International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.05 pp 712-722, 2016

New Spectrophotometric method for the Determination of Chloramphenicol in Pharmaceutical Preparations Based on Schiff Base Reaction with P Dimethylamino benzaldehyde as Reagent

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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 reaction between CAP and p-Dimethylamino benzaldehyde (PDAB) as reagent to formed a yellow Schiff base compound after reducing nitro group in drug into amino group by used a concentrated HCl and zinc dust. yellow compound was shown a maximum absorption at 436.5nm.Beers law was obeyed in the concentration rangeof 0.1-12µg.mL^{-1} with a molar absorptivity (1.79×10^{4})L.mol^{-1}.cm^{-1}, and sandell's sensitivity (1.8×10^{-2})µg.cm^{-2}, respectively. The analytical parameters were optimized as the fallowing: The best temperature is (1-60 °C), the reaction completed directly with addition PDAB to drug and the best volume of PDAB solutionis3.5mL. Limit of detection (LOD),and limit of quantification (LOQ)are0.037ppm,and0.124ppm, respectively, there coveries range 98.02%-100.6%. The method was successfully applied to the analysis of the(CAP) inits pharmaceutical preparations (Eye drops ,Ointments and Capsules).

Key words: Drugs, Chloramphenicol(CAP) , p-Dimethylaminobenzaldehyde (PDAB),Schiff base , 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]

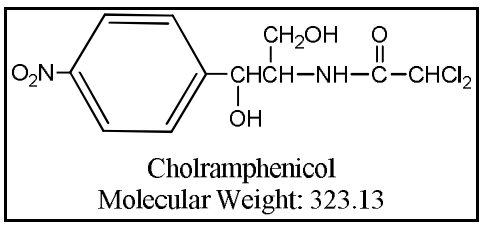


Fig. 1: Chemical structure of chloramphenicol

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 in 1947 . 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 [(2, 4,5, 6)]. Chloramphenicol is distributed to body fluids and all tissues such the central nervous system also cerebrospinal fluid there for 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[(7)].

The adverse effects of this compound have led to restrict its use in both human and veterinary medicine[(8)].

Several methods have been used for determination of chloramphenicol in pharmaceutical dosage form different spectrophotometric methods[(9-18)], chromatographic methods[(14-18)], electrochemical methods[(8-7,19-21)], flow-injectionanalysis methods[(22-23)].

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 PDAB reagent to form a yellow Schiff base compound and measured the absorbance of yield yellow colored by use UV-Visible Spectrophotometer.

Experimental

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 of reduced 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 reducedby 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 filtered to 100 ml in a calibrated flask and diluted with methanol to the mark.

Stock Solution of (0.01 M) PDAB

It was prepared by dissolving 0.1492 g of PDAB reagent in 100 ml beaker with 5 ml of methanol with stirring after that the solution was transferred to 100 ml volumetric flask and its volume was completed to the mark with same solvent .

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

Preliminary studies

In this study, the Schiff base reaction of CAP with PDAB was formed yellow compound that showed a maximum absorbance at 436.5 nm where as a maximum absorbance of pure CAP was 276 nm against the solution blank. These result were showed in (Fig. 2 and Fig. 3).

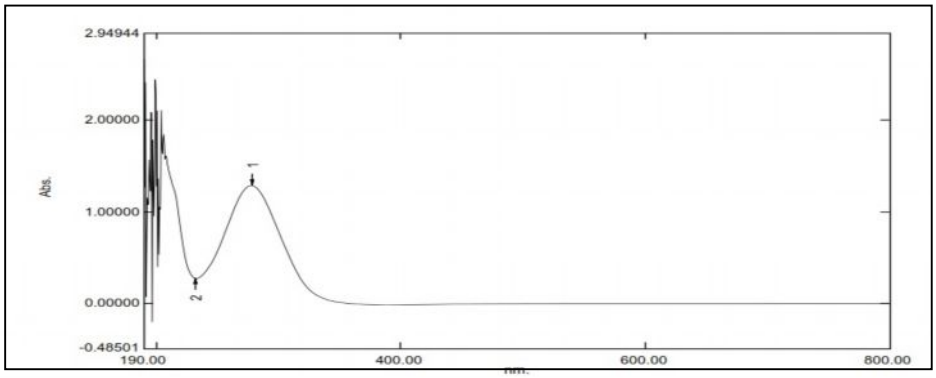


Fig. 2: Absorption spectra of pure CAP against distilled water. λ max = 276

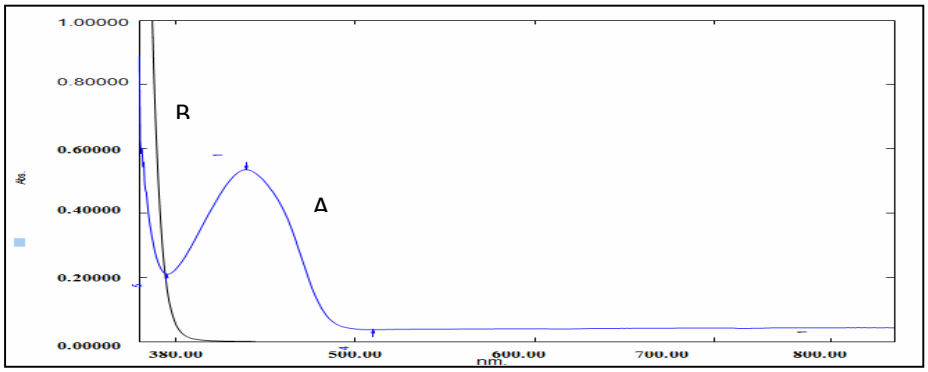
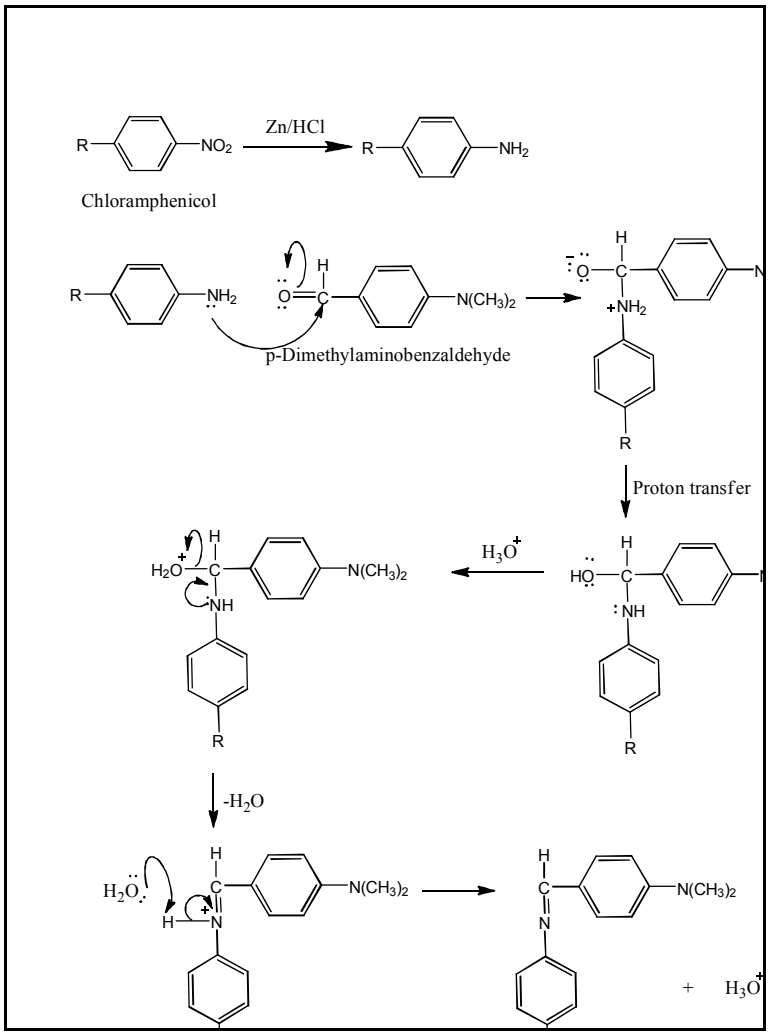


Fig. 3: Absorption spectra of A. CAPR-PDAB against reagent blank λ max = 436.5. and B. reagent blank measured against distilled water.

The mechanism of Schiff base reaction was suggestion in this study showed in scheme (1) agreement with that was found in litterateurs[24]



Scheme 1: Proposed mechanism of the reaction between CAPR and PDAB

Optimum condition of reduction process .

Effect of concentration hydrochloric acid volume.

The effect of conc. hydrochloric acid volume was studied by using different volumes 0.25-15mL of conc. hydrochloric acid .There sults were shown in (Fig.4) 0.5m Lof acid was selected as preferred volume that required for reduction process.

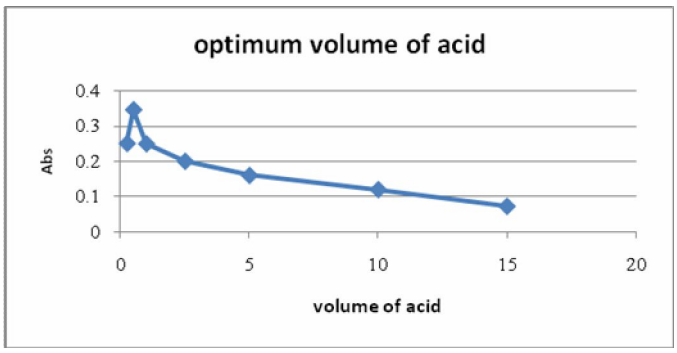


Fig. 4: Effect of conc. hydrochloric acid volume on the reduction Process. Mentioned of the weight of zinc dust (0.2 g) at (5 min) of reaction and (50 ^{o}C)

Effect of zinc dust amount

Different amounts of zinc dustwereused0.1-0.5g .The results showed in(Fig. 5) .it was obvious that0.3 g of zinc dust gave the maximum absorption, denoted the reaction of the reduction of nitro group containing in drug was completed reducing to amino group .

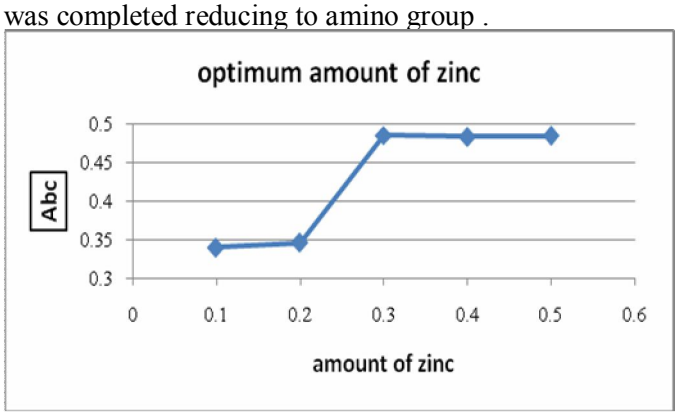


Fig. 5: Effect of zinc dust amount that required for reduction process. Mentioned of the volume of conc. Hydrochloric acid (0.5 ml) at (5 min) of reaction and (50 ^{o}C)

Effect of temperature and time.

The effect of temperature and time were tested by using different temperature 40-70^{o}C and different time 2.5 –15 min and the results were shown in(Fig.6) and (fig 7).50 ^{o}Cand 5 min were selected as preferred temperature and time that required in reduction process.

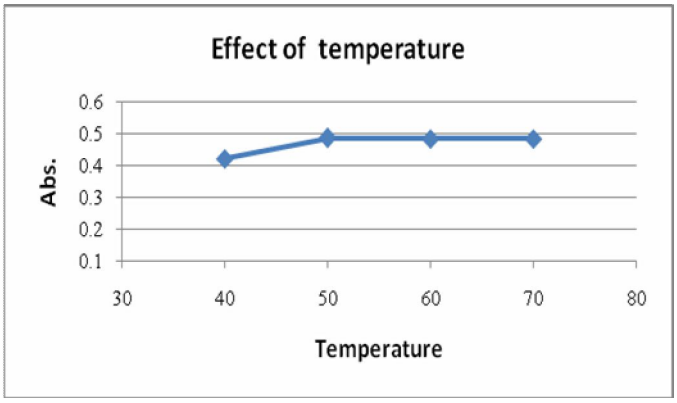


Fig. 6: The effect of temperature that required for reduction process. Mentioned of the weight of zinc dust (0.3 g) and the volume of conc. Hydrochloric acid (0.5 ml) at (5 min) of reaction .

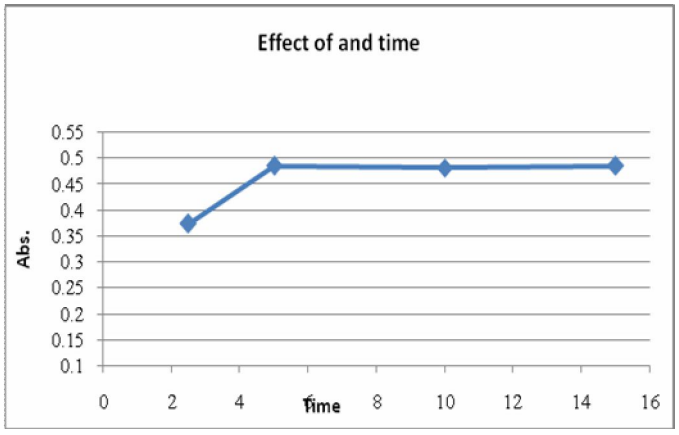


Fig. 7: Effect of time that required for reduction process. Mentioned of the weight of zinc dust (0.3 g) and the volume of conc. Hydrochloric acid (0.5 ml) at (50 ^{o}C)

Effect of solvent type .

The effect of different solvents on the development of reaction between CAPR and PDAB Schiff base were studied , the results were obtained shown in (Fig.8) the methanol gave the best result.

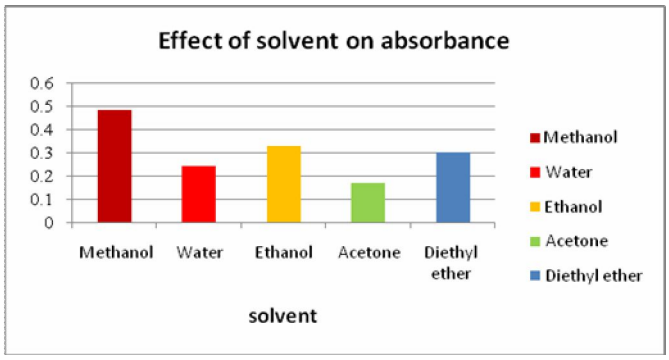


Fig. 8: The effect solvent that required for Schiff base reaction .

Effect of acids.

The effect of different acids on the development of reaction between CAPR and PDAB Schiff base were studied , the results were obtained shown in (Fig.9) without acid gave the best result.the acids were effected on the development of Schiff base color that may be (the reaction between RCAP with PDAB does not completed) therefor the Schiff base reaction gave the best result without any additions of acids .

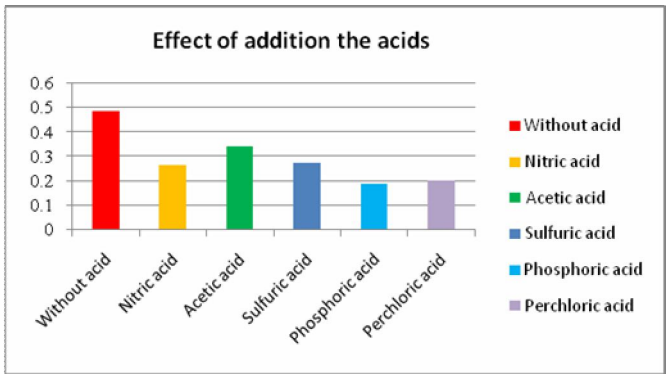


Fig. 9: The effect acid that required for Schiff base reaction

Effect of volume of PDAB(0.01 M)

DifferentvolumeofPDABweretested0.5-4.5mL and the results are shown in (Fig. 9)3.5 mL of it was used for Schiff base reaction with CAPR to give best colored product.

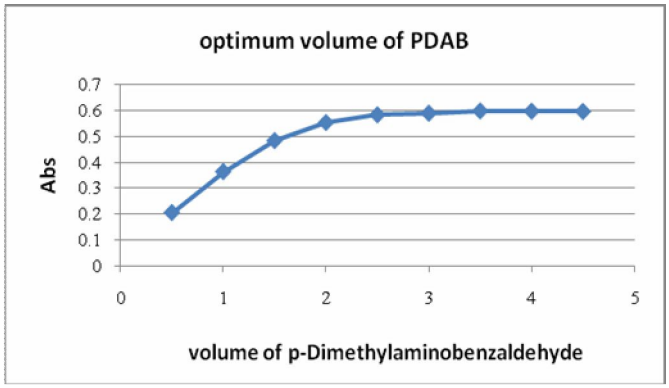


Fig. 9: The effect of reagent (PDAB)volume

Effect the temperature and the time

The effect of temperature and time were studied on the reaction between CAPR and PDAB the absorbance of Schiff base product were measured at each individual time and temperature and the results were shown in (Fig. 10), (20 ^{o}C)and 5 min – 60 min were selected as preferred result that required in the Schiff base reaction .

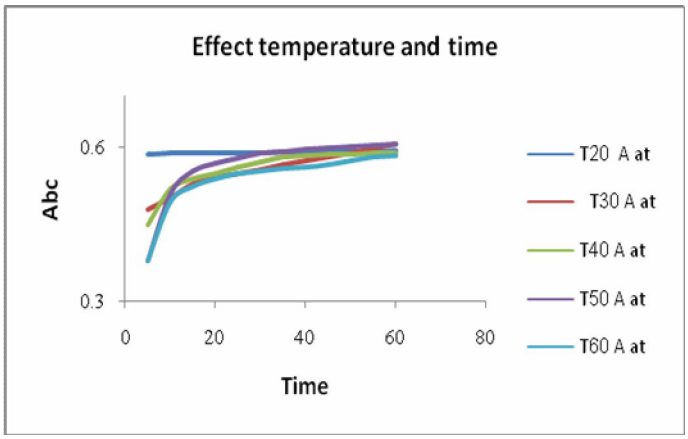


Fig. 10: The effect of temperature and time on stability of product

Calibration curve and sensitivity

(Fig.11) explain the calibration curve of Schiff base product that obey the Beer law in the range of (0.1-12 ppm)of the concentration of CAP at 436.5 nm.

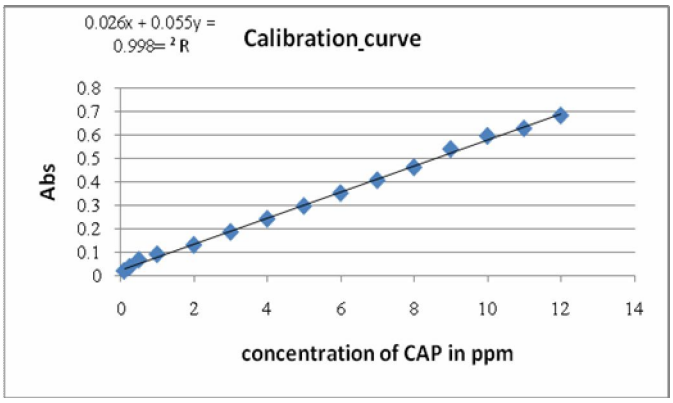


Fig. 11: Calibration curve of CAP

Other parameters such (Sandell's sensitivity, Molar absorptivity,Limit of detection LOD and limit of quantification LQD) were calculated and the results shown in Table 1.

Table 1:Analytical characteristics of proposed method

Value	Parameter
y = 0.0555x + 0.0264	Regression equation
0.1 – 12	Beer's law limits (µg ml^{-1})
0.9982	R^2 value
0.0555	Slope
1.79 * 10^{4}	Molar absorptivity (l.mol^{-1}. cm^{-1})
1.8 * 10^{-2}	Sandell's sensitive , S (µg cm^{-2})
0.037	LOD (µg.ml^{-1})
0.124	LOQ (µg.ml^{-1})

Nature and stability constant of the Schiff base product .

The stoichiometry of the product was studied by applying the continuous variation method and molar ratio method[25]The results are shown in (Fig. 10,11)for the ratio between the CAPR to PDAB is 1:1.The stability constant of the Schiff base product is (2.65 x 10^6)L.mol^{-1}.cm^{-1}.

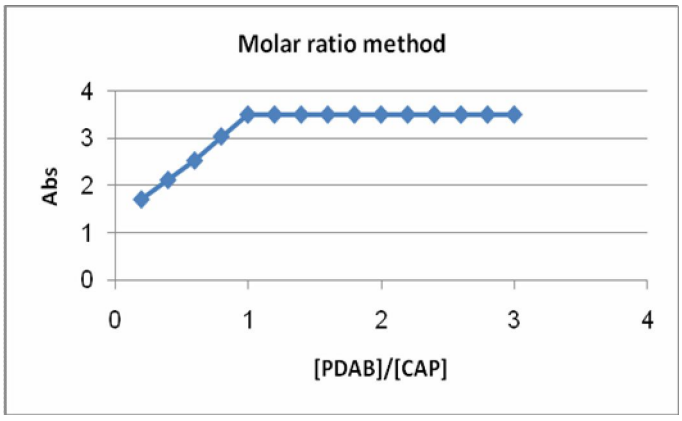


Fig. 10: molar ratio methods of colored product

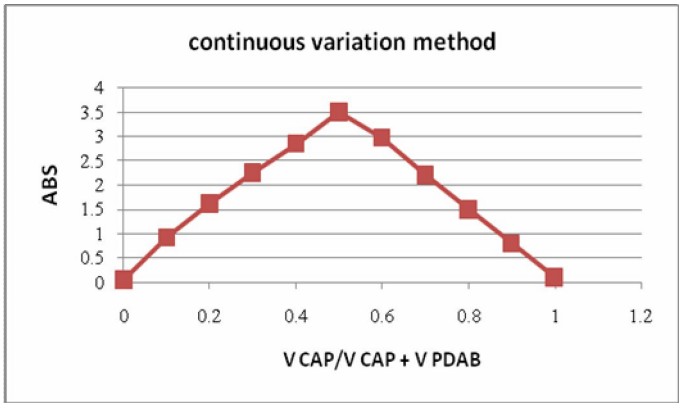


Fig. 11: continuous variation of colored product

Accuracy and precision of the proposed method

The accuracy and precision (Relative Error E% , Recovery percent (Rec %) and Relative standard deviation percent (RSD %)).were tested by apply proposed method by reading the absorbance to different sample and find the concentrations and calculated (E% ,Rec % and RSD %) .

Table 2: Accuracy and precision of proposed method

R.S.D%	Rec%	E%	Conc. Of CAPR in ppm		NO
			Found	Taken	
1.22	98.02	-1.98	1.470	1.5	1
0.52	98.64	-1.36	3.452	3.5	2
0.36	100.6	0.6	7.545	7.5	3

Pharmaceutical applications

The method was applied on different pharmaceutical preparations (eye drops, ointments and capsules) by using three concentration of each one. The results are shown in Table 4. A good recoveries and the results of this method were compared with official method [1]in Table 5 that showed there is no significant different between the official method and the proposed method.

Table 4: Pharmaceutical applications for CAP using the proposed method

No	Type of assay	Drug taken in (mg)	Drug taken in (ppm)		Drug found in (ppm)	Drug found in (mg)	average	Error%	Rec%
1	Eye drop	50	1	10	10.191	50.955	51.062	2.124	102.124
			2	7	7.056	50.400			
			3	3	3.11	51.832			
2	Ointment	50	1	10	9.813	48.096	48.601	-2.798	97.202
			2	7	6.75	48.211			
			3	3	2.912	48.529			
3	Capsule	250	1	10	9.939	248.468	253.207	1.283	101.283
			2	7	7.056	251.995			
			3	3	3.11	259.159			

Table 5: Application of the proposed and official methods to determination of CAP in pure and dosage forms

standard method				
$\sum(x-\bar{x})^2$	$(x-\bar{x})^2$	$(x-\bar{x})$	$\bar{x}$	x
7.07×10^{-3}	3.42×10^{-4}	-0.02	7.56	7.541
	3.42×10^{-4}	0.02		7.578
	3.19×10^{-3}	0.06		7.616
	3.19×10^{-3}	-0.06		7.503
Proposedmethod				
$\sum(x-\bar{x})^2$	$(x-\bar{x})^2$	$(x-\bar{x})$	$\bar{x}$	x
1.51×10^{-3}	3.60×10^{-5}	-0.01	7.544	7.538
	5.76×10^{-4}	-0.02		7.52
	9.00×10^{-4}	0.03		7.574

Values						
F	**S_2^2**	**S_1^2**	**S_2**	**S_1**	**t**	**S_{1-2}**
3.12	7.56×10^{-4}	2.36×10^{-3}	0.03	0.05	0.92	0.04

Conclusion

A simple, sensitive, rapid spectrophotometric method for determination of CAP drug. It is based on Schiff base reaction between CAP and PDAB yield yellow colored product that exhibits a maximum absorption t436.5nm. 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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