Spectrophotometric Determination of Chloramphenicol in Pharmaceutical Preparations

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Abstract

Two simple, rapid and sensitive spectrophotometric methods have been developed for the determination of chloramphenicol (CAP) in pure form and in pharmaceutical preparations. The proposed methods are based on a coupling reaction between reduced chloramphenicol (by zinc powder and concentrated hydrochloric acid) with chromatropic acid or phenol in the presence of ammonium hydroxide to form an intense red violet or yellow color, water-soluble dyes that are stable and have a maximum absorption at 515 nm using chromatropic acid and 432 nm on using phenol. The calibration graphs were linear over the concentration ranges of 0.52 -12 µg mL^{-1} and 0.4- 18µg mL^{-1} with a limit of detection (LOD) of 0.1334 µg mL^{-1} and 0.0873 µg mL^{-1} for chromatropic acid and phenol, respectively. The proposed methods were successfully applied to the determination of CAP in ointment and eye drops.

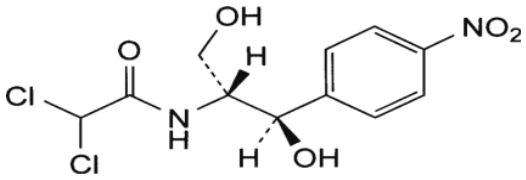
Keywords: chloramphenicol, chromatropic acid, phenol spectrophotometry, diazotization–coupling reaction.

الخلاصة

تم تطوير طريقتين تحليليتين للتقدير البسيط و السريع و الحساس للكلورامفنيكول بصورته النقية وفي المستحضرات الصيدلانية. اعتمدت الطريقة الاولى على تفاعل الازدواج بين الكلورامفنيكول المختزل (باستخدام الزنك مع حامض الهيدروكلوريك المركز) و المؤزوت مع كاشف حامض الكروماتروبك والثانية على كاشف الفينول في الوسط القاعدي لتكوين صبغة حمراء بنفسجية او صفراء مستقرة وذائبة في الماء اعطت أقصى امتصاص عند طول موجي 515 نانومتر للكاشف حامض الكروماتروبك و432 نانومتر لكاشف الفينول0 يشير الرسم البياني للامتصاص مقابل التركيز بان قانون بير ينطبق ضمن المدى 0,52 – 12 مكغم $مل^{-1}$ و 0,4– 18 مكغم $مل^{-1}$ وبحد كشف 0,1334 مكغم $مل^{-1}$ و 0,0873 مكغم $مل^{-1}$ للكاشفين حامض الكروماتروبك والفينول على التوالي. تم تطبيق الطرق المقترحة بنجاح في تقدير الكلورامفنيكول في المستحضرات الصيدلانية المرهم وقطرات العين.

Introduction

Chloramphenicol (CAP) is 2,2 dichloro- N-[(1R,2R)-2-hydroxy-1-hydroxymethyl-2-(4-nitrophenyl)ethyl] acetamide, with molecular structure $C_{11}H_{12}Cl_2N_2O_5$ (323.13g.mol^{-1}) and its chemical structure:



It is a white, grayish-white or yellowish-white, fine crystalline powder or fine crystals, needles or elongated plates, freely soluble in methanol, ethanol, butanol, ethyl acetate, acetone, and in propylene glycol, slightly soluble in water, and ether, insoluble in benzene and petroleum ether, it melts at 150.5–151.5°C [1].

CAP is a bacteriostatic antimicrobial. It is considered a prototypical broad-spectrum antibiotic, alongside the tetracyclines. CAP is effective against a wide variety of Gram-positive and Gram-negative bacteria, including most anaerobic organisms. It is widely used because it is inexpensive and readily available [2]. It is used by local application for the treatment of a variety of infections of the skin, ear and eye including trachoma [3].

Various methods have been reported for the determination of CAP in pharmaceutical preparations, including high performance liquid chromatography [4], LC-Mass spectrometry [5- 6], Polarography [7], chemiluminescence [8-9], Fluorescence [10], enzymatic method [11],chemometry [12], colorimetry [13-14], thin layer chromatography [15], Voltametry [16], spectrophotometry and FIA Spectrophotometry [17- 23].

The aim of the present work was to develop a spectrophotometric methods for determination of CAP based on coupling reaction between diazotized CAP with either chromatropic acid or phenol in the presence of ammonium hydroxide. The analytical procedure is safe, simple, fast and accurate. It has been satisfactorily applied to the determination of CAP in pure and in pharmaceutical preparations.

Experimental

Apparatus

All spectral and absorbance measurements were carried out on a Shimadzu UV- Visble-260 digital double-beam recording spectrophotometer (Tokyo-Japan), using 1-cm quartz cells.

Reagents:

All chemicals used were of analytical reagent grade. CAP standard material was provided from the state company for drug industries and medical appliances (SDI) Sammara-Iraq.

Chloramphenicol (CAP) solution (500 µg mL^{-1}= 1.547 × 10^{-3}M) [24]

Was prepared by dissolving 0.0500 g of CAP in ethanol transferred into 50 mL volumetric flask, and diluted to the mark with the same solvent. The solution was transferred into a beaker of 125 ml. A 20 mL of distilled water, 20 mL of concentrated hydrochloric acid (11.64 N) and 3 g of zinc powder were added. The beaker was allowed to stand for 1hr. at room temperature, then the solution was filtered into 100 mL volumetric flask,

washed the residue with distilled water, and diluted to the mark volume with distilled water to obtain 500 $\mu g.mL^{-1}$ of CAP reduced solution. More dilute solutions were prepared daily by appropriate dilution using distilled water.

Sodium nitrite (1.547×10^{-3}M)

Was prepared freshly by dissolving 0.0535g of $NaNO_2$ in small amount of distilled water then completed to 500 mL with the same solvent.

Chromatropic acid (3.1×10^{-4}M)

Was prepared by dissolving 0.0113g of chromatropic acid in distilled water then completed the volume to 100 mL with the same solvent.

Phenol(0.1%)

Was prepared by dissolving 0.1g of phenol with distilled water then completed the volume to 100 mL with the same solvent.

Ammonium hydroxide (4M)

Was prepared by diluting 149.7 mL of 13.36M of concentrated ammonium hydroxide with distilled water in 500 mL volumetric flask.

Diazotized chloramphenicol (3.1×10^{-4}M) reagent solution

Was prepared by transferring 20 mL of 500$\mu g.mL^{-1}$CAP into 100 mL volumetric flask then added 20 mL of 1.547×10^{-3}M sodium nitrite, shaked well and completed to 100 mL in a volumetric flask with distilled water.

More dilute solutions were prepared fresh daily by dilution of the stock solution with distilled water.

Solutions of pharmaceutical preparations

1- **Eye drops samples - 10 mL (0.5% chloramphenicol/ 0.005% cetrimide-SDI, Sammara, Iraq):**

The contents of three bottles of eye drops were mixed. An aliquot corresponding to 50 mg of CAP (10 mL) was diluted to 50 mL with ethanol in a volumetric flask. This solution was transferred into 125 mL beaker and was reduced as described above and diluted to 100mL volume with distilled water to obtain 500 $\mu g.mL^{-1}$ of CAP reduced solution.

2- **Ointment samples - 5 gm (Betaphenicol sterile ophthalmic / 0.5% chloramphenicol 0.2 % betamethasone - Delta for medicaments, Syria):**

The contents of five tubes of ointment were mixed. An accurately weighed amount of ointment equivalent to 50 mg of CAP was extracted three times with 10 mL of ethanol. The solution was filtered and diluted into a 50 mL volumetric flask with ethanol. This solution was transferred into 125 mL beaker and was reduced as described above and diluted to 100mL volume with distilled water to obtain 500 $\mu g.mL^{-1}$ of CAP reduced solution.

General procedure for calibration

Method A

An increasing volumes (0.13 - 3 ml) of 100 $\mu g.ml^{-1}$ (3.1×10^{-4}M) diazotized CAP were transferred into a series of 25 mL standard flask. A 4 mL of chromatropic acid (3.1×10^{-4}M) and 2 ml of 4 M ammonium hydroxide were added and shaked well . The contents of the flasks were diluted to the mark with distilled water, mixed well and left for 15 min at room temperature , the absorbance of the red violet dye formed was measured at 515 nm against a reagent blank containing all materials except CAP. A calibration graph was constructed and the regression equation was calculated for the optimization of conditions and in all subsequent experiments, a 2 ml of 100 $\mu g.mL^{-1}$ of diazotized CAP in a final volume of 25 mL was used.

Method B

An increasing volumes (0.1 -4.5 ml) of 100 µg.ml^{-1} (3.1 x 10^{-4}M) diazotized CAP were transferred into a series of 25 mL standard flask. A 1mL of phenol (0.1%) and 1.5 ml of 4 M ammonium hydroxide were added and shaked well . The contents of the flasks were diluted to the mark with distilled water, mixed well and left for 15 min at temperature (10-15^{o} C), the absorbance of the yellow dye formed was measured at 432nm against a reagent blank containing all materials except CAP. A calibration graph was constructed and the regression equation was calculated for the optimization of conditions and in all subsequent experiments, a 2 ml of 100 µg.mL^{-1} of diazotized CAP in a final volume of 25 mL was used.

Results and Discussion

Absorption spectra

Factors affecting on the sensitivity and stability of the colored products resulting from the coupling reaction between diazotized CAP and chromatropic acid(method A) and phenol(method B) in alkaline medium were carefully studied. A typical spectrum for the formed azo dyes were measured versus reagent blank which has negligible absorbance at λmax 515 nm (method A) and 432 nm(method B), Fig.(1).

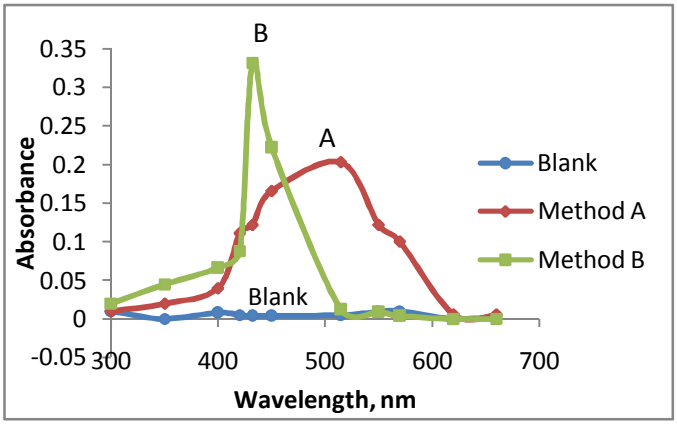


Fig.(1): Absorbance spectra of CAP (8µg mL^{-1}) - azo dyes and reagent blank. The reagent blank measured against distilled water

A: azo dye by method A

B: azo dye by method B

Optimization of the experimental conditions

Effects of various parameters on the absorption intensity of the formed products were optimized

The coupling reaction of diazotized CAP with both chromatropic acid(method A) and phenol(method B) were formed in alkaline medium. Therefore, the effects of different alkaline solutions (4 M) were studied such as sodium hydroxide, sodium carbonate, potassium hydroxide and ammonium hydroxide. It was found that ammonium hydroxide was the most suitable alkaline medium for a maximum absorbance and was used in all subsequent experiments in both methods A and B.

The effect of different volumes of ammonium hydroxide (4 M) was studied on the maximum absorbance by varying the volume of ammonium hydroxide solution between (1-5) mL. A volume of 2 mL , 1.5 mL ammonium hydroxide (4 M) for methods A and B respectively were enough to obtain the maximum absorbance.

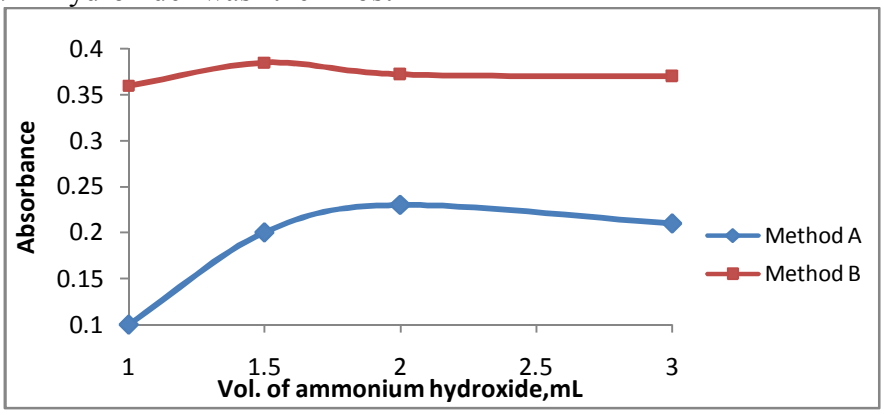


Fig.(2): Effect of the volume of ammonium hydroxide (mL).

Similarly, the effect of the reagents (chromatropic acid (3.1×10^{-4} M) and phenol(0.1%) were studied in the range of (0.5-6) mL .The greatest absorbance intensity were obtained with 4 mL and 1mL of the reagents for method A and B respectively, Fig.(3).

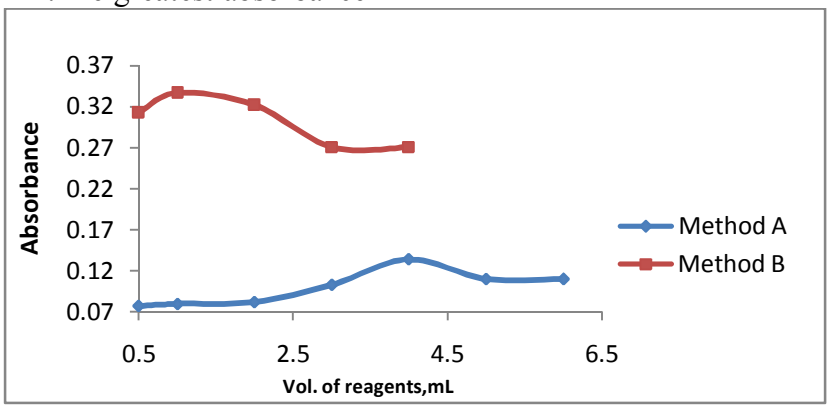


Fig.(3): Effect of the volume of reagents(mL).

Effect of order of addition

Different orders of addition of the reagents were examined and it was found that the order of addition of reagent by mixing chromatropic acid (method A) and phenol (method B) as cited under general procedure was optimum and was used in all subsequent experiments.

Effect of reaction time

In spite of the rapid color development (formed immediately) the color intensity reached a maximum after diazotized CAP solution had been reacted with chromatropic acid(method A),phenol(method B) and ammonium hydroxide for 15min, therefore a 15 min development time was selected as optimum in the general procedures. The color obtained was stable for 2 hr.

Structure of the products

The stoichiometry of the reaction between diazotized CAP (3×10^{-4}M) and chromatropic acid (3×10^{-4} M) (method A) and phenol (3×10^{-4} M)(method B) were investigated using continuous variation method. The result obtained in Fig.(4) shows that a (1:1) was formed between diazotized CAP with chromatropic acid (method A) and phenol(method B) at 515 nm and 432 nm respectively.

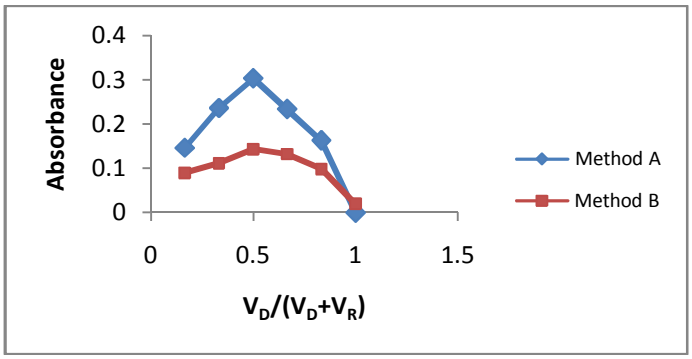
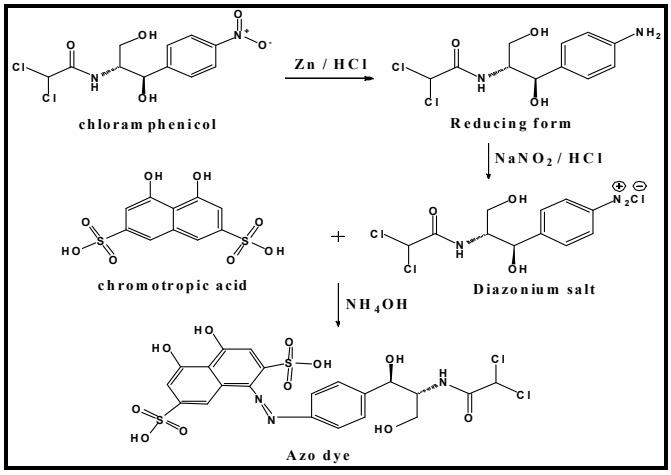
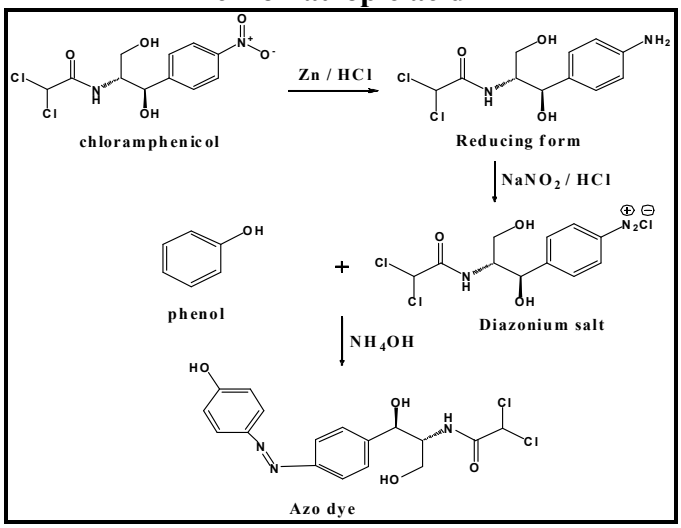


Fig.(4): Continuous variation plot.

The development of the reactions occur in two steps: in the first step the reaction of reduced chloramphenicol with sodium nitrite producing the diazo compound. In the second step, the diazo compound in alkaline medium coupled with chromatropic acid (method A) and phenol (method B) produced a compounds that were monitored at 515nm and 432nm respectively. The reaction schemes are given below:



Scheme 1: proposed mechanism of the reaction between diazotized CAP with chromatropic acid



Scheme 2: proposed mechanism of the reaction between diazotized CAP with phenol.

The apparent stability constant in methods A and B were calculated by comparing the absorbance of a solution containing stoichiometric amount of diazotized CAP (3×10^{-4}M) and chromatropic acid (3×10^{-4} M) (method A) or phenol(3×10^{-4}) (method B) (A_S) with that of a solution containing a five-fold excess of chromatropic acid or phenol reagents (A_m) (method A and B)respectively and according to analytical procedure. The average stability constant was (K)= 2.064×10^5 L $mol^{-1}$and 3.0913×10^5 L. mol^{-1} in method A and B respectively where [$K = (1-\alpha) / \alpha^2 C$; $\alpha = (A_m - A_s) / A_m$][25].

Analytical characteristics of spectrophotometric method

For the proposed methods, a calibration graphs were obtained by the procedure described previously and a series of standard solutions were analyzed in triplicate to test the linearity, Fig.(7). The molar absorptivity (ε), the Sandell's sensitivity (S), the slope (a) and the intercept (b) were determined and are included in Table 1. The accuracy and precision of the proposed methods were tested by analyzing five replicate

of diazotized CAP using the proposed spectrophotometric methods for three different concentrations of diazotized CAP. The values of relative standard deviation RSD% and relative error E_{rel}% are summarized in the same table. These values indicate high accuracy and precision of the proposed methods. As well as to LOD, the author capable to calculate limit of quantitation (LOQ). Therefore, the underlined lines could be replaced with the following:

The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the formula: Kσ/ S where K= 3 and 10 for LOD and LOQ, respectively. σ is the standard deviation of the blank and S is the slope of the calibration curve[26].

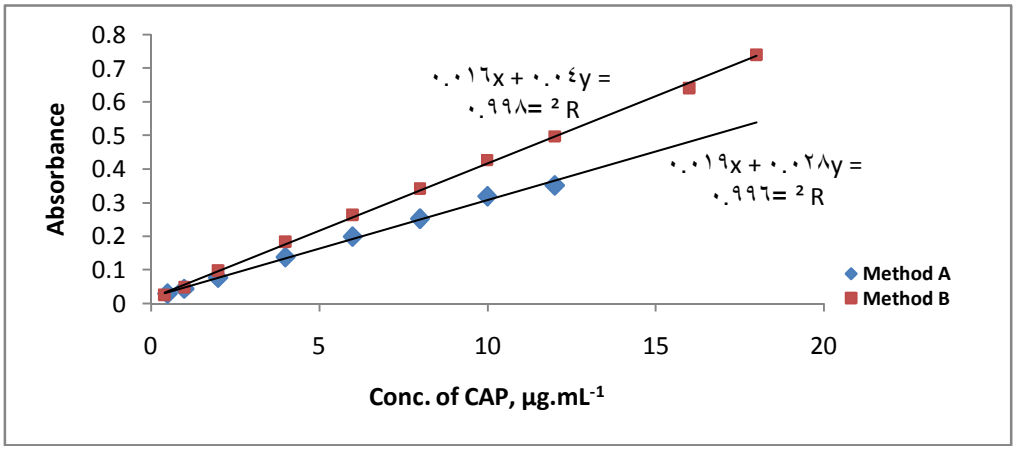


Fig.(7): Calibration graph of CAP.

Table (1): Analytical parameters of spectrophotometric method

Parameters	Method A	Method B
$\lambda_{max\ (nm)}$	515	432
Linearity range, µg ml^{-1}	0.52-12	0.4-18
Molar absorbtivity (L mol^{-1} cm^{-1})	1.241×10^{4}	1.491×10^{4}
Sandell's sensitivity(µg $.cm^{-2}$)	26.035×10^{-3}	21.670×10^{-3}
Regression equation	y = 0.0288x + 0.0195	Y= 0.04X+0.0168
Correlation coefficient (r)	0.9979	0.9994
Limit of detection (µg $ml^{-})^{1}$	0.1334	0.0873
Relative standard deviation (RSD%)*	1.905	1.276
Average of recovery%	100.188	100.169
E_{rel} %	0.188	0.169
Stability (hr.)	2	2
Molar ratio (D:R)	1:1	1:1
Color	Red violet	Yellow

Pharmaceutical application

The proposed methods were compared successfully with the British Pharmacopeia[1] for both pure CAP and the pharmaceutical preparations . For all preparations examined, the assay results of the proposed methods were in good agreement with the declared content. The results in table 2 are in accordance with those obtained by the official method[1] and by applying F-test and t- test, at 95% confidence level. The calculated values for F(2.363, 3.251) and t (0.115, 0.046) for method A and B respectively did not exceed the critical values of $F_{4,4}$ = 9.605 and t = 2.306 (n_1+n_2-2= 8). This confirm that there is no significant

differences between the proposed methods with the official method with respect to precision and accuracy in the determination of CAP in pharmaceutical preparations.

Table 2- Application of the proposed and official methods for the determination of CAP in pharmaceutical formulation

Pharmaceutical preparation	Proposed methods					Official method Recovery (%)
	Present Conc. (μgml^{-1}) in both methods (A and B	Method A		Method B		
		Rec.* (%)	R.S.D* (%)	Rec.* (%)	R.S.D* (%)	
Cetrimide eye drop	4	100.95	1.347	101.325	0.918	100.6
	6	101.533	1.302	101.400	0.841	
Betaphenicol ointment	4	98.075	0.569	98.025	1.162	98.67
	6	98.466	1.951	97.667	0.677	

***average of four determinations**

The standard additions method was used to confirm the direct procedure results presented. It involves adding increment volumes (0-2) mL of standard solution of 100 $\mu g.mL^{-1}$ to a fixed volume sample(0.5 mL of 100$\mu g.mL^{-1}$) and employing the conditions described under procedure. It gave a good accuracy and precision (Table 3) and indicated that by using either the direct procedure or standard additions method, there was no interferences from other ingredient present in the pharmaceutical preparation used.

Table 3- Application of the standard additions method and official methods for the determination of eye drop and ointment containing CAP.

Pharmaceutical preparation	[CAP] depend on st. additions*		Standard additions method		Official method
			Method A	Method B	
	Method A	Method B	Recovery, %	Recovery, %	Recovery, %
Cetrimide eye drop	2	2.01	100	100.5	100.6
Betaphenicol Ointment	1.95	1.97	97.5	98.5	98.67

***Standard additions**

Conclusion

The proposed methods were found to be very simple, rapid, low cost, and fairly selective than some of the reported methods. They had an advantage of being accurate, did not require the removal of excipients, any chemical sample pretreatment, temperature control, pH control, solvent extraction step, and expensive reagents and solvents. The proposed methods were applied to the analysis of CAP in pharmaceutical formulations and can be used for the routine analysis .

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