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A new spectrofluorimetric method for the determination of chloramphenicol in pharmaceutical preparations based on fluorescence quenching of methanol

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Abstract

A new, simple, rapid, sensitive, selective, and accurate method for the spectrofluorimetric determination of Chloramphenicol (CAP) in different pharmaceutical preparations. Chloramphenicol as active antibiotic is widely used in the treatment the diseases. The spectrofluorimetric method is based on the chloramphenicol affect the fluorescence quenching of methanol, methanol is fluorescence solvent with the wavelength at λ_{ex}. 273 and λ_{em}. 322 nm. The working range is 1-10 µg mL-1. Limit of detection (LOD), and limit of quantification (LOQ) are 0.21 ppm, and 0.65 ppm, respectively, the recoveries range 99.8%-101.4%. The method was successfully applied to determine the (CAP) quantity in its pharmaceutical preparations (Eye drops, Ointments and Capsules).

Keywords: Chloramphenicol (CAP), fluorescence quenching, methanol, Pharmaceutical preparation.

Introduction

Chloramphenicol is 2,2-dichloro-N-[(1R,2R)-2-hydroxy1(hydroxymethyl)-(4nitrophenyl) ethyl] acetamide.($C_{11}H_{12}Cl_2N_2O_5$) Fig. 1 A white, greyish-white or yellowish-white, fine, crystalline powder or fine crystals, needles or elongated plates , a little soluble in water, freely soluble in alcohol and in propylene glycol, the melting point of this drug 149 °C to 153 °C .(1)

Figure1: Chemical structure of chloramphenicol

Chloramphenicol is a broad-spectrum antibiotic. It is active against diseases caused by aerobic and anaerobic gram-positive also gram-negative organisms (2). Chloramphenicol was initially obtained from Streptomyces Venezuelae in 1947. Chemical processes soon synthesized it and the commercial product now is all-synthetic (3). Chloramphenicol is one of the few natural nitro compounds, an active inhibitor of protein synthesis of microbial. It usually binds to the 50S subunit of the ribosome of the bacterial and inhibits the formation of the peptide bond (2, 4-6). Chloramphenicol distribute to body fluids and all tissues such as the central nervous system and cerebrospinal fluid therefore the concentration of chloramphenicol in brain tissue usually be equal to that in serum due to the drug transfer through cell membranes readily (2). Chloramphenicol is an antimicrobial agent with restricted use. It use to combat serious infections where other antibiotics are ineffective. Because of its risk to cause cancer, aplastic anemia, and carcinogenic properties, its use in human and veterinary medicine limited by its toxicity (7,8).

Several methods have been used for the determination of chloramphenicol in pharmaceutical dosage forms such as spectrophotometric methods (9-16), chromatographic methods (16-20), electrochemical methods (20-22), flow-injection analysis methods (23،24).

In this paper, a spectrofluorometric method was developed to determine chloramphenicol in its pharmaceutical preparations.

Methanol is a fluorescence solvent and the fluorescence spectrum of methanol was recorded. The fluorescence spectrum of a methanol solution that was prepared by nine to one ml methanol to water was shown excitation and emission maximum wavelength at λ_{ex}. 273 and λ_{em}. 322 nm, when CAP was added to methanol case quench in the fluorescence intensity of methanol as shown in Figure (2).

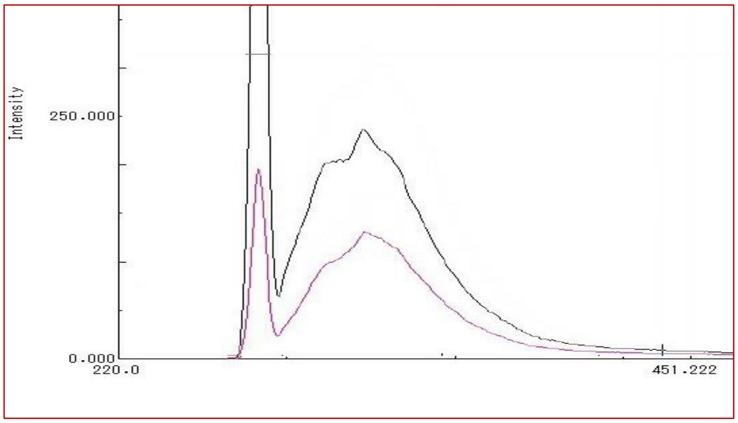

Figure 2: The fluorescence spectrum of methanol, the higher intensity is without chloramphenicol and the lower intensity is with 10 µg mL^{-1} chloramphenicol.

Method

Apparatus

UV-Visible Spectrophotometer, double-beam, Shimadzu model UV-1800 PC (Japan) with quartz cell of 1 cm path length was used for all spectral and absorbance measurements.

Reagents

All reagents and chemicals used without further purification and freshly prepared.

Standard solution methanol (9:1) methanol to water

Water affects the intensity of methanol fluorescence. When determining chloramphenicol, 1 ml of chloramphenicol of different concentrations is added to nine ml of methanol to obtain 10 ml of solution in a 10 ml volumetric flask. Therefore, and because chloramphenicol solution contain water, a standard methanol solution was prepared by diluting 90 ml of methanol with 10 ml of distilled water in a 100 ml volumetric flask to achieve 9:1 methanol to water ratio.

Chloramphenicol solution (10 µg mL^{-1})

100 mL of a 10 µg ml^{-1} chloramphenicol solution was prepared by dissolving 0.01 g of chloramphenicol in 5 mL of methanol and the volume was completed to the mark with distilled water in a 10 mL volumetric flask. 1 mL of this solution contains 0.05 mL of methanol, and this volume does not affect the ratio of methanol to water in the final volume of the measured solution.

Pharmaceutical preparations of chloramphenicol

(i) **Eye drops:** Prepared by mixing two tubes (50 mg, 10 mL each) and then diluting the solution to 50 mL with distilled water (equivalent to 100 mg (0.1 g) in 50 mL). 5 mL of this solution (equivalent to 10 mg (0.01 g) in 5 mL) was transferred into a 100 mL volumetric flask and the volume was completed to the mark using distilled water.

(ii) **Ointment:** Prepared by dissolved two tube of ointment (1 %, 5 g, equivalent to 100 mg of CAP) in 50 ml of petroleum ether and extracted three time with the 15 ml D.W. The total extracts were filtered and completed to 50 ml with distilled water. 5 ml from this solution was transferred into a 100 mL volumetric flask and the volume was completed to the mark using distilled water.

(iii) **Capsules (250 mg):** Ten capsules (250 mg) were mixed and weighed, and 10 mg of the mixture was transferred into a 100-mL beaker and dissolved with 5 mL of methanol. Other preparation steps are described in the chloramphenicol paragraph above.

Results

Preliminary studies

In this study, the fluorescence and intensity spectrum of methanol without chloramphenicol was investigated at a ratio of 9:1 methanol to water and it was found that the excited and emitted wavelengths are λ_{ex}. 273 and λ_{em}. 322 nm and the intensity is 220. Figure 2 above.

Excitation is expected due to the excitation of the lone-pair electron on the OH group in methanol (25), and quenching may occur due to the formation of hydrogen bonds between chloramphenicol and some methanol molecules that may prevent the excitation of the lone-pair electron. (26)

Optimal Conditions. .

Effect of pH

In this part, the effect of pH was investigated by measuring the fluorescence intensity signal at different pH to find the best intensity. From the result shown in Figure 3 below, the intensity is quenched when the pH is below or above 7. At normal pH, methanol is in a mixture with its dissociated form of it (CH_3OH to CH_3O^-). A pH lower than 7 leads to methanol protonation and an energy level change between the ground and excited state of the lone-pair electron of the OH group while if the pH is higher than 7, it leads to an increase in the form of CH_3O^-. This may lead to the expectation that the intensity will increase due to the increase in the negative charge on the O atom but the remaining cation of the base acts as an impurity and increases the collisions of the methanol molecules, thus the photon energy is transferred to the impurity or lost as heat. (27)

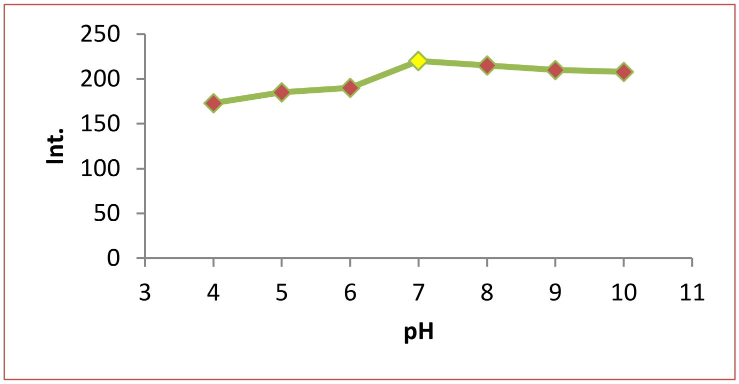

Figure 3: Effect of pH on the fluorescence intensity of methanol.

Effect of heating

In this part, the effect of temperature was investigated by measuring the fluorescence intensity signal at different temperatures to find the best temperature that can be used.

From the result shown in Figure 4 below, the intensity is quenched when the temperature is increased above 30 °C. An increase in temperature leads to an increase in the kinetic energy of the methanol molecules and, as a consequence, an increase in the number of collisions of molecules. Generally, an increase in the collision of particles causes the energy of the photons to be lost as heat. (28)

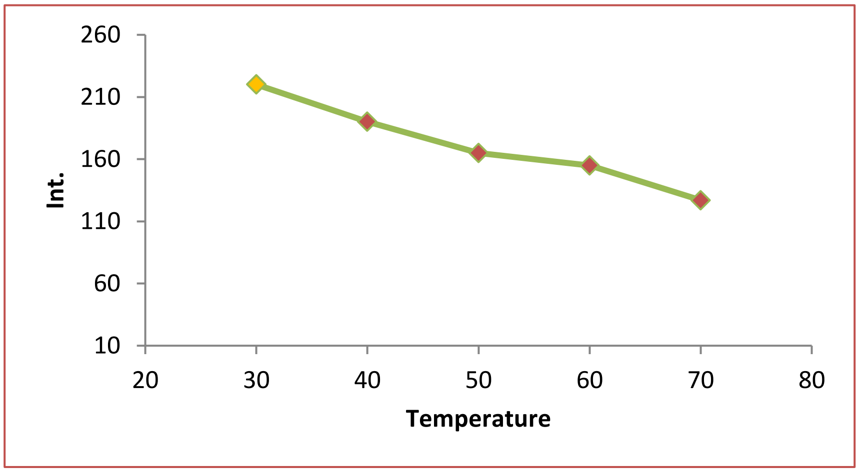

Figure 4: Effect of the temperature on the fluorescence signal of methanol.

Calibration Curve for CAP

According to the optimized conditions that established via method, 1 ml of a numerous concentration of chloramphenicol solution ranging from (1-10) ppm were added to 9 ml of methanol and the intensity at a λ_{em} is 322 nm was measured.

Linear calibration curve was established by plotting the amount of quenching of fluorescence intensity versus the concentration of CAP. Table (1) and figure (5) represent the calibration curve, and the analytical parameters of merit are summarized in Table (2).

Table (1). Amount of intensity quenching of methanol at different concentrations of CAP. where, I_0 is intensity of methanol without CAP. I_x is intensity of methanol with (x) concentration of CAP. I_0-I_x is Amount of intensity quenching of methanol at (x) concentrations of CAP.

ppm	1	2	3	4	5	6	7	8	9	10
I_0	220	220	220	220	220	220	220	220	220	220
I_x	214	200	190	178	169	159	143	135	125	110
I_0-I_x	6	20	30	42	51	61	77	85	95	110

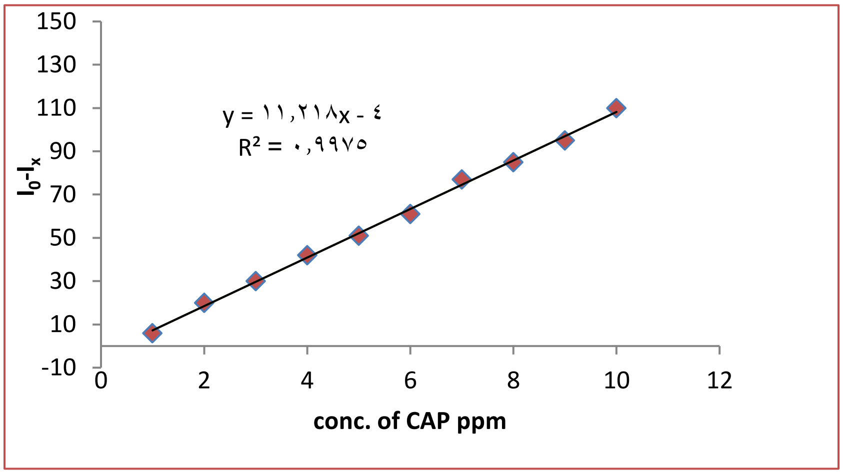

Figure 3.2.13: Calibration curve.

Figure 5: calibration curve of the method, working range is (1-10) ppm for CAP.

Sensitivity of method

LOD and limit of quantification LQD which were calculated in this method by the equations which was mentioned in paragraphs (2-1-3-3) and other information that has been obtained from the calibration curve was included in the table (3-2-8).

Table (3-2-8). Method validation of the Spectroflourometric determination of CAP.

parameter	value
Regression equation y = absorbance x = concentration (ppm)	**y = 11.155x - 4.102**
Beer's law limits (µg ml^{-1})	**1-10**
R^2 value	**0.9977**

Slope	**11.155**
LOD ($\mu g.ml^{-1}$)	**0.21**
LOQ ($\mu g.ml^{-1}$)	**0.65**

3-2-4 Accuracy and precision

In this method accuracy and precision was calculated by use three parameters, Relative Error E%, Recovery percentage (Rec %) and Relative standard deviation percent (RSD %).

Tables (3-2-9) and (3-2-10) below illustrate the results which found from reading the intensity of colored product to three concentrations of drug and calculating the concentrations from the calibration curve.

Table (3-2-9). Concentration (ppm) of CAPR found by applying the method.

NO	CAPR presents	int	CAPR found	Average
1	1.5	13.67	1.59	**1.55**
		12.88	1.52	
		13.16	1.55	
2	3.5	35.69	3.57	**3.53**
		35.02	3.51	
		35.21	3.52	
3	7.5	79.74	7.52	**7.54**
		80.12	7.55	
		80.02	7.54	

Note. Each measured was an average of three readings

Relative Error E%, Recovery percentage (Rec %) and Relative standard deviation percent (RSD %) were calculated with the method by using results above and equations, which it was mentioned in paragraph (2-1-4).

Table (3-2-10). Values of parameters of accuracy and precision.

NO	Present	Found	E%	Rec%	R.S.D%
1	1.5	1.55	3.62	103.62	**2.31**
2	3.5	3.53	0.94	100.94	**0.88**
3	7.5	7.54	0.48	100.48	**0.23**

3-٢-٥ Analytical applications

The solutions of the assay (eye drop, Ointment, Capsule) were prepared and the intensity of the amount taking from reducing solution was measured and amount of CAP in all types of an assay was calculated. (Paragraph 2-2-5). Relative Error E%, Recovery percentage (Rec %) also were calculated. Table (3-2-11) below illustrates the results.

Table (3-2-11). Application of the method for determination of CAP in pharmaceutical preparations.

No	Type of assay	Drug present from assay mg		Drug taken From Solution ppm	Drug found in Solution ppm	Drug found in assay mg	average	E%	Rec%
1	Eye drop	50	1	7	7.076	50.542	49.87	0.249-	99.751
			2	5	4.952	49.522			
			3	3	2.974	49.562			
2	Ointment	50	1	7	7.032	50.229	8.917	-2.165	97.835
			2	5	4.872	48.724			
			3	3	2.868	47.799			
3	Capsule	250	1	7	7.101	253.608	253.537	1.415	101.41
			2	5	5.010	250.524			
			3	3	3.078	256.477			

The proposed method was successfully in determination CAP in pharmaceutical preparations with good values of recovery presents (96.5%-100.6%).

3-3-6 t – test and F- test

t – Test and F- test were calculated by using equations in paragraph (2-1-9) to comparison between the data which obtained from method 1 and from this method. Table (3-2-12) below illustrates the results.

Table (3-2-12). Results of t – test and F- test

Standard method						
x	$\overline{x}$	$(x-\overline{x})$	$(x-\overline{x})^2$	$\sum(x-\overline{x})^2$		
7.541	٧,٥٦٠	$-1.85*10^{-2}$	$3.42*10^{-4}$	**$7.07*10^{-3}$**		
7.578		$1.85*10^{-2}$	$3.42*10^{-4}$			
7.616		$5.65*10^{-2}$	$3.19*10^{-3}$			
7.503		$-5.65*10^{-2}$	$3.19*10^{-3}$			
Proposed method						
x	$\overline{x}$	$(x-\overline{x})$	$(x-\overline{x})^2$	$\sum(x-\overline{x})^2$		
7.516	٧,٥٣٦	-0.020	$3.87*10^{-4}$	**$6.21*10^{-4}$**		
7.55		0.0143	$2.05*10^{-4}$			
7.541		0.005	$2.84*10^{-5}$			
parameters						
S_{1-2}	**t**	**S_1**	**S_2**	**S_1^2**	**S_2^2**	**F**
0.039	**1.489**	**0.049**	**0.018**	**0.002**	**0.0003**	**7.953**

The proposed method has given high reliability ratio with standard method because the values of t and F less than tabulated values ($t < 2$, $F < 19$)

Table (3-1). Has been explained a comparison in the parameters among the three methods.

Discussion

The results clearly showed that the fluorescence of methanol is sensitive to the presence of chloramphenicol, and its quenching effect was used as the basis of the analytical method. Neutral pH and room temperature were found to be the most suitable conditions, since both acidic and alkaline media, as well as elevated temperatures, caused a significant decrease in signal stability.

The calibration curve demonstrated excellent linearity within the concentration range of 1–10 µg/mL with high sensitivity, reflected in low LOD and LOQ values. Recovery studies indicated accurate and reproducible results, and the method was successfully applied to various pharmaceutical dosage forms with results consistent with standard methods. The quenching mechanism is most probably related to hydrogen bonding interactions between chloramphenicol and methanol molecules, which limit fluorescence emission.

These findings confirm that the proposed method is reliable and can serve as an alternative analytical approach, particularly in laboratories with limited resources.

Conclusion

A new spectrofluorimetric method was developed and validated for the determination of chloramphenicol in pharmaceutical preparations. The method is simple, rapid, inexpensive, and highly sensitive compared with many conventional techniques. Due to its accuracy, reproducibility, and low cost, it can be recommended as an effective alternative to other more sophisticated and expensive methods for routine quality control analysis of chloramphenicol.

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