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New Spectrophotometric Determination of Chloramphenicol in Pharmaceutical Preparations Based on Condensation Reaction with 1,2-Naphthoquinone-4-Sulfonic Acid (1,2 NQS) as Reagent

Abbas Noor Alshirifi* and Dheyaa Yahaia Alhameedi

Department of Chemistry, Faculty of Science, University of Babylon, Hilla, Iraq

Abstract : A new simple, rapid, sensitive, selective, and accurate method for the spectrophotometric determination of Chloramphenicol (CAP)in different pharmaceutical preparations. Chloramphenicol as active antibiotic is widely used in the treatment the diseases. The spectrophotometric method is based on the condensation reaction between CAP and 1,2 naphthoquinone-4-sulfonic(1,2 NQS) as reagent to formed an orange-red product after reducing nitro group in drug into amino group by used a concentrated HCl and zinc dust . Orange-red product was showed a maximum absorption at 489nm. Beers law was obeyed in the concentration range of 1-9µg.mL^{-1} with a molar absorptivity (1.86 * 10^{4})L.mol^{-1}.cm^{-1},and sandell's sensitivity (1.73* 10^{-2}) µg.cm^{-2}, respectively. The analytical parameters were optimized as the following: It was found the time for completed reaction was (10 min) at temperature (70 ^{0}C)in bicarbonate solution, and the best volumeof0.01 mol. L^{-1} of 1,2 NQS solutionis1mL.Limit of detection (LOD), and limit of quantification (LOQ)are0.068 ppm, and 0.207 ppm, respectively, the recoveries range 98.52%-100.66%.The method was successfully applied to the analysis of the (CAP)in its pharmaceutical preparations(Eye drops ,Ointments and Capsules).

Key words: Drugs, Chloramphenicol (CAP), 1,2-naphthoquinone-4-sulfonic(1,2 NQS),condensation reaction, 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.1A 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]

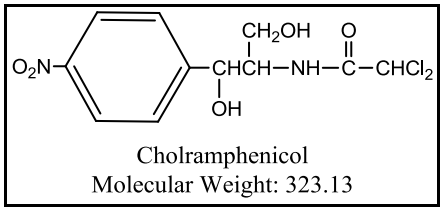


Fig. 1: Chemical structure of chloramphenicol

Chloramphenicol is a broad-spectrum antibiotic It is active against the diseases caused by aerobic and anaerobic gram-positive also gram-negative organisms[2].Chloramphenicol was initially obtained from Streptomyces Venezuelae in1947. it was soon synthesized by chemical processes and the commercial product now is all synthetic [3]. Chloramphenicol is one of the few natural nitro compounds, active inhibitor of protein synthesis of microbial. It usually binds to the 50S subunit of ribosome of the bacterial and inhibits formation the peptide bond[4]. Chloramphenicol is distributed to body fluids and all tissues such the central nervous system also cerebrospinal fluid therefor 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 is used 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 is limited by its toxicity[5].

The adverse effects of this compound have led to restrict its use in both human and veterinary medicine[6].

The reaction between drug and reagent is condensation reaction but there are Severaltype of reactionhave beenusedforformation colored drug compound for using in spectrophotometricdetermination such as[7-27].

In this method was develop for the reducing of nitro group of drug CAP by concentrated hydrochloric acid and zinc dust and then reaction with NQS reagent to form anorange-red compound and measured the absorbance of yieldorange-red colored by use UV-Visible Spectrophotometer .

Experimental

Apparatus

UV-Visible Spectrophotometer,double-beam, Shimadzumodel UV-1800 PC (Japan) with quartz cell of 1 cmpath length was used for all spectral and absorbancemeasurements.

Reagents

All reagentsand chemicals usedwithoutfurther purification and freshlyprepared.

Standardsolutionofreduced chloramphenicol (CAPR)100 µg.mL^{-1}

Reduced chloramphenicol(RCAP) solution (100 ppm) was prepared by dissolving of 0.01 g of its pure form with 5 ml of methanol in 100 ml beaker and was reduced by using 0.3 g zinc powder and 0.5 ml of conc. hydrochloric acid and kept aside for 5 min in bath water at 50 ^{o}C with stirring for complete reduction. The reduced solution was leted for 15 min for cooling after that was filtered to 100 ml in a calibrated flask and diluted with D.W to the mark.

Stock Solution of (0.01 M) NQS

It was prepared by dissolving 0.26 g of it in 100 ml beaker with 20 ml of D.W with stirring after that the volume was completed to the 100 ml with the same solvent after transferred it to volumetric flask.

Stock Solution of (0.01 M) Sodium bicarbonate

It was prepared by dissolving 0.84 g of it in 100 ml beaker with 20 ml of D.W with stirring after that the volume was completed to the 100 ml with the same solvent after transferred it to volumetric flask.

Pharmaceutical preparations of chloramphenicol

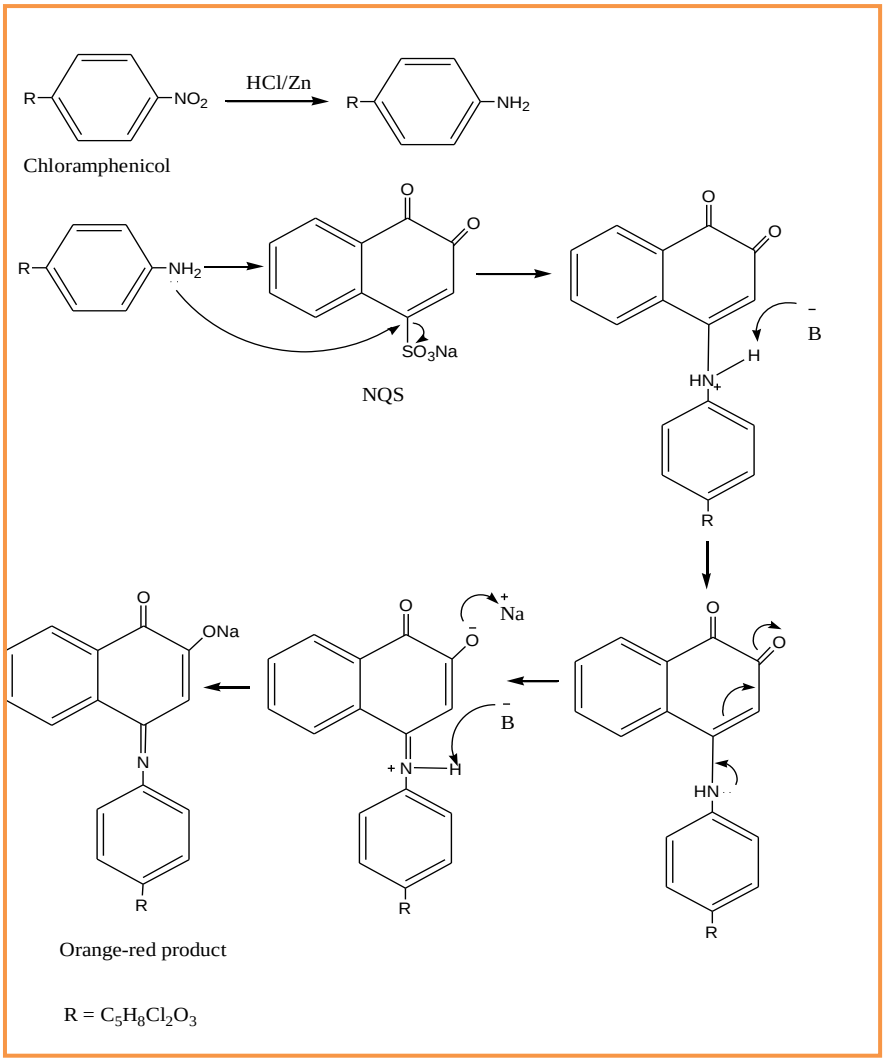
(i) **Eye drops:** It was prepared by mixed tow tube (50 mg ,10 ml each) then the solution was diluted to 50 ml with methanol (equivalent to 100 mg (0. 1 g) in 50 ml). 5 ml from this solution was transferred (equivalent to 10 mg (0.01 g) in 5 ml) to 100 ml beaker . Reducing solution of (100 ppm) CAPR was prepared by the way which was explained previously .

(ii) **Ointment (1 % , 5 g) ,** equivalent 50 mg of CAP**.** Two tube of ointment (1 % , 5 g) , equivalent to 100 mg of CAP was dissolved 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 methanol. 5 ml from this solution was transferred (equivalent to 10 mg (0.01 g) in 5 ml) to 100 ml beaker . Reducing solution of (100 ppm) CAPR was prepared by the way which was explained previously.

(iii) **Capsules (250 mg) .**ten capsules (250 mg) was took ,mixed and weighted .from the mixture was transferred 10 mg to 100 ml beaker and dissolved with 5 ml of methanol. Reducing solution of (10 ppm) CAPR was prepared by the way which was explained previously.

Results and discussion

The mechanism of condensation reaction was suggestion in this study showed in scheme (2) agreement with that was found in litterateurs [23-25]



Scheme 2: Proposed mechanism of the reactionbetween CAPR and NQS

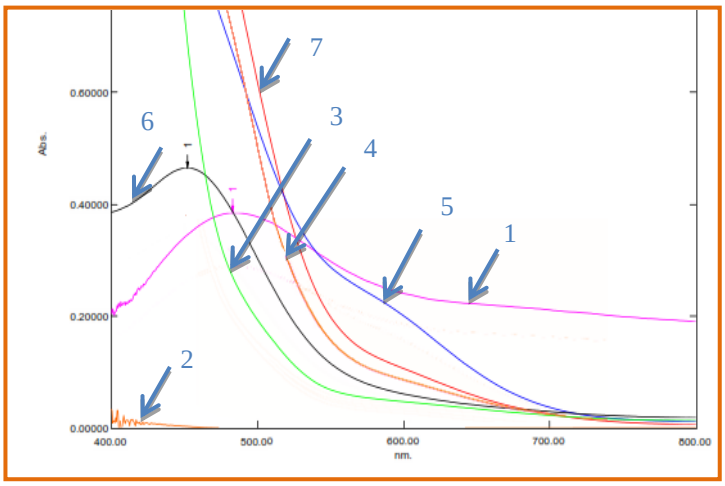
Optimum conditions[26-36]

General process

One ml of NQS (0.01 M) as regent was added to desert of CAPR (100 ppm) in 10 ml volumetric flask then 0.5 ml of sodium bicarbonate (0.1M) as base. The solution was heated at 70 ^{0}C in bath water for 10 min to form orange-red product. The colored solution was cooled and completed the volume with water to the mark.

Effect of type of alkaline medium

Althoughthat the reaction occurs in alkaline medium, but same alkaline solution using it causes development color of the blank and its absorbance interaction with region of absorbance of product of CAP. Therefore, the best alkaline medium can be used in reaction those that cause little development of color of the blank. Therefor the best alkaline medium with the smallest absorbance of blank was studded by added (1 ml, 0.1M) of different alkaline solutions . Absorbance of a colored solution formed was measured at 489 nm against distilled water (D.W), as blank in each time.Figure (1) explain the result.



1- Product

2- $NaHCO_3$ without heating

3-$NaHCO_3$ with heating

4- KOH with heating

5- $Na_2CO_3$with heating

6-NaOHwith heating

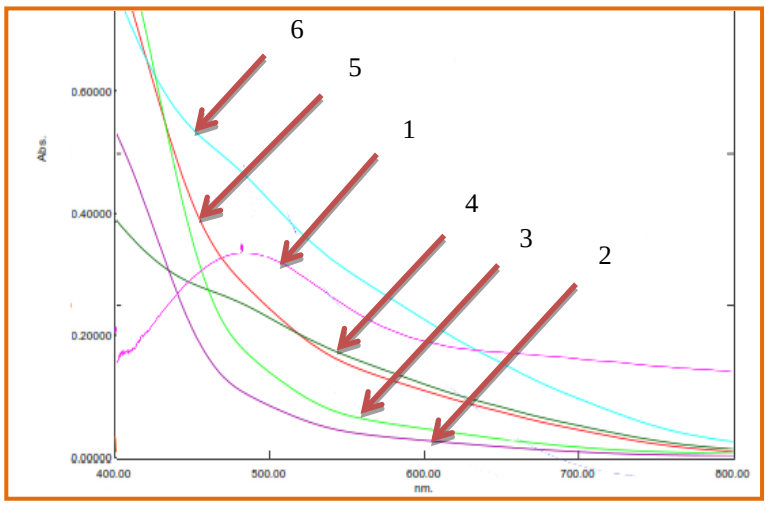
7- $NH_4OH$with heating

Figure (1). Absorbance and λ max of blank at different types of alkaline media at 70 ^{0}C

Figure(1) shows that the best alkaline media was sodium bicarbonate with smallest absorbance at 489 nm. But the absorbency of blank of other alkaline solutions at 489 nm were obtained . The absorbance must be measured at 70 ^{0}c because the changed of temperature cause the changed of the absorbance of blank therefore the blank must be heated at a same optimum temperature of the product solution.

Effect of buffers

When was measured optimum volume of sodium bicarbonate the pH of the solution was distinguish and was (8), Therefore the effect of buffer solutions was studied by completing the solution of colored product with certain buffer.



1, 2- Product and blank without buffer

3- Blank with (NH4Cl +NH3) buffer

4- Blank with (KH_2P0_4+NaOH) buffer

5- Blank with (CH3COONH4 +NaOH) buffer

6- Blank with (Na2HPO4 + HCl) buffer

Figure (2).Absorbance of blank at different types of buffer.

On the other hand, using the buffer solution was effect also on the development the color and absorbance of blank at 489 nm. Therefore the effect of buffer solution on blank also studied. The absorbance of

the solutions were measured at 489 nm. The study show the absorbance of product was decrease and the absorbance of blank was increase. Figure (2) explains the result.

From the figure (2) shows that buffer solutions reason increased absorbance of blank at same λ max(489 nm) of colored product. Therefor using sodium bicarbonate only without buffer gave the best condition of an alkaline medium to form colored product.

Effect of volume of sodium bicarbonate

The optimum volume of 0.1 mol.L^{-1}sodium bicarbonate solution was studied where was prepared a series of solutions contain a different volume of sodium bicarbonate with fixing the volume of drug (0.5 ml) and volume of NQS (1 ml). The absorbance against suitable blank was measured for each solution at λ_{max} 489 nm.figure (3) explains the result.

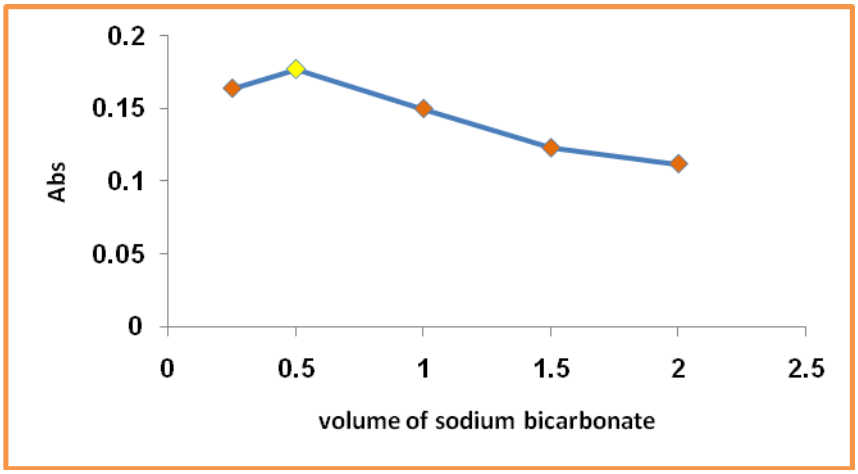


Figure (3).Absorbance of colored product at different volume of sodium bicarbonate.

Effect of solvent

Effect of solvents was studied by completing the volume of colored product to the mark o volumetric flask with different solvent. The absorbance against suitable blank was measured for each solution at λ_{max} 489. Figure (4) explains the result. Water solution was selected due to givea high value of absorbance of colored product.

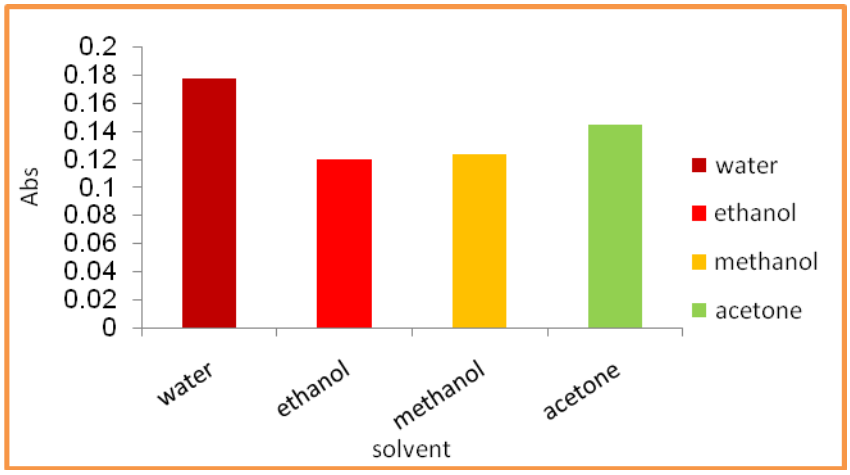


Figure (4): Absorbance of colored product at different solvents.

Effect of volume of NQS (0.01 M)

The optimum volume of NQSwas studied by preparing a series of solutions contain a different volume of reagent (0.5-2.5) with fixing the volume of CAPR (0.5 ml) and volume of sodium bicarbonate (0.5 ml). The absorbance 0f colored product against suitable blank was measured for each solution atλ_{max} 489. Figure (5) explains the result.

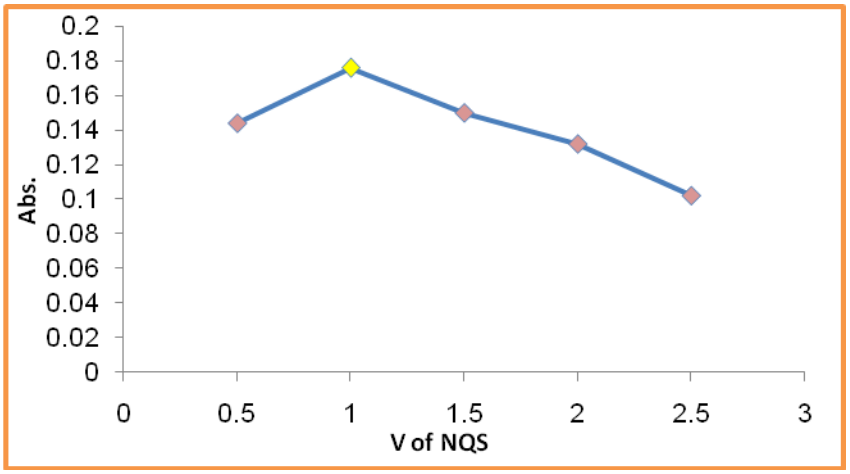


Figure (5).Absorbance of colored product at different volume of NQS.

The optimum volume of NQS can be used in reaction was 1 ml. This volume was fixing in all subsequent experiments.

Effect temperature and time of heating.

The optimum temperature of heating also was studied by preparing a series of solutions contain (0.5 ml) of CAPR, 0.5 ml of sodium bicarbonate and 1 ml of NQS. After that, the solution was heated at different temperature for 10 min toform colored product.

Also the optimum time of heating was studied by the same procedure above but with fixing temperature in 70 ^{0}C and heated for different time. The absorbance against suitable blank was measured for each solution at λ_{max} 489 nm. Figure (6) and Figure (7) explain the results.

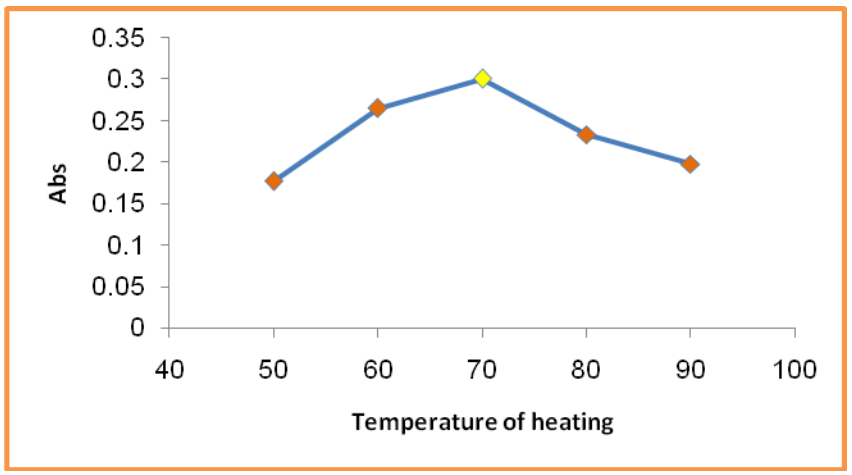


Figure (6).Absorbance of colored product at different temperature of heating.

The optimum temperature which can be used in development of colored reaction was 70 ^{0}C, this temperature fixing in all subsequent experiments.

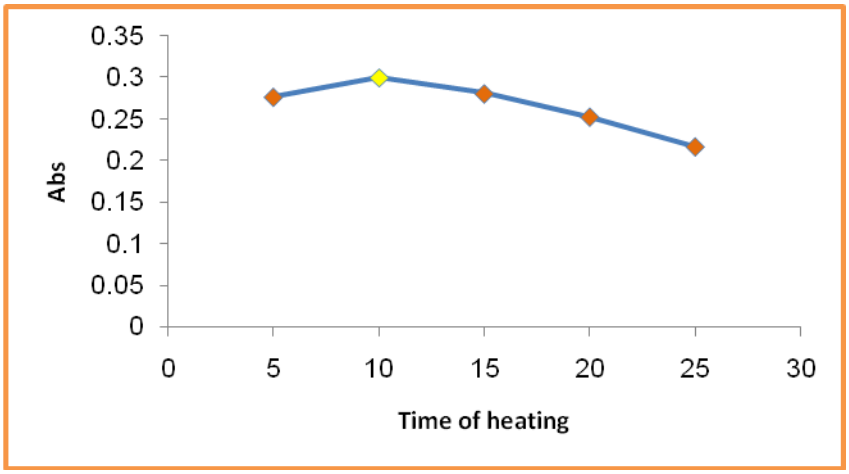


Figure (7).Absorbance of colored product at different temperatures of heating.

From the Figure (7) 10 min.isthe optimum time of heating at 70 ^{0}c to develop the color of the resulting solution CAPR- NQS .

This time fixing in all subsequent experiments.

The order of addition

The order of addition was studied by providing three solutions in 10 ml volumetric flask contain 0.5 ml CAPR, 0.5 ml sodium bicarbonate and 1 ml NQS but in a different sequence in addition. The absorbance of colored product was measured against suitable blank at 489 nm. Figure (8) explains the results.

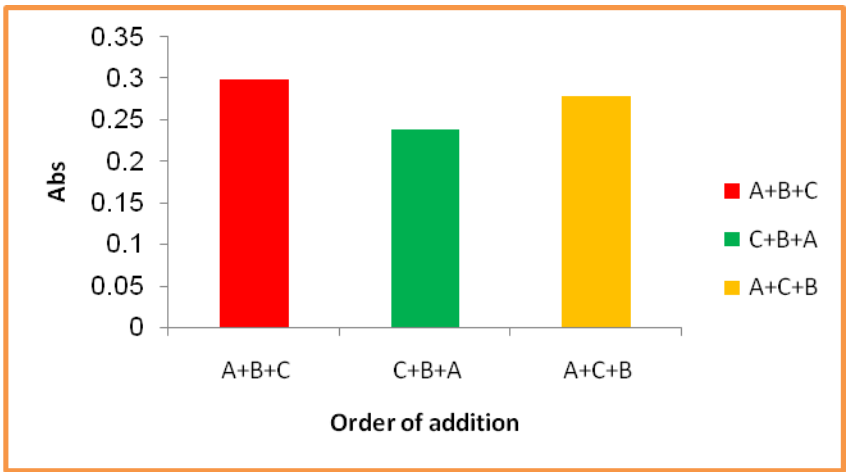


Figure (8).Absorbance of colored product in different order of addition.

Where, A is (CAPR), B is (sodium bicarbonate), C is (NQS).

The optimum order of addition was CAPR + sodium bicarbonate + NQS.

Effect of time of stability

The effect of time of stability of the colored product (CAPR- NQS) also was studied. After prepare the solution of colored product by applying optimum conditions the absorbance of colored product was measured each five min. figure (9) explains the result.

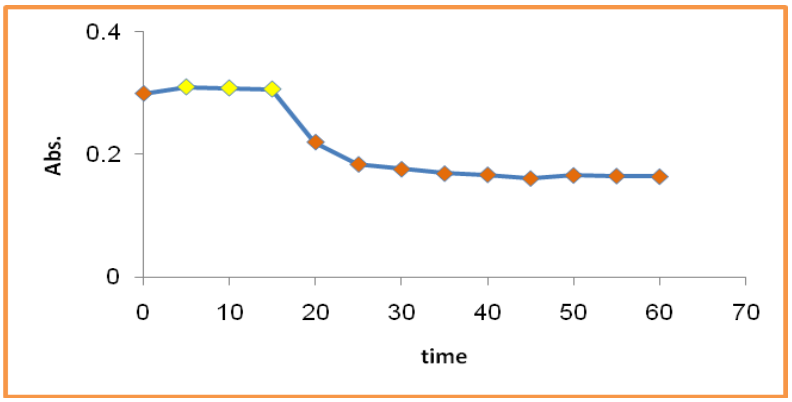


Figure (9).The absorbance of colored product at a different time.

From figure (9), the absorbance wasstablefor 15 minutesand then beginsto reduce. Therefore, the optimum time for measuring the absorbance was duringthe first fifteenminutes.

Calibrationcurve

After knowing the optimum conditions of the method the calibration curve was plotted by took different concentrations of CAPR and measured the absorbance of colored product, which was formed by reacting drug with reagent. Figure (10) show results and Calibrationcurve.

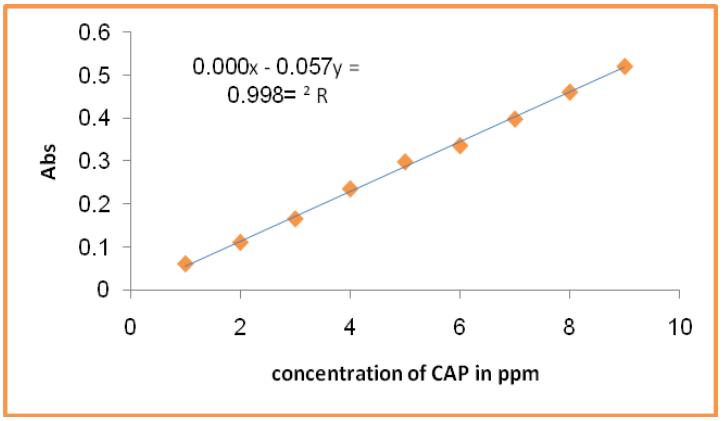


Figure (10).The calibrationcurve of CAPA-NQS product.

Sandell'ssensitive, Molar absorptivity, Limit of detection LOD, limit of quantification LQD which were calculated in this method by the equations which was mentioned inlitterateurs and other information that have been obtained from the calibration curve was included in the table (1).

Table (1). Analytical values of statistical treatments of the calibration curve.

value	parameter
y = 0.0577x - 0.0006	**Regression equation (ppm)**
1 – 9	**Beer's law limits (µg ml^{-1})**
0.9983	**R^2 value**
0.0577	**Slope**
$1.86 * 10^4$	**Molar absorptivity ($l.mol^{-1}. cm^{-1}$)**
$1.73* 10^{-2}$	**Sandell's sensitive**
0.068	**LOD (µg.ml^{-1})**
0.207	**LOQ (µg.ml^{-1})**

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

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

Average	CAPR found	Abs.	CAPR presents	NO
1.477	1.483	0.085	1.5	1
	1.466	0.084		
	1.483	0.085		
3.655	3.649	0.21	3.5	2
	3.667	0.211		
	3.649	0.21		
7.549	7.532	0.434	7.5	3
	7.549	0.435		
	7.566	0.436		

Note. Each measured was an average of three readings.

Relative Error E%, Recovery percentage (Rec %) and Relative standard deviation percent (RSD %) were calculated by the method by using results above and equations which was mentioned inlitterateurs.

Table (3). Values of parameters of accuracy and precision.

R.S.D%	Rec%	E%	Found	Present	NO
0.677	98.517	1.483-	1.477	1.5	**1**
0.274	104.448	4.448	3.655	3.5	**2**
0.230	100.659	0.659	7.549	7.5	**3**

Molar ratio method and continues method (job)

By using the molar ratio method and continues method (job), the Stoichiometry and reaction mechanism were known. By use the procedure which was mentioned inlitterateurs.

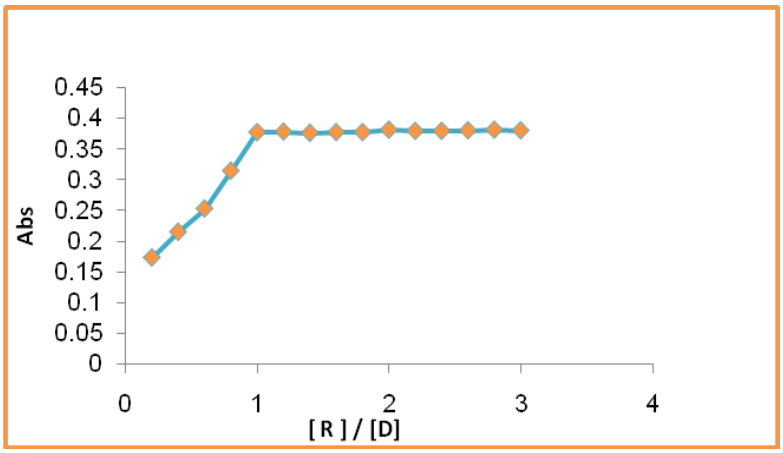


Figure (11).Molar ratio method.

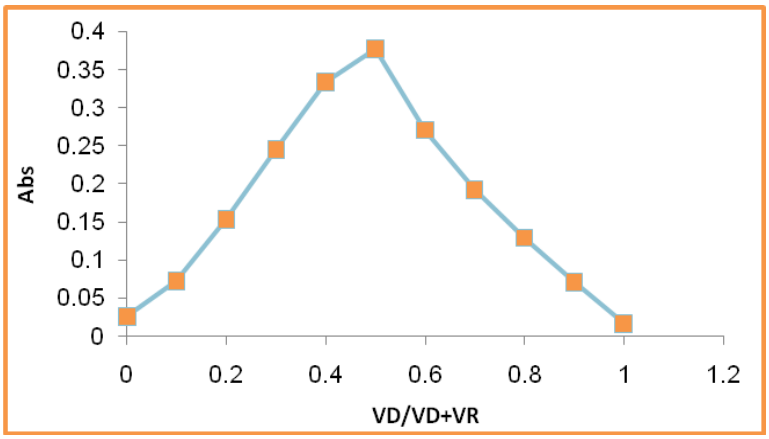


Figure (12).Job'smethod.

Both methods have proven that theratio betweenthe drug,andthe reagent was 1:1 (D 1: 1 R). Figures (11) and (12) below illustrate the results.

Stability constant of complex

Stability constantof the product was calculated to the drug in the final colored solution by measuring the absorbance and using suitable equations which it was mentioned in inlitterateurs. Table (4) below illustrates the results.

Table (4). Value of stability constant

K	**α**	**AM**	**As**	**CAPR (mol L^{-1})**	**CAPR ppm**
1.88×10^{6}	1.23×10^{-1}	0.171	0.15	3.1×10^{-5}	**10**

Analytical applications

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

Table (5).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	7.081	50.6	51.7	3.3	103.3
			2	5	5.192	51.9			
			3	3	3.147	52.5			
2	Ointment	50	1	7	6.856	49.0	48.075	-3.9	96.1
			2	5	4.915	49.2			
			3	3	2.766	46.1			
3	Capsule	250	1	7	6.977	249.2	257.319	2.9	102.9
			2	5	5.296	264.8			
			3	3	3.095	257.9			

The proposed method was successfully in determination CAP in pharmaceutical preparations with good values of recovery presents (96.1%-103.3%).

t-Test and F- test

t – Test and F- test were calculated by using equations in inlitterateursto comparison between the data which obtained from standard method() and from this method. Table (6) below illustrates the results.

Table (6). Results of t – test and F- test

Standard method					
$\sum(x-\overline{x})^2$	$(x-\overline{x})^2$	$\overline{x-(x)}$	$\overline{x}$	x
$7.07*10^{-03}$	$3.42*10^{-04}$	$-1.85*10^{-02}$	7.560	7.541
	$3.42*10^{-04}$	$1.85*10^{-02}$		7.578
	$3.19*10^{-03}$	$5.65*10^{-02}$		7.616
	$3.19*10^{-03}$	$-5.65*10^{-02}$		7.503
Proposed method				
$\sum(x-\overline{x})^2$	$(x-\overline{x})^2$	$\overline{x-(x)}$	$\overline{x}$	x
$5.78*10^{-04}$	$2.89*10^{-04}$	-0.017	7.549	7.532
	0000	0.000		7.549
	$2.89*10^{-04}$	0.017		7.566
parameters						
F	S_2^2	S_1^2	S_2	S_1	t	S_{1-2}
8.153	0.0003	0.002	0.017	0.049	0.658	0.039

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)

Conclusion

A simple, sensitive, rapid spectrophotometric method for determination of CAP drug. It is based on condensation reaction between CAP and NQS yield orange-red colored product that exhibits a maximum absorption 489 nm. The proposed method was applied successfully for the determination of drug in its pharmaceutical preparations. The results obtained from this study gives good agreements and comfortable method for the determination of CAP in different pharmaceuticals preparations .

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