SPECTROPHOTOMETRIC DETERMINATION OF CEFACLOR IN PHARMACEUTICAL PREPARATIONS

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A simple spectrophotometric method is proposed for the determination of cefaclor. The method involves alkaline hydrolysis of the drug in ammonia buffer solution at pH 10 to yield diketopiperazine-2,5-dione derivative and subsequent measurement at 340 nm. Beer's law is obeyed in the concentration range 1.8 - 55 μg/mL. The proposed method was successfully applied to the determination of cefaclor in pharmaceutical formulations.

Keywords: cefaclor; hydrolysis; pharmaceuticals.

INTRODUCTION

Cefaclor (Figure 1), an orally active cephalosporin in clinical practice, belongs to the group of β-lactam antibiotics[1].

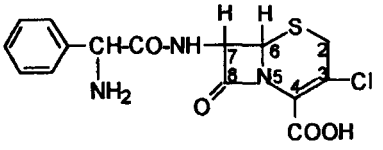
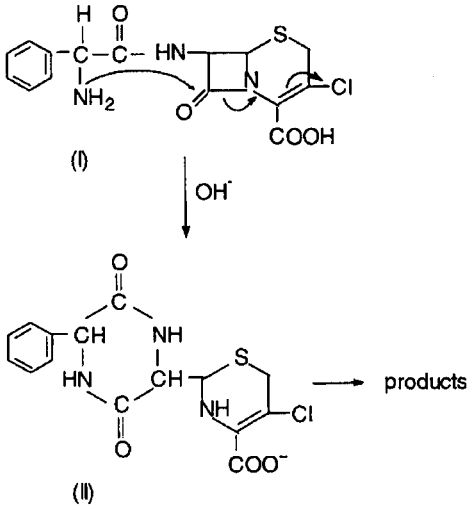


Figure 1. Chemical structure of Cefaclor.

Its antibacterial activity is dependent on the presence of the β-lactam functionality that can be hydrolyzed under aqueous conditions[2]. This hydrolytic instability leads to chemical degradation and the formation of piperazine-2,5-dione derivative via intramolecular nucleophilic attack of the primary amine from the side chain on the β-lactam moiety at neutral or slightly alkaline medium, as shown in the Scheme 1[3,4,5].



Scheme 1.

Numerous analytical procedures have been reported for their determination in its pure form, in pharmaceutical preparations and in biological fluids. The USP XXII recommends the hydroxylamine colorimetric assay[6]. HPLC methods were described for the determination of cefaclor in human plasma and urine[7,8]. Also iodometric determination of cefaclor has been reported[9]. A spectrofluorimetric method is described for the determination of cefaclor in formulations and biological fluids[10].

The aim of the present work is to investigate the behaviour of cefaclor upon alkaline hydrolysis in an attempt to develop a simple UV spectrophotometric procedure for determining cefaclor in pharmaceutical forms by means of its hydrolysis product.

EXPERIMENTAL

Apparatus and Reagents

A Diode Array spectrophotometer Hewlet Packard model 8452A and a 1 cm quartz cell were used in all absorbance measurements. All reagents were of analytical grade. Cefaclor (monohydrate) was obtained from Ely Lilly, Brazil. Cefaclor standard solutions were freshly prepared using demineralized water from a Milli-Q system (Millipore). Britton-Robinson (B-R) buffer (pH 2-12) was prepared by mixing 0.4 mol/L orthophosphoric acid, 0.4 mol/L acetic acid and 0.4 mol/L boric acid with the apropriated amount of 0.4 mol/L sodium hydroxide solution. The ammonia buffer (pH 8-11) was prepared by mixing 0.4 mol/L ammonium hydroxide and 0.4 mol/L ammonium chloride. Ammonium hydroxide 2 mol/L was used at pH 12.0.

Analysis of authentic samples

An aliquot of cefaclor solution (368 μg/mL) was transferred to a 10 mL standard flask containing ammonia buffer or B-R buffer. The solution was maintained for 30 min at room temperature and the absorbance was measured at 340 nm against a blank prepared without cefaclor. The calibration curve was obtained through the same procedure, using cefaclor standard solutions.

Analysis of dosage forms

Capsules. The contents of a commercial capsule Ceclor® 500 mg from Eli Lilly were weighed and diluted in demineralized water. The solution was shaken and filtered under vacuum. The solution was then diluted to 100 mL in demineralized water. An aliquot of this solution to give an analyte concentration of about 350 μg/mL was transferred into a 10 mL volummetric flask containing ammonia buffer and analysed as for authentic samples.

Suspension. An aliquot of 1 mL of a well mixed suspension of Ceclor® 250 mg/5mL was pipetted into 25 mL of demineralized water. The mixture was shaken for 10 minutes and the

extract was filtered under vacuum. The filtered portion of the solution was diluted to 100 mL of water. Aliquots of the test solution were then diluted in ammonia buffer pH 10 and analysed as for authentic samples.

RESULTS AND DISCUSSION

A typical absorption spectra of $1x10^{-4}$ mol/L cefaclor in Britton-Robinson (B-R) buffer pH 10 is shown in Figure 2 (Curve I). Two well resolved bands with absorption maxima at λ= 264 nm (peak A)[9,11,12] and 214 nm (peak C) attributed to O=C-N-C=C- and -C=C-COO- groups[10], respectively. This behaviour is observed in all the pH range 2-11. The Curve II of the Figure 2 shows the corresponding spectrum obtained after 30 min of heating time at 60°C. The peak at 264 nm (peak A) disappeared, as a result of C-N bond cleavage in the four-membered amido ring. This behaviour is similar to degradation of all cephalosporins[4,12]. However, a new absorption peak at 340 nm (peak B) is observed in the UV absorption spectrum, which decreases with increase of the time heating (60 min) and increase of temperature (> 80°C). Also, at room temperature the same spectrum was obtained for $1x10^{-4}$ mol/L of cefaclor at pH 12.0 indicating an spontaneous hydrolysis of the drug.

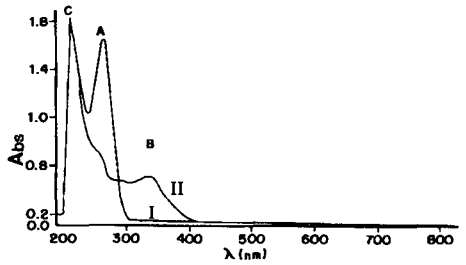


Figure 2. Absorption spectra of $1x10^{-4}$ mol/L cefaclor in Britton-Robinson buffer pH 10. I) room temperature II) - after 30 min of heating at 60°C.

The pH influence on the hydrolysis reaction of cefaclor was monitored at room temperature (25°C) by following the loss of the characteristic UV-VIS absorbance band at 264 nm and appearance of maximum absorption in longer wavelengths (λ= 340 nm) between 0 and 2 h of reaction time in solution. Figure 3 (Curve a) shows that the peak at 264 nm is stable up to pH 10 in fresh solution of B-R buffer and the peak at 340 nm is defined only at pH 12.0 (Curve b). This behaviour is changed after 2 hours; when the absorbance is markedly diminished in pH values higher than 10 and the occurence of the peak at 340 nm is well defined even in pH 11.

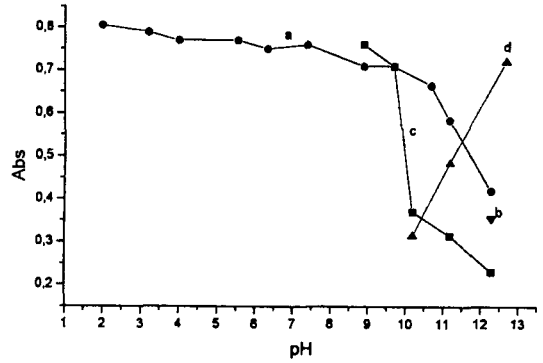


Figure 3. Effect of pH on the absorbance of $1x10^{-4}$ mol/L cefaclor at: (a) λ = 264 nm, Britton-Robinson buffer; (b) λ = 340 nm, Britton-Robinson buffer; (c) λ = 264 nm, Ammonia buffer; (d) λ = 340 nm, Ammonia buffer.

As described in previously studied cefaclor degradation mechanism[3], the peak B (340 nm) can be attributed to the piperazine-2,5-dione derivative (compound II; scheme I) that it is the principal degradation product in cephalosporins containing α-amino groups during hydrolysis in neutral or alkaline medium. The formation mechanism is attributed to an intramolecular aminolysis involving the $-NH_2$ group attack to the side chain at C_7 on the β-lactam carbonyl function (scheme I). The structure of this product was confirmed by NMR[3,2] and HPLC studies[12]. The UV-VIS spectrophotometric identification after alkaline hydrolysis has also been found for cephalexin, containing α-amino group that promotes spontaneous hydrolysis in alkaline medium[14].

Although it is known that cefaclor has two ionizable groups with ionization constants (pK_{a1} =1.5 and pK_{a2} = 7.1) for the dissociation of carboxilic and the α-amino group[4] respectively, the formation of the piperazine derivative occurs relatively slowly at room temperature in B-R buffer solution. Nevertheless, the degradation of cephalosporins and penicillins is directly influenced by parameters such as pH, temperature, metal ions, buffer concentration and composition. Therefore, the hydrolysis reaction of cefaclor was investigated in ammonia buffer.

A solution of cefaclor ($1x10^{-4}$ mol/L) in ammonia buffer pH=11, within 30 min of reaction has shown the complete absence of the absorption peak at 264 nm and the occurrence of a peak at 340 nm higher than the one in B-R buffer, as shown Curve y of Figure 4. For comparison, the spectrum obtained in ammonia buffer pH 8 shows absence of any absorption band at 340 nm (Curve x of Figure 4). The influence of the buffer composition was investigated between pH 8-12 (ammonia buffer) and the results are shown in the Curve c and d of Figure 3. Comparable absorbance peaks at 264 nm for cefaclor $1x10^{-4}$ mol/L in B-R buffer (Curve a) and ammonia buffer (Curve c) are seen for pH values up to 9. However, at pH ≥ 10 a sudden decrease of the peak at 264 nm (Curve c) is observed in ammonia buffer than that one verified in B-R buffer, followed by concomitant linear increase of the peak at 340 nm (Curve d). This behaviour is indicative that the aminolysis reaction is accelerating the drug decomposition.

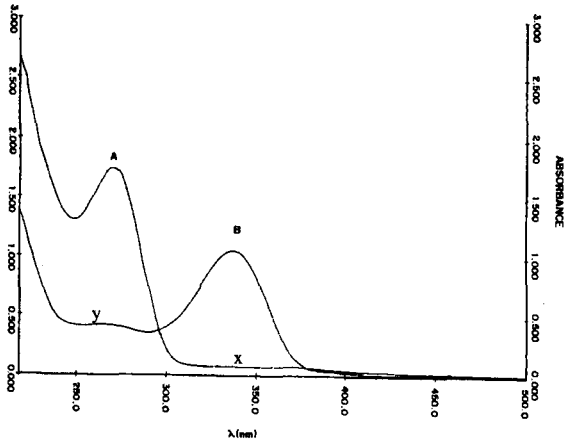


Figura 4. Absorption spectra of $1x10^{-4}$ mol/L cefaclor after 30 min in Ammonia buffer. (x) pH 8.0 and (y) pH 11.

Cephalosporin reactions in aqueous solution containing ammonia, or other sources of amino groups at pH 11, have been reported to accelerate the formation of compounds, whose UV-VIS spectra resembled the one product resulting from the action of a β-lactamase enzyme. Taking into account that in this case, the β-lactam ring of a cephalosporin is split, but there is no extensive fragmentation of the molecule[3]. The product responsible for a maximum absorption at 340 nm could be used for analytical purpose.

The aminolysis reaction was followed for $1x10^{-4}$ mol/L cefaclor in ammonia buffer pH 10 at room temperature by monitoring the decreasing absorbance peak at 264 nm (Curve a, Figure 5) and the increasing absorbance at 340 nm (Curve b, Figure 5) as a function of time. In these conditions the piperazine-2,5-dione is formed spontaneously after 30 min in solution, and the signal is still stable at least up to 2 hours.

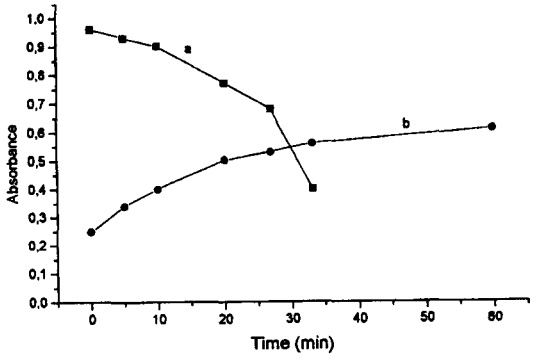


Figura 5. Effect of reaction time upon $5x10^{-5}$ mol/L cefaclor degradation in Ammonia buffer pH 11. (a) λ = 264 nm and (b) λ = 340 nm.

The effect of heating time on the cefaclor aminolysis for $1x10^{-4}$ M of the drug in ammonia buffer (pH 10) has shown that the absorbance at 264 nm is rapidly decreased after heating at 60°C, but the peak at 340 nm is smaller and not well defined as at room temperature. This behaviour suggests that in high temperatures the β-lactam compound is exposed to rather rash conditions promoting other degradation of the molecule[3]. So, as mentioned before, ammonia buffer (pH 10), at room temperature and 30 min of reaction are the best conditions to follow the primary degradation product, which is a piperazine-2,5-dione derivative.

For analytical purposes, the UV band in 340 nm was selected and a linear relation between absorbance and cefaclor concentration was observed under the specified reaction conditions. For analytical determination the calibration curve method was used for the pure drug in the concentration range of 1.8 μg/mL to 55 μg/mL, which is shown in Figure 6. Beer's law was obeyed over all range investigated. The regression equation calculated from the calibration graph was: A= 0.05001 + 0.02625 C, where A = absorbance andC = concentration (μg/mL). To examine the precision of the procedure, five replicate determination were carried out on the same solution containing 55 μg/mL of cefaclor and a coefficient of variation of 1.89% was obtained. Cephalosporins free from the α-amino group did not interfere in the proposed method.

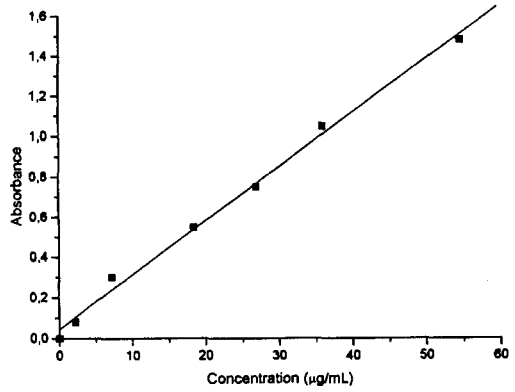


Figura 6. Calibration graph of Cefaclor in Ammonia buffer pH 10. λ = 340 nm.

Commercial pharmaceutical dosage forms of the studied drug were analyzed using the proposed procedure and compared with the official method[6].

Thus, CECLOR® capsules and CECLOR® suspension were satisfactorily analyzed by applying the standard addition technique. The absorption spectra obtained for both samples do not show any change by excipient formula in relation to that obtained for pure drug. At 340 nm, samples with different concentrations of the drug in capsules and suspension showed a rectilinear relationship between absorbance intensity and the claimed concentration.

Ceclor capsules and Ceclor suspension were spiked with authentic samples of pure Cefaclor and analyzed by the proposed procedure. Their mean percentage recoveries are shown in Table 1.

Table 1. Determination of Cefaclor in bulk form and in pharmaceutical formulation using the proposed method.

Sample	Amount taken *	Amount found *	Recovery
	μg/mL	μg/mL	%
Cefaclor (pure drug)	36.79	36.72	99.8
Ceclor capsules (500 mg)	36.79	36.18	98.4
Ceclor suspension (250 mg)	36.79	36.09	98.1

* Average of three determinations.

Table 2. Analysis of some dosage forms containing Cefaclor by the proposed and official method.

Sample	Proposed method	Official method
	Recovery ± SD %	Recovery ± SD %
Ceclor - capsules	98.40 ± 0.80	98.35 ± 0.27
Ceclor - suspension	98.08 ± 0.89	98.08 ± 0.20

CONCLUSION

The above reported experimental results demonstrate that cefaclor can be determined by means of its hydrolysis product, 2,5-dione-piperazine derivative, which produces a characteristic UV spectrum with a maximum absorption at 340 nm.

The proposed method proved to be simple, economic, accurate and can therefore, be applied to the determination of cefaclor in the pure form and in pharmaceutical preparations. Taking into consideration that the direct spectrophotometric method for cefaclor does not find a practical application because of the lack of specificity, since all compounds containing β-lactam ring absorb in the range 250-270 nm, the present method, however, is enough selective for cephalosporins containing free α-amino group. In addition, the major advantage of the proposed method is that the procedure does not require extraction step or great number of chemicals.

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REFERENCES

1. American Pharmaceutical Association; *The United States Pharmacopeia*, XXII, Washington DC 1990.
2. Baertschi, S. W.; Dorman, D. E.; Occolowitz, J. L.; Spangle, A.; Collins, M. W.; Wildfeuer, M. E. and Lorenz, L. J.; *J. Pharm. Sci.* **1993**, *82*, 622.

3. Villanova, B.; Donoso, J.; Munoz, F. and Garcia-Blanco F.; *Helv. Chim. Acta* **1996**, *79*, 1793.
4. Dimitrovska, A.; Stojanoski, K. and Dorevski, K.; *Intern. Pharm.* **1995**, *115*, 175.
5. Indelicato, M. J.; Norvilas, T. T.; Pfeiffer, R. R.; Wheeler, J. W. and Wilham, L. W.; *J. Med. Chem.* **1974**, *17*, 523.
6. Mays, D. L.; Bangert, F. K.; Cantrell, W. C. and Evans, W. G.; *Anal. Chem.* **1975**, *47*, 2229.
7. Kovach, P. M.; Lantz, R. J. and Brier, G.; *J. Chromatogr.* **1991**, *567*, 129.
8. Nakata, M. C.; *J. Chromatogr.* **1982**, *228*, 429.
9. Yamana, T. and Tsuji, A.; *J. Pharm. Sci.* **1976**, *65*, 1563.
10. Aly, F. A.; Hefnawy, M. M. and Belal, F.; *Anal. Lett.* **1996**, *29*, 117.
11. Chauvehe, R. R.; Flynn, E. H.; Jackson, B. G.; Lavagnino, E. R.; Morin, R. B.; Müller, R. A.; Pioch, R. P.; Roeske, R. W.; Ryan, C. W.; Spencer, J. L. and Van Heyningen, E.; *J. Am. Chem. Soc.* **1962**, *84*, 3401.
12. Lorens, L. J.; Bachore, F. N. and Olsen, B. A.; *J. Chromatogr. Sci.* **1992**, *30*, 211.
13. Nakashima, E.; Tsuji, A.; Nakamura, M.; Yamana, T.; *Chem. Pharm. Bull.* **1985**, *33*, 2098.
14. Li, Q. and Chen, S.; *Anal. Chim. Acta* **1993**, *282*, 145.