solution and the reaction time).The conditions of these variables were optimized via face centered central composite design while other experimental parameters were maintained unchanged at their optimum values that obtained by following the univariate approach. Table [2] shows the uncoded values of each of the studied variable.

Table [2]: The CCD for three independent variables (uncoded) together with their corresponding values of absorbance for (20 μg.mL^{-1}) CAP.

Exp. No.	Vol. of SNP sol. (mL)	Vol. of 6% Na_2CO_3 sol. (mL)	Reaction time (min)	Abs.
1	2.5	0.6	10	0.424
2	4.0	1.0	20	0.400
3	1.0	1.0	0.0	0.432
4	1.0	0.6	10	0.364
5	2.5	0.6	10	0.412
6	1.0	0.2	20	0.346
7	2.5	1.0	10	0.395
8	2.5	0.6	10	0.417
9	2.5	0.6	10	0.426
10	2.5	0.6	10	0.431
11	2.5	0.2	10	0.423
12	2.5	0.6	20	0.339
13	4.0	0.2	20	0.150
14	4.0	0.2	0.0	0.000
15	2.5	0.6	0.0	0.423
16	1.0	0.2	0.0	0.491
17	1.0	1.0	20	0.167
18	4.0	1.0	0.0	0.370
19	4.0	0.6	10	0.368
20	2.5	0.6	10	0.432

Statistical 12 software (Stat. Soft. Inc., release 2013) was used to estimate the values of coefficients the for the response surface equation. the results are expressed in Table [3].

Table [3]: ANOVA table for the second order polynomial linear-quadratic model.

Variable	*Regression coefficient*	*Standard error of coefficient*	*t-value*	*P*
Constant	0.56849	0.01280	0.39722	0.45424
SNP vol.	-0.05095	0.01177	-0.07743	-0.02497
(SNP vol.)^2	-0.027929	0.02245	-0.11283	-0.01281
Na_2CO_3 vol.	-0.13474	0.01177	0.00917	0.06163
(Na_2CO_3 vol.)^2	-0.12386	0.02245	-0.06983	0.03019
Reaction time	-0.00137	0.01177	-0.05763	-0.00517
(Reaction time)^2	-0.00048	0.02245	-0.09783	0.00219
SNP vol.× Na_2CO_3 vol.	0.17875	0.01316	0.07793	0.13657
SNP vol.×Reaction time	0.00492	0.01316	0.04443	0.10307
Na_2CO_3 vol.×Reaction time	-0.00750	0.01316	-0.05932	-0.00068

Optimum values for the studied variables were 2.04 mL for the volume of (SNP) solution,0.85 mL for the volume of 6%Na_2CO_3 solution and 2.33 minutes for the reaction time. Figure [5] shows the plots of response surface for couples of variables while maintaining the third variable constant.

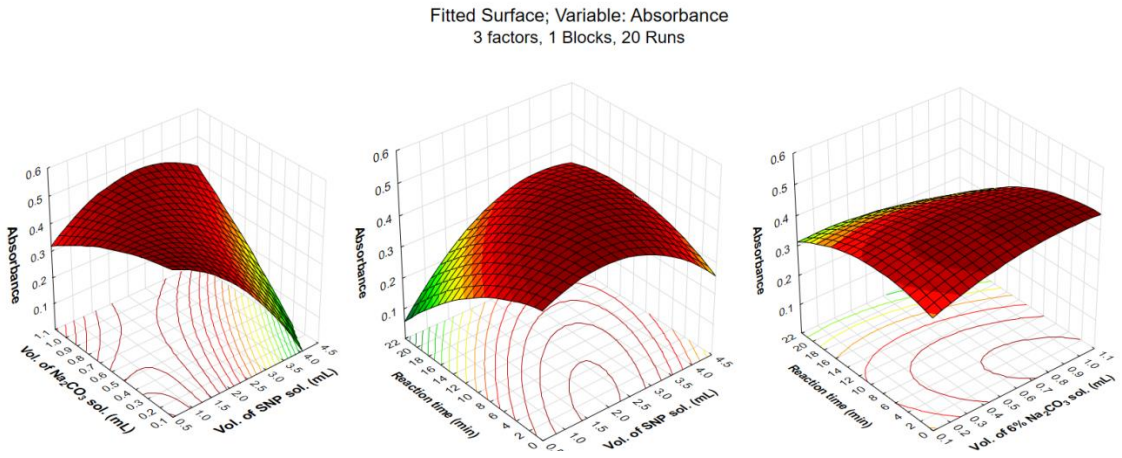
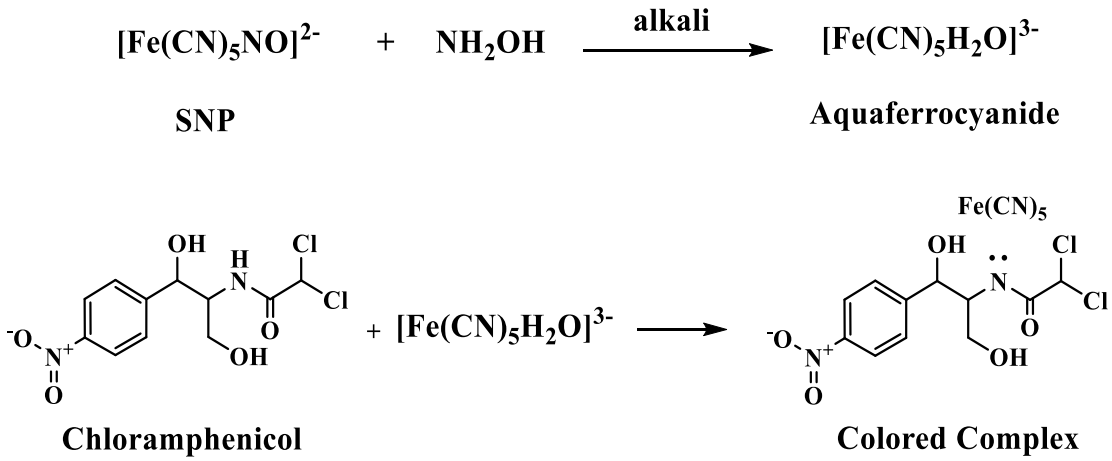


Figure [5]: The plotsabsorbance response surface for chloramphenicol- SNPcomplex against of any pair of variables (keeping third variable constant at its optimum value).

The proposed mechanism for the reaction between CAP and SNP isgiven in scheme (1).



Scheme [1]: The suggested reaction mechanism for charge-transfer reaction between CAP and sodium nitroprusside.

Calibration curves and analytical data

Table [4]: Spectral and statistical data for spectrophotometric determination CAP under conditions obtained via univariate and DOE methods.

Parameter	***Univariate condition***	***multivariate condition***
λmax (nm)	451.0	
Color	Red	
Linearity range (µg.mL^{-1})	1.0-25.0	0.5-26.0
Regression equation	y = 0.0252[CAP µg/ml] – 0.0066	y = 0.0277[CAPµg/ml]+0.0699
Slope (mL.µg^{-1})	0.0252	0.0277
Correlation coefficient (r)	0.9995	0.9991
Molar absorptivity (L.mol^{-1}.cm^{-1})	8.142 x 10^{3}	8.949 x 10^{3}
Sandell's sensitivity (µg.cm^{-2})	3.968 x 10^{-2}	3.610 x 10^{-4}
Detection limit (µg.mL^{-1})	0.350	0.210
Quantification limit (µg.mL^{-1})	1.166	0.7

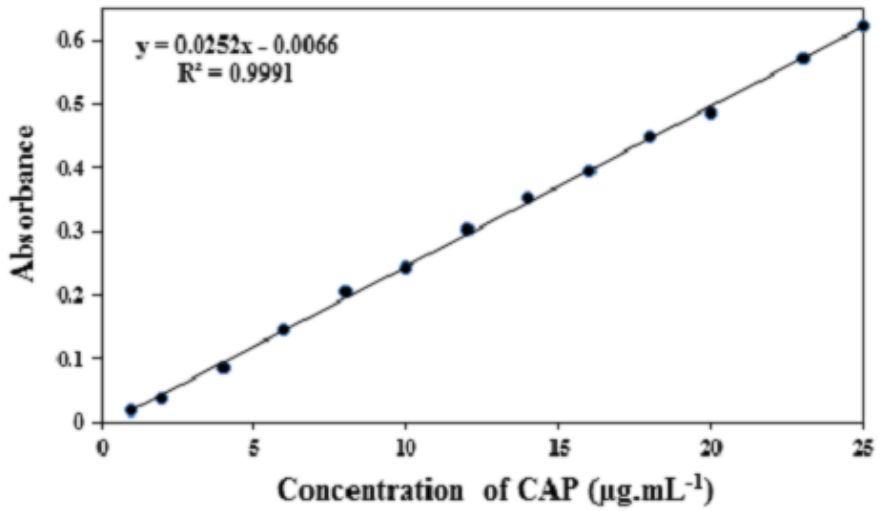


Figure [6]: Calibration curve for the determination of CAP under optimal conditions obtained by univariate optimization.

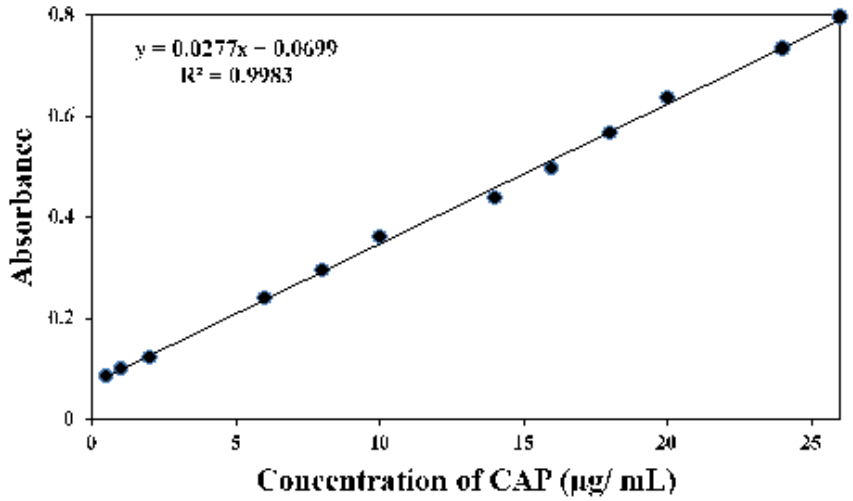


Figure [7]: Calibration curve for the determination of chloramphenicol under optimum condition obtained by DOE.

Accuracy and precision

The accuracy and the precision of the proposed method were established by analyzing five replicates for three concentration levels of the pure drug. The values of relative error and coefficient of variation (C.V %) were determined, Table [5].

Table [5]: The accuracy and precision of the method.

	Conc. of Chloramphenicol ($\mu g.mL^{-1}$)		***Relative Error %***	***C.V* %***
	Taken	***Found****		
For univariate	4.000	3.99	-0.250	0.355
	12.000	12.07	0.583	0.117
	24.000	23.915	-0.354	0.059
For DOE	4.000	4.021	0.525	0.334
	12.000	12.056	0.466	0.111
	24.000	24.107	0.445	0.055

*****Average of five determinations.

2-3-5 Interference study

To assess the analytical potential of the suggested procedure, the effect of the presence of the commonly used excipients (viz; sucrose, glucose, lactose, and starch) were examined by carrying out the determination of 20.0 $\mu g.mL^{-1}$ of chloramphenicol in the presence of above compounds. The results presented in Table [6] indicate no interferences were found from any of the excipients studied in determination of chloramphenicol.

Table [6]: The effect of the presence of the excipients(1000 µg.mL^{-1})on the analysis of 20.0 µg.mL^{-1} of chloramphenicol.

Excipients	**Concentration (µg.mL^{-1})**	**Chloramphenicol Conc. Taken (20.0 µg.mL^{-1})**	
		(Conc. Found* µg.mL^{-1})	***Recovery*%***
Sucrose	1000	20.11	100.55
Glucose		19.97	99.85
Lactose		19.71	98.55
Starch		19.88	99.4

*Average of three determinations.

2-3-6 Application on pharmaceutical Sample

The developed method was successfully used for assaying chloramphenicol in pharmaceutical samples. The results showed in Table [7] were satisfactory.

Table [7]: Application of the proposed method under conditions obtained via univariate optimization for chloramphenicol determination in pharmaceutical samples.

Sample	***Conc. taken (µg.mL^{-1})***	***Conc.* found (µg.mL^{-1})***	***Recovery %***	***C.V* %***
Phenicol (eye drop) API- Jordan	20.00	19.77	98.85	0.072
Chloroper (eye drop) COOPER- European Union	20.00	19.26	96.33	0.073
Chloramphenicol Capsule SDI- Iraq	20.00	20.04	100.2	0.070

*****Average of three determinations.

دراسة التقدير الطيفي للكلورامفنيكول بشكله النقي وفي المستحضرات الصيدلانية

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الخلاصة

تم تقدير الكلورامفنيكول طيفيا بواسطة تكوين معقد انتقال الشحنة الملون الذي اظهر اعلى امتصاص عند الطول الموجي 445 نانوميتر بعد تفاعل الامين الثانوي مع صوديوم نايتروبروساید بوجود الهيدروكسيل امين في محيط قاعدي. وقد تم ايجاد الضروف التجريبية الفضلى التي تؤثر على تكوين معقد انتقال الشحنة بالطريقة الكلاسيكية بنمط المتغير الواحد مع بقاء المتغيرات الاخرى ثابتة وطريقة تصميم التجربة. الطريقة المقترحة بسيطة وحساسة لتقدير الدواء على مدى من التراكيز يتراوح بين (1.0-25.0 $\mu g.mL^{-1}$) وكانت قيمة معامل الامتصاص المولي مساوية الى (8.142×10^3 $L.mol^{-1}.cm$) و ($r = 0.9995$). ولقد تمتأكد صلاحية هذه الطريقة من خلال ايجاد معادلة الانحدار والضبط والدقة وحدود الكشف. كما تم تقدير الكلورامفنيكول بنجاح في المستحضرات الصيدلانية بواسطة هده الطريقة المطورة بدقة وتغطية مقبولة.

الكلمات المفتاحية: تعين الكلورامفينكول،الطيف الضوئي، تصميم التجربة المركزي.