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Development of Indirect Spectrophotometric Method for Quantification of Cephalexin in Pure Form and Commercial Formulation Using Complexation Reaction

Muhammad Naeem Khan*, Sundus Kalsoom, Rahana Hussain, Zarbad Shah and Muhammad Saadiq

Department of Chemistry, Bacha Khan University, Charsadda, KPK, Pakistan.

*Corresponding Author Email: mnaeemchemist@yahoo.com

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Abstract

A simple, accurate and indirect spectrophotometric method was developed for the quantification of cephalexin in pure form and pharmaceutical products using complexation reaction. The developed method is based on the oxidation of the cephalexin with Fe^{3+} in acidic medium. Then 1, 10-phenanthroline reacts with Fe^{2+} and a red colored complex was formed. The absorbance of the complex was measured at 510 nm by spectrophotometer. Different experimental parameters affecting the complexation reactions were studied and optimized. Beer's law was obeyed in the concentration range 0.4 -10 μgmL^{-1} with a good correlation of 0.992. The limit of detection and limit of quantification were found to be 0.065 μgmL^{-1} and 0.218 μgmL^{-1}, respectively. The method have good reproducibility with a relative standard deviation of 6.26 % (n = 6). The method was successfully applied for the determination of cephalexin in bulk powder and commercial formulation. Percent recoveries were found to range from 95.47 to 103.87 % for the pure form and 98.62 to 103.35 % for commercial formulations.

***Keywords*:** Cephalexin, Spectrophotometry, Pharmaceutical formulations, 1, 10-Phenonthroline.

Introduction

Cephalexin (7-[(aminophenyl acetyl) amino]-3-methyl-8-oxo-5-thia-l-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid) is a semisynthetic β-lactam antibiotic, which belongs to the group of cephalosporin antibiotics. Cephalexin is a first-generation cephalosporin antibacterial used for the handling of vulnerable infections including those of respiratory tract, urinary tract and skin [1]. Cephalexin is an active broad spectrum antibiotic that targets both Gram positive and Gram negative bacteria [2]. Cephalexin is an effective cephalosporin and shows a broad spectrum of antibiotic activity, weak bond capability to blood protein, no metabolites, and low toxicity and to be quickly absorbed following oral administration to give a high serum levels and urine concentration. Therefore, the cephalexin is widely used for clinical chemotherapy [3,4]. The extensive use of cephalexin make a clinical and pharmacological study needs a simple, fast and sensitive analytical method to determine the drug in commercial formulations and biological samples.

A number of methods have been described in the literature for the determination of cephalexin. These include chromatographic methods [5-9], fluorometry [10-12], flow injection analysis [13,14], atomic absorption [15] and electroanalytical methods [16]. Limited spectrophotometric techniques are presented for the determination of cephalexin [17-20]. The first spectrophotometric method is based on the reaction of cephalexin with sodium 1, 2-naphthoquinone-4-

sulfonate (NQS) and measured the absorbance at 475 nm. The second spectrophotometric method is based on the reaction of cephalexin with ninhydrin reagent giving blue colored chromogen, which was measured at 576 nm. These spectrophotometric methods involve prolonged procedures or have a narrow range of calibration curves. The aim of the present attempt was to develop a simple, accurate, selective and reproducible method for the determination of cephalexin in pure form and pharmaceutical preparations. The developed method is more sensitive than the published methods and is free from such experimental variables such as heating or extraction step.

Materials and Methods

Instruments

A UV-Visible spectrophotometer SP-1800 (721 G) equipped with 1-cm matched glass cell was used for absorbance measurement. A digital analytical balance and a digital water bath, labacon, model LWB-104 was also used.

Materials and reagents

Analytical or high grade purity reagents were used. $FeCl_3$. $6H_2O$ and H_2SO_4, 95-97% extra pure (Riedel-deHaën, Germany) and 1,10-phenanthroline (S.A, Barcelona Spain) were used. Standard reference cephalexin was gifted by Saydon Pharmaceutical Industry (Pvt.) Ltd., Peshawar, Pakistan. Commercial formulation of cephalexin (Ceporex capsules 250 mg, manufactured by GlaxoSmithKline) Pakistan was purchased locally. 0.003 $molL^{-1}$ Fe^{3+} fresh solution was prepared by dissolving 0.04 g iron chloride hexahydrate in volumetric flask and diluted to 50 mL with distilled water. 0.02 $molL^{-1}$ 1,10-phenanthroline solution was prepared by dissolving 0.198 g of reagent in 5.0 mL of methanol and diluting up to 50 mL with distilled water.

Standard solution

Standard stock solution of cephalexin (100 μgmL^{-1}) was prepared by dissolving 0.005 g of standard cephalexin in distilled water by heating it for few minutes at 75 ^{o}C and after cooling diluted to 50 mL with distilled water. Working standard solutions of the required concentration were freshly prepared by dilution.

Sample Solution

The contents of five capsules of Ceporex containing 250 mg of cephalexin were weighed and mixed. The average mass of the powder in one capsule was calculated. Proper amount of the drug powder equivalent to 0.01 g of cephalexin was dissolved in distilled water by heating it for few minutes at 75 °C, filtered, transferred to a 100 mL volumetric flask and made the volume to the mark with distilled water. A working sample solution of 5 μgmL^{-1} was prepared from stock sample solution by dilution with distilled water.

General procedure

Proper volumes of cephalexin standard stock solution to produce a final concentration of 0.4–10 μgmL^{-1} were taken in Erlenmeyer flasks. Then 1.0 mL of Fe^{3+} (0.003 $molL^{-1}$) solution and 1.0 mL of 1, 10-phenanthroline (0.02 $molL^{-1}$) was added. The mixture was heated at 100 °C for 20 minutes on water bath and after cooling transferred to volumetric flasks and made the volume up to 10 mL with distilled water. The absorbance was measured at 510 nm on a spectrophotometer against a reagent blank.

Results and Discussion

When varying amount of cephalexin drug are reacted with a known and fixed amount of Fe^{3+} in acidic medium, proportionate amount of Fe^{3+} will be used for the oxidation of the drug, and there will a concurrent fall in Fe^{3+} concentration and a concomitant increase in the concentration of Fe^{2+}. The amount of Fe^{2+} can be determined by using 1, 10-phenanthroline. This property has been used for the determination of cephalexin in pharmaceutical preparations. The absorption spectra of the colored species show maximum absorbance at 510 nm (Fig. 1). The absorbance is

linearly dependent on the concentration of cephalexin drug studied, forming the basis for the determination.

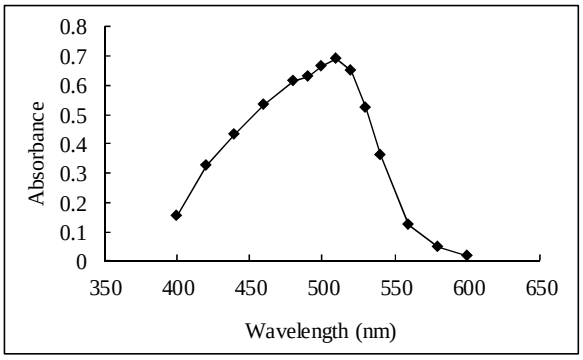

***Figure 1*. Absorption spectra for the colored product**
***Conditions;* 10 µgmL^{-1} cephalexin, 1.0 mL of Fe^{3+} (0.003 molL^{-1}), 1.0 mL of 1,10 phenanthroline (0.02 molL^{-1}), heated at 100 °C for 20 min, diluted to 25 mL**

Optimization of reaction parameters

Different experimental parameters affecting the complexation reaction were carefully studied.

Effect of heating temperature and time

The effect of temperature in the range of 60-100 °C and heating time from 5 to 25 minutes was studied. Maximum color product was formed when the reaction mixture was heated for 20 minutes at 100 °C (Fig. 2, 3).

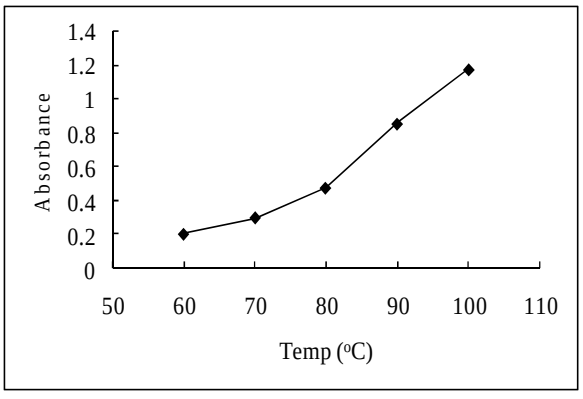

***Figure 2*. Effect of temperature on absorbance**
***Conditions;* 10 µgmL^{-1} cephalexin, 1.0 mL of Fe^{3+} (0.003 molL^{-1}), 1.0 mL of 1,10 phenanthroline (0.02 molL^{-1}), heated at 60-100 °C for 20 min, diluted to 25 mL**

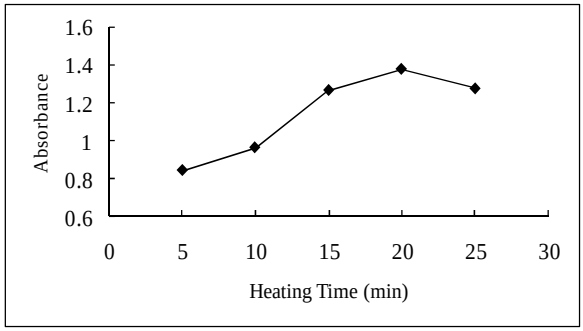

***Figure 3*. Effect of heating time on absorbance**
Conditions: 10 µgmL^{-1} cephalexin, 1.0 mL of Fe^{3+} (0.003 molL^{-1}), 1.0 mL of 1,10 phenanthroline (0.02 molL^{-1}), heated at 100 °C for 5-25 min, diluted to 25 mL

Effect of concentration and volume of Fe^{3+} solution

The effect of concentration of Fe^{3+} was also studied in the range of 0.001 to 0.005 molL^{-1}. It was observed that maximum color formation was occurred with 0.003 molL^{-1} Fe^{3+} (Fig. 4). The effect of volume of 0.003 molL^{-1} Fe^{3+} was also studied and it was found that 1.0 mL was found to produce maximum absorbance.

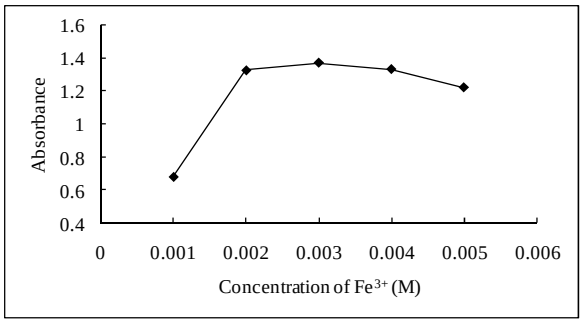

***Figure 4*. Effect of Fe^{3+} concentration on absorbance**
Conditions; 10 µgmL^{-1} cephalexin, 1.0 mL of Fe^{3+} (0.001-0.005 mol L^{-1}), 1.0 mL of 1, 10 Phenanthroline (0.02 molL^{-1}), heated at 100 °C for 20 min, diluted to 25 mL

Effect of concentration and volume of 1, 10-phenanthrolin solution

The effect of concentration of 1,10-phenanthroline was investigated in the range of 0.006 to 0.05 molL^{-1}. Maximum absorbance was observed with 0.02 molL^{-1} (Fig. 5). The effect of volume of 1,10-phenanthroline solution was also investigated and it was found that maximum colored formation was produced with 1.0 mL of 1,10-phenanthrolin.

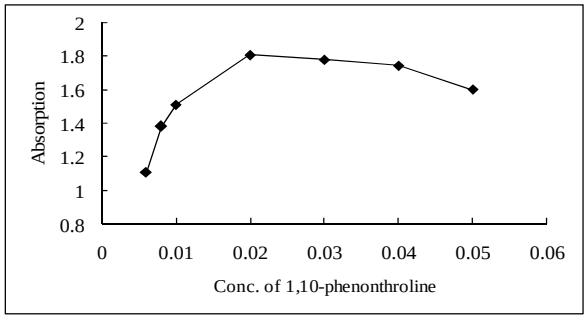

***Figure 5*. Effect of concentration of 1, 10-phenonthroline**
Conditions; 10 µgmL^{-1} cephalexin, 1.0 mL of Fe^{3+} (0.003 molL^{-1}), 1.0 mL of 1,10 phenanthroline (0.006-0.05 molL^{-1}), heated at 100 °C for 20 min, diluted to 25 mL

Stability

The stability of the reaction product was studied by measuring the absorbance after 10 minutes intervals up to 120 minutes. It was investigated that no change in absorbance of the reaction product was observed. Thus, the reaction product is stable and will not affect the result of analysis even if the absorbance of the reaction product is measured after 2 hours of dilution (Fig. 6).

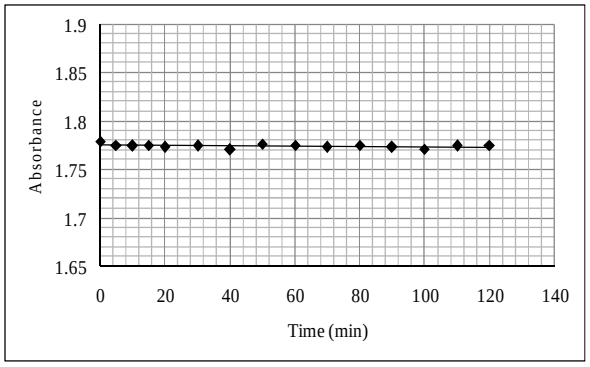

***Figure 6*. Effect of time on stability of reaction product after dilution**
Conditions; 10 µgmL^{-1} cephalexin, 1.0 mL of Fe^{3+} (0.003 molL^{-1}), 1.0 mL of 1,10 phenanthroline (0.02 molL^{-1}), heated at 100 °C for 20 min, diluted to 25 mL

Effect of interferences

The interferences effect from commonly used excipients in pharmaceutical preparations of cephalexin was investigated (Fig.7). These excipients include sucrose, starch and glucose. Under the same experimental conditions, these excipients were added to a known amount of drug (cephalexin 0.4 µgmL^{-1}), in different concentration in the ratio of 1:1, 1:2, 1:4, 1:6, 1:8 and 1:10. The mixtures containing drug and excipients were analyzed by the proposed method. None of these common excipients was found to cause interference.

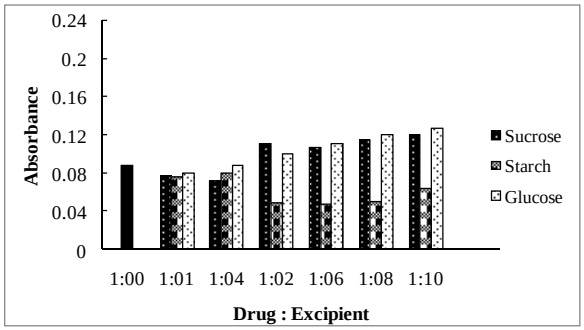

***Figure 7*. Effect of common excipients on determination of cephalexin by the proposed method**

Analytical figures of merit

Under the optimized conditions of the proposed method, Beer's law was obeyed in the concentration range 0.4-10 µg mL^{-1} with a good correlation of 0.992 (Fig. 8). The LOD and LOQ values were calculated and found to be 0.0654 µgmL^{-1} and 0.218 µgmL^{-1}, respectively. The analytical parameters are given in Table 1. The sensitivity of the method is compared with other reported methods (Table 2), which show that sensitivity of the present method is superior to those of previously reported methods.

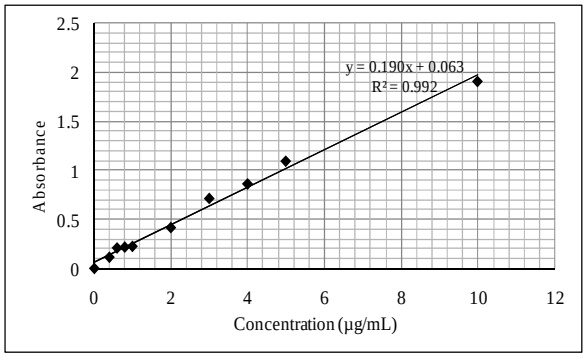

***Figure 8*. Effect of concentration of cephalexin on absorbance**
Conditions; 0.4 - 10 µgmL^{-1} cephalexin, 1.0 mL of Fe^{3+} (0.003 molL^{-1}), 1.0 mL of 1,10 Phenanthroline (0.02 molL^{-1}), heated at 100 °C for 20 min, diluted to 25 mL

***Table 1*. Analytical parameters for the spectrophotometric determination of cephalexin.**

Parameters	Value
λ_{max} (nm)	510
Linear range (μgmL^{-1})	0.4-10
Limit of detection (μgmL^{-1})	0.0654
Limit of quantification (μgmL^{-1})	0.218
Regression equation (y)	Y=0.190X+ 0.063
Slope (b)	0.190
Intercept (a)	0.063
Correlation coefficient (r^2)	0.992
Standard deviation (μgmL^{-1})	0.0218
Relative standard deviation (%)	6.26

***Table 2*. Comparison of the present method and other reported methods for the determination of cephalexin levels.**

Methods	Linear range	Limit of Detection (LOD)	References
Spectrofluorimety	0.04–0.4 μgmL^{-1}	7.76 ngmL^{-1}	[12]
Atomic absorption spectroscopy	5-50 μgmL^{-1}	1.66 μgmL^{-1}	[15]
Spectrophotometry	5–60 μgmL^{-1}	1.22 μgmL^{-1}	[18]
Spectrophotometry	1.5–10 μgmL^{-1}	0.90 μgmL^{-1}	[19]
HPLC	10-110, 10–120 μgmL^{-1}	2.236, 2.090 μgmL^{-1}	[21]
Spectrophotometry	0.4–10 μgmL^{-1}	0.065 μgmL^{-1}	Present method

Reliability of the method

The precision of the method was checked by evaluating cephalexin in pure form and pharmaceutical preparations at three different concentrations in triplicate within the Beer's law range. The results are summarized in Table 3 for pure form and Table 4 for dosage form. The percentage recoveries obtained ranged from 95.47 to 103.87% for the pure form and 98.62 to 103.35% for dosage form. The RSD value was found to be satisfactory (RSD ≤ 6.5) showing good reproducibility of the method. Accuracy of the method was calculated by the standard addition method using one brand of capsule (ceporex containing 250 mg of cephalexin). Specific concentrations of standard cephalexin solution were added to commercial formulation and investigated by the described procedure. Percent recoveries were calculated and ranged from 99.01–102.13 % (Table 5).

***Table 3*. Evaluation of accuracy and precision of the proposed method using solutions of pure cephalexin.**

Amount taken (μgmL^{-1})	Amount found (μgmL^{-1})	% Recovery ± RSD	Confidence limit
0.4	0.402	100.5 ± 9.98	100.50 ± 0.24%
0.6	0.5728	95.47 ± 12.21	95.47 ± 0.30%
0.8	0.8310	103.87 ± 2.16	103.87 ± 0.05%
Mean =		92.31	
± SD =		13.41	
t-test =		0.9920 (4.303)	

Results are the averages of three separate analyses; RSD = Relative standard deviation

***Table 4*. Evaluation of accuracy and precision of the proposed method for cephalexin determination in pharmaceutical preparation.**

Pharmaceutical preparations	Amount taken (μgmL^{-1})	Amount found (μgmL^{-1})	% Recovery ± RSD
Ceporex capsules 250 mg	0.4	0.401	100.00 ± 9.98
	0.6	0.591	98.62 ± 3.93
	0.8	0.827	103.35 ± 7.98

Results are the averages of three separate analyses; RSD = Relative standard deviation

***Table 5*. Evaluation of recovery test of cephalexin in commercial formulation (capsules) by the proposed method.**

Sample	Sample (μgmL^{-1})	(μgmL^{-1}) added	(μgmL^{-1}) found	% Recovery ± RSD
Ceporex capsules 250 mg	0.4	0.4	0.81	102.13 ± 7.75
		0.6	1.01	101.0 ± 3.59
		0.8	1.22	99.01 ± 5.72

Results are the averages of three separate analyses; RSD = Relative standard deviation

Application

The proposed method has been effectively applied for the determination of cephalexin in commercial formulation. The result of the proposed method obtained showed close agreement with the label claims without any interference. The results were statistically compared using student's t-test for accuracy (Table 6).

***Table 6*. Determination of cephalexin in commercial formulations.**

Brand name	Active ingredient (mgcap^{-1})		t-test value (4.303)
	Labeled value	*Found value*	
Ceporex capsules 250 mg	250	250.43	-7.45

Results are the averages of three separate analyses; RSD = Relative standard deviation

Conclusion

A sensitive, simple and selective spectrophotometric method was developed for determination of cephalexin in pure form and commercial formulation. Compared with HPLC and LC/MS methods, the proposed method is simple and does not need any pretreatment or lengthy procedure. The developed method was found to have a wider linear range and can be used as an alternative to HPLC for the determination of cephalexin in industrial and research institution laboratories.

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