

Indirect Spectrophotometric Determination of Cephalexin in Pharmaceutical Formulations

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Abstract: Simple, reliable and sensitive indirect spectrophotometric method was developed for the determination of cephalexin in pharmaceutical products without pretreatment. The method was based on the fading effect of the cephalexin on developed color product resulted from the bromination reaction of NBS with *p*-anisidine in acidic medium, subsequently vanished colored species was measured at 522 nm. Cephalexin acts in accordance with the Beer's law, under optimum conditions, at concentration ranges 1.5-10.0 µg/mL ($R = 0.9949$) with detection limit of 0.90 µg/mL. The relative standard deviation and error percent for 3 replicate determinations were found to be 2.2% and 1.75%, respectively. The proposed method was applied to the determination of cephalexin in different pharmaceutical products. A comparison of the method reported is made with standard official method and can be used as a convenient analysis method for routine quality control.

Keywords: Indirect spectrophotometric, Cephalexin, Pharmaceutical products

Introduction

Cephalexin (Table 1), a semisynthetic derivative of cephalosporin, is known to have antibacterial action against gram-positive and gram-negative bacteria. Cephalexin is a potent cephalosporin and exhibits a broad spectrum of antibiotic activity, weak bond ability to blood protein, no metabolites, low toxicity and to be rapidly absorbed following oral administration to give a high serum levels and urine concentration. The drug, therefore, is widely used for clinical chemotherapy^{1,2}.

The widespread use of cephalexin make a clinical and pharmacological study requires fast and sensitive analytical methods to determine the drug in pharmaceutical formulations and serum samples. Literature survey reveals a great variety of methods for the estimation of this compound, including chromatography³⁻⁷, spectrophotometry⁸⁻¹³, fluorometry¹⁴⁻¹⁵, flow injection analysis^{16,17}, atomic absorption¹⁸ and electroanalytical methods^{19,20}.

The aim of the present study was to develop simple, sensitive and rapid methods for spectrophotometric determination of cephalexin based on its effect on the color fading reaction of *N*-bromosuccinimide (NBS) and *p*-anisidine in acetic acid medium.

Table 1. Chemical structure of cephalexin

Molecular formula	Molecular weight	Chemical structure
$C_{16}H_{17}N_3O_4 \cdot H_2O$	365.4 g/mol	

Experimental

Unless otherwise stated, all chemicals and reagents were analytical grades. Distilled water (DW) was used throughout the work. All laboratory reagents were freshly prepared and protected from the light.

Standard cephalexin monohydrate solution

Highest purity drug form obtained from Awamedica Drug Manufacturing (Erbil, Iraq) was made up in DW to a concentration of 1000 $\mu\text{g/mL}$, by dissolving 0.1 g of the solid primary standard in a sufficient amount of DW and diluted to 100 mL in a volumetric flask with DW. Desired concentrations were obtained by diluting the stock solution during an investigation.

The N-bromosuccinimide (NBS) solution

4.5×10^{-2} mol/L was prepared by dissolving 2.0 g of NBS (BDH) in 250 mL DW.

A solution of p-anisidine

A solution of p-anisidine (BDH) with a concentration of 6.5×10^{-3} mol/L was prepared by dissolving 0.08 g of the reagent in 100 mL DW.

Acetic acid (AA)

0.1 mol/L stock solution was prepared by diluting 0.58 mL of acetic acid (BDH) (1.05 g/mL, 99.8%) to 100 mL in a volumetric flask. This stock solution was diluted as required with DW.

Pharmaceutical formulations

Oral suspension products

- *Lexine* (Hikma Pharmaceuticals- Jordan), labeled to contain 250 mg cephalexin monohydrate per packet.
- *Rivalexin* (Reva Pharma -Egypt), labeled to contain 125 mg cephalexin monohydrate per packet.
- *Pharmexin* (Pharma International -Jordan), labeled to contain 250 mg cephalexin monohydrate per packet.
- *Cephalexin Asia* (Asia Pharmaceutical Industries -Syria), labeled to contain 250 mg cephalexin monohydrate per packet.

Capsule products

- *Lexin* (Hikma Pharmaceuticals- Jordan), containing cephalexin monohydrate equivalent to 500 mg per capsule.
- *Pharmexin* (Pharma International-Jordan), 500 mg cephalexin monohydrate per capsule.
- *Cephalexin Asia* (Asia Pharmaceutical Industries -Syria), 500 mg cephalexin monohydrate per capsule.

Sample preparation

Capsule products

Contents of 20 capsules were emptied, thoroughly mixed in a mortar and an amount of the sample equivalent to about 0.1 g of cephalexin was weighed accurately and dissolved in a sufficient amount of warm distilled water. Shake and use an ultrasonic bath to homogenize and dissolve the sample solution. Filter off the residue through Whitman No. 1 filter paper and wash with water. The filtrate and washing were diluted with distilled water and made up to 100 mL volumetric flask.

Oral suspension products

Accurately weigh a quantity of powder equivalent to 0.1 g cephalexin and treated as described under capsules.

Apparatus

Spectral measurements were carried out on a CECIL CE 3021 UV-Vis digital spectrophotometer, while other measurements were carried out with a UV-Vis spectrophotometer model 7305 (UK) furnished with 1 cm quartz cuvette.

Recommended procedure

To a 25 mL volumetric flask, containing 1.5 mL AA and 3.0 mL NBS solution, aliquot volumes of the standard or sample cephalexin solution was added, so that the final concentration was in the range of 1.5-10.0 $\mu\text{g/mL}$. The flask was shaken for complete reaction and solution homogenization. Subsequently, 5 mL *p*-anisidine solution was added and the mixtures were allowed to stand for 3 min, then the solutions were diluted to the mark with DW and the absorbance was measured against DW at 522 nm (A_s). The blank reaction was performed according to the same procedure without addition of cephalexin and the change in absorbance was labeled as A_0 . A standard curve showing the difference in absorbance between A_s and A_0 (ΔA) vs. cephalexin concentration was obtained.

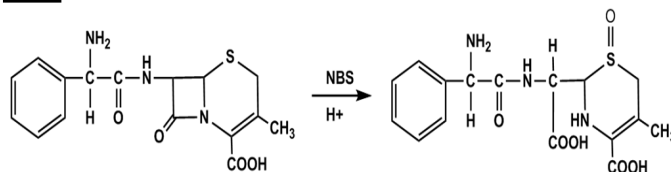
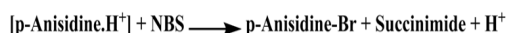
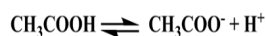
Results and Discussion

This method was based on the reaction of NBS with aromatic amines in an acidic medium to form a brominating colored product. Cephalexin is let to react with a known excess of NBS in acidic media²⁰⁻²². The unreacted NBS found in excess over cephalexin is quantified by adding *p*-anisidine solution (Figure 1) and monitoring the absorbance of the solution at $\lambda_{\text{max}}=522$ nm. This caused a proportional decrease in the concentration and absorbance of formed color in the mixture by an increase in concentration of cephalexin (Figure 2).

Effect of reaction variables

Acid concentration

The effects of various types of acids, *i.e.*, sulfuric, hydrochloric, phosphoric, nitric and acetic acid, of similar concentration were studied and maximum differences in the absorbance (ΔA) were obtained with the selected AA solution. The effect of acidity was studied by varying the volume of the employed 0.1 mol/L AA solution between 0.5-3.0 mL. The results showed that there is an increase in the absorbance signal (ΔA) as the acid volumes are increased up to 1.5 mL, beyond this volume the increments light intensity was inconsiderable and thus a 1.5 mL of volume of 0.1 mol/L acid shows a good option of these variables.

Part 1**Part 2**

Violet color product
Maximum Absorption
at 522 nm

Figure 1. Suggested mechanism of NBS with cephalexin and *p*-anisidine

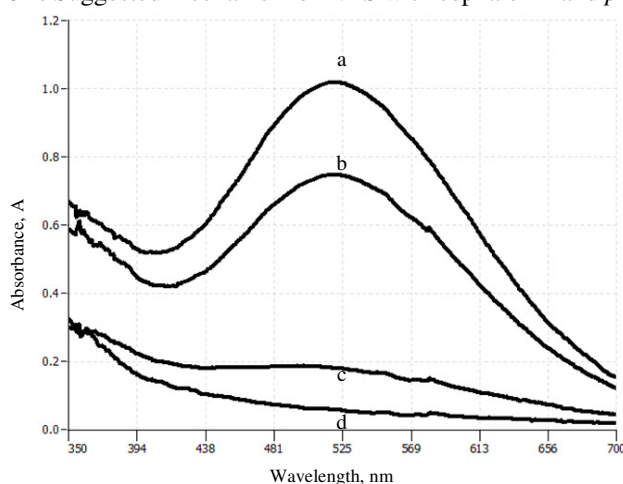


Figure 2. Absorption spectra of the reaction mixture against water in the presence of different concentration of cephalexin: NBS, 5.4×10^{-3} mol/L; Acetic acid, 6.0×10^{-3} mol/L; *p*-anisidine, 1.3×10^{-3} mol/L; cephalexin: a, 0.0; b, 3.0; c, 9.0; d, 10.0 µg/mL

Reagents concentration effect

When the effect of various concentrations of NBS solution were examined, 3.0 mL of 4.5×10^{-2} mol/L solution was found enough to develop the color to its full differences (ΔA) in intensity between that of the blank (A_0) and in the presence of drug standard (A_s) (Figure 3). Thus this concentration was considered to be optimum for subsequent works. In another study, the effect of the *p*-anisidine concentration on the reaction rate is shown in Figure 4. When *p*-anisidine concentration was increased in the range 2.6×10^{-4} to 1.82×10^{-3} mol/L, the difference in the absorbance (ΔA) increased. A concentration of 1.3×10^{-3} mol/L of *p*-anisidine in the final solution was selected. At higher reagent concentrations the absorbance of the solution became too high and almost remained constant.

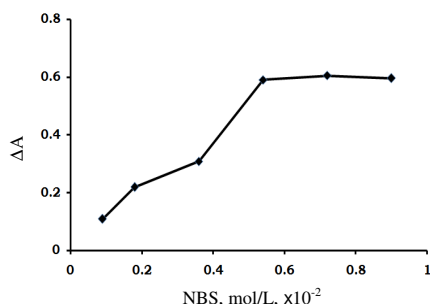


Figure 3. Effect of NBS concentration

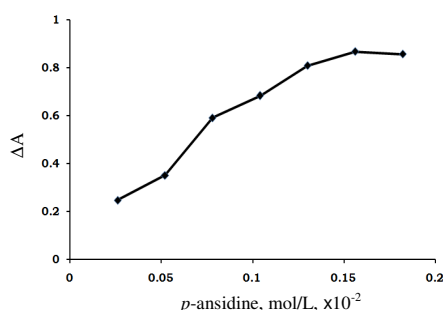


Figure 4. Effect of *p*-anisidine reagent concentration

Influence of time and sequences of addition

Effect of time intervals between each addition was determined by following the differences in the absorbance before and after addition of the cephalixin (ΔA). The results of this investigation predicate that direct mixing of acid-NBS-cephalexin and 3 min time standing of the previous reaction product with *p*-anisidine was gives maximum differences in the absorbance.

The order of adding reagents during reaction process has important role in accuracy of results and enhancement of absorbance, accordingly, the effect of order of addition was performed by following the color intensity and maximum absorbance differences on changing the sequences of addition of drug, acid and reagent. The best condition was obtained with the sequences of “acid-NBS-drug-*p*-anisidine” for the highest absorbance differences.

Study of the effect of time on color stability

To study the effect of time on stability of color, 1.5 mL AA was mixed with 3.0 mL NBS solution in 25 mL volumetric flask. Then 5 mL *p*-anisidine solution was added and the mixture was stand for 3 min, then the solutions were diluted to the mark with DW. The absorbance of the solution was noted between 1.0-100 minutes. The results demonstrate (Figure 5) that the color was stable for at least 30 min, after this time, the electronic absorption bands are found shifted towards shorter wavelength with maximum absorbance at 460 nm.

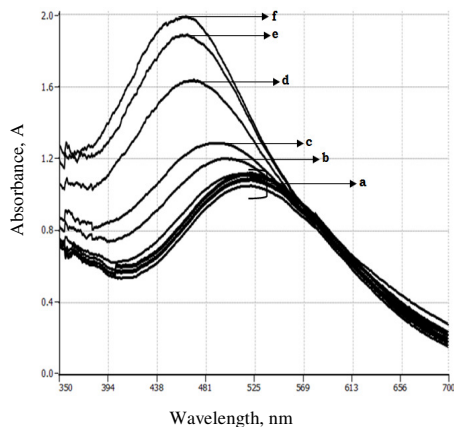


Figure 5. Influences of different time on the stability of the developed color; a-1-30 min, b-45 min, c-50 min, d-60 min, e-80 min, f-100 min

Study of interferences by common excipients

Effect of common excipients normally present in the cephalixin drugs products, such as glucose, fructose, talc, starch and dicalcium phosphate, were examined to evaluate the selectivity of the present method. A quantitative assessment of the tolerable amounts of these compounds was given in Table 2. Generally, those excipients did not interfere under applied experimental conditions and process of separation was not required.

Table 2. Results of interferences study on the determination of 15 µg/mL cephalixin.

	MAC*, µg/mL	Cephalexin, µg/mL		Recovery %	TCR***
		Added	Found**		
Glucose	50	15	15.20	101.33	3.33
Fructose	50	15	15.12	100.80	3.33
Sucrose	50	15	14.89	99.26	3.33
Starch	50	15	14.80	98.66	3.33
Ca ²⁺	100	15	15.10	100.66	6.67
CO ₃ ²⁻	100	15	14.95	99.66	6.67

*Maximum allowable concentrations, **Mean of three replicate analyses, ***TCR: Tolerable Concentration Ratio with no interferences (Interferent (µg/mL) / Cephalexin (µg/mL))

Calibration graph

A calibration graph was constructed by plotting ΔA versus cephalixin concentrations in µg/mL of final solution using developed method under the optimal experimental conditions (Table 3). The calibration graph was linear in the range of 1.5-10.0 µg/mL of cephalixin (Figure 6). Each point in the calibration graph was the average of three replicates. The experimental limit of detection (3Sb/m, three times the blank standard deviation divided by the slope of the equation) was 0.90 µg/mL. The relative standard deviation and error percent for 3 replicate determinations were found to be 2.2% and 1.75%, respectively.

Table 3. Optimal experimental conditions, spectral characteristics and statistical data

Parameters	Data
Final concentration of NBS, mol/L	5.4×10^{-3}
Final concentration of <i>p</i> -anisidine, mol/L	1.3×10^{-3}
Final AA concentration, mol/L	6.0×10^{-3}
Calibration graph range, µg/mL	1.5-10.0
Molar absorptivity, L/mol.cm $\times 10^3$	39.103
Sandell sensitivity, µg/cm ²	8.9
Detection limit, µg/mL	0.90
Slope (a)	0.102
Intercept (b)	0.008
Correlation coefficient (R)	0.997

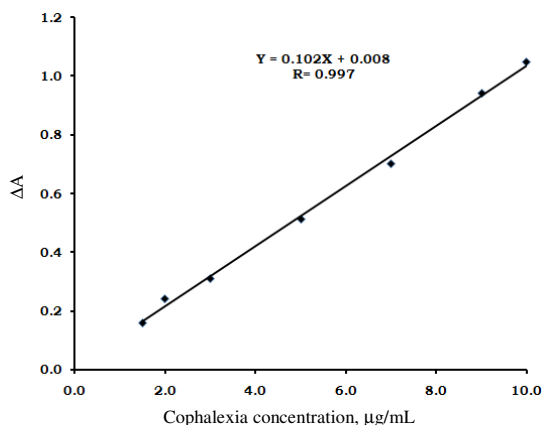


Figure 6. Calibration graph obtained by the developed method

Applications

The reliability of the proposed method for the assay of typical cephalexin dosage forms was tested by analyzing cephalexin in commercially available drug formulations supplied in different dosage forms. The concentration of cephalexin was calculated using the corresponding calibration equation (Table 4).

Table 4. Results and comparison of analysis for cephalexin in pharmaceutical preparations

Samples trade name	Cephalexin monohydrate (mg/ capsule or 5 mL of oral suspension)			E% (II)	
	Labeled amount	Detectable amount (I)		Method 1	Method 2
		Method 1	Method 2		
1-Capsule products					
-Lexin (Hikma Pharmaceuticals-Jordan).	500	500.1±0.02	500.2±00.1	0.02	0.04
-Pharmexin (Pharma International-Jordan).	500	499.2±0.03	498.6±0.05	-0.16	-0.28
-Cephalexin Asia (Asia Pharmaceutical Industries -Syria).	500	500.2±0.02	499.7±0.04	0.04	-0.06
2-Oral suspension products					
-Lexine (Hikma Pharmaceuticals-Jordan)	250	249.1±0.04	247.3±0.04	-0.36	-1.08
-Rivalexin (Reva Pharma -Egypt).	125	123.9±0.03	123.7±0.06	-0.88	-1.04
-Pharmexin (Pharma International -Jordan).	250	248.8±0.05	247.5±0.03	-0.48	-1.00
-Cephalexin Asia (Asia Pharmaceutical Industries –Syria).	250	249.7±0.06	248.4±0.04	-0.12	-0.64

Method 1= the present developed method, Method 2= official method, (I) Mean value of three determinations ± standard deviations (SD). (II) Relative standard error between the Method 1 as well as Method 2 and claimed manufacturer's values.

Statistical analysis of the results obtained by the proposed procedures compared with those of the official method²³ are given in showed comparable accuracy (*t*-test) and precision (*F*-test), since the calculated values of *t*-and *F*-tests at 95% confidence limits were less than the theoretical data, indicating no significant difference between the two methods.

Conclusion

A simple method for the determination of cephalexin is described. The method is based on the bleaching effect of the cephalexin on violet color species resulted from the bromination reaction of NBS with *p*-anisidine, subsequently faded colored species was measured at 522 nm. The developed method does not involve any stringent reaction conditions and offers the advantages in speed, high simplicity and reliability. The proposed method was applied to the determination of cephalexin in different pharmaceutical products. A comparison of the method reported is made with standard official method and can be used as a convenient analysis method for routine quality control.

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