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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF CEFACLOR AND CEPHALEXIN USING FLUORESCENCE DETECTION FOLLOWING PRECOLUMN DERIVATIZATION

S. ÖZKIRIMLI, Z. CESUR, G. ÇAPAN, N. ERGENÇ, A. GÜRSOY

SUMMARY

Quantitative determination of two cephalosporin antibiotics cefaclor and cephalexin was performed by high performance liquid chromatography using phenylglycine as the internal standard. The method is based on the reaction of cephalosporin derivatives containing an ∝-amino group with dansyl chloride. The proposed method gives reliable and accurate results for pharmaceutical preparations.

ÖZET

Sefalosporin grubu antibiotiklerden sefaklor ve sefaleksininin yüksek performanslı sıvı kromatografisi ile miktar tayini yapılmış, fenilglisin internal standard olarak kullanılmıştır. Yöntem ∝-amino grubu taşıyan sefalosporinlerin dansil klorür ile reaksiyonuna dayanmaktadır. Önerilen yöntem farmasötik preparatlar için güvenilir ve doğru sonuçlar vermektedir.

Key words: Quantitative determination, HPLC, cefaclor and cephalexin, dansyl chloride

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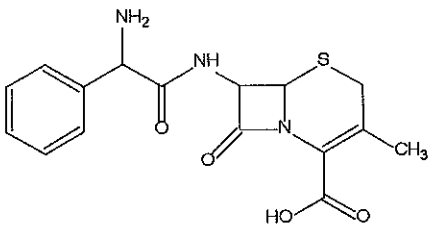
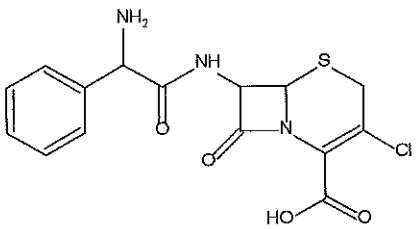
INTRODUCTION

Quantitative determination of β-lactam antibiotics is one of the more difficult areas of pharmaceutical research conventionally being performed either by microbiological assay and / or a variety of physicochemical methods. In recent years high performance liquid chromatography (HPLC) has become the preferred technique for the quantitation of β-lactams. UV absorbance and fluorescence detection methods have been used, the latter is favoured due to its inherently greater sensitivity. For those β-lactams which lack a chromophore the greatest sensitivity is normally achieved at wavelengths 230-265 nm (1-3). To enhance detection properties and selectivity precolumn or post column derivatization techniques which employ fluorogenic agents have been developed. Fluorescamine (4) and o-phtaldialdehyde (5) have been used in post column derivatization procedures. Precolumn derivatization methods also involve the derivatization of the degradation products of β-lactams with mercury II chloride (6), imidazole-mercury II chloride (7) or 1-hydroxybenzotriazole-mercury II chloride (8). 4-(2-cyanoisoindolyl)phenylisocyanate (9) and fluorescamine (10) have been employed as precolumn derivatization reagents for ∝-aminocephalosporins. Dansyl chloride (DNS-Cl) (I) has been widely used in the HPLC analysis of amino acids (11,12). We now report the application of DNS-Cl to the precolumn derivatization and quantitation of two cephalosporin antibiotics cefaclor and cephalexin.



Cephalexin



Cefaclor

RESULTS AND DISCUSSION

The proposed method is based on the reaction of cephalosporin derivatives cephalexin and cefaclor containing an ∝-amino group with DNS-Cl. The dansyl derivatives **III** and **IV** were separated and determined by HPLC using fluorescence detection (λ excitation 360 nm; λ emission 530 nm). The effect of the reaction time on the formation of the dansyl derivatives, **III** and **IV**, was investigated (Figure 1). The standard derivatization time was determined as 30 min, at which the peak height reached a maximum (remained constant for 3h). The maximal and constant peak height was obtained in the presence of about 15 fold excess of DNS-Cl (Figure 2). Under these conditions, the retention times of the dansyl derivatives, **II, III** and **IV**, were 6, 7.8 and 8.1 min, respectively (Figure 3).

The statistical analysis of the HPLC determination of **III** and **IV** are presented in Table 1. The results obtained for the pharmaceuticals by HPLC are given in Table 2. As the matrix of the suspensions and cephalexin tablets do not interfere with HPLC determinations, the proposed method gives reliable and accurate results.

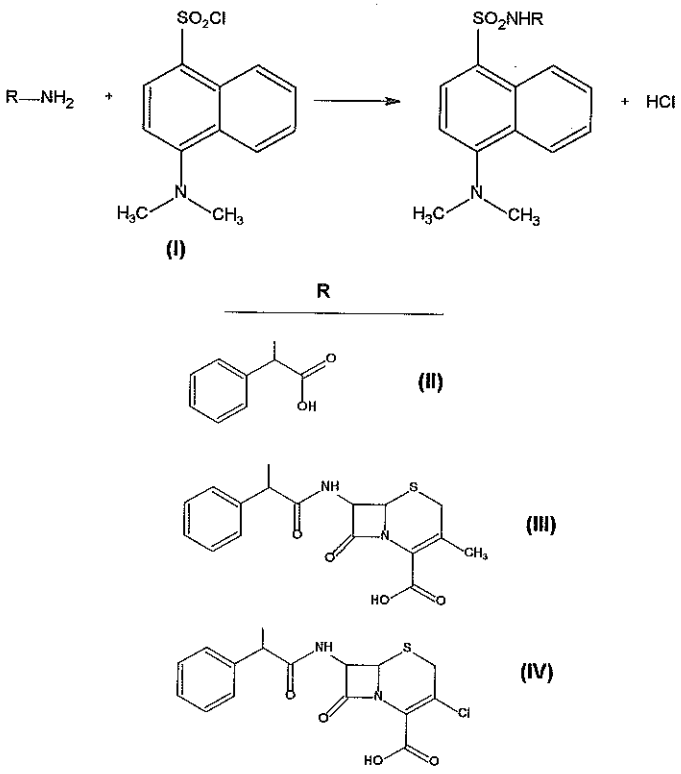


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Table 1. Stetistical analysis of the determination of cephalexin and cefaclor by HPLC

Drug	Concentration range(C) ($\mu g\ ml^{-1}$)	Regression equation	r
Cephalexin	40.32-94.09	*$y=1.25x10^{-2}C-1.96x10^{-1}$	0.999
Cefaclor	30.27-100.9	$y=9.6x10^{-3}C+1.68x10^{-3}$	0.999

*y : Peak height ratio (A/IS)

Table 2. Assay of cephalexin and cefaclor in pharmaceuticals by HPLC

Pharmaceuticals	Amount of label claim	HPLC Mean recovery %± SD*
Cephalexin (tablet)	500 mg / tablet	110.33 ± 1.05
Cephalexin (tablet)	1000 mg / tablet	97.56 ± 0.33
Cephalexin (suspension)	250 mg / 5 ml	101.41 ± 2.79
Cefaclor (capsule)	250 mg / capsule	93.19 ± 1.29
Cefaclor (suspension)	250 mg / 5 ml	103.27 ± 1.99

* Mean and standard deviation for five determinations; percentage recovery from the label claim amount.

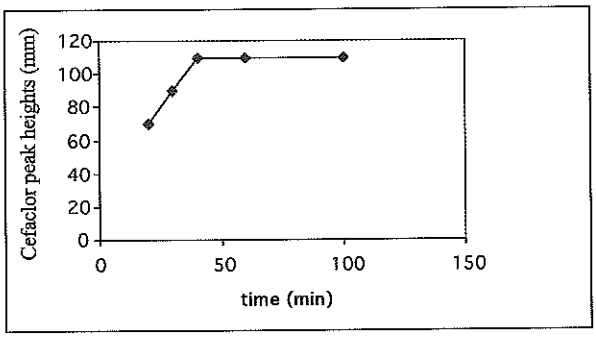

Figure 1. Effect on reaction time on the precolumn derivatization of cefaclor

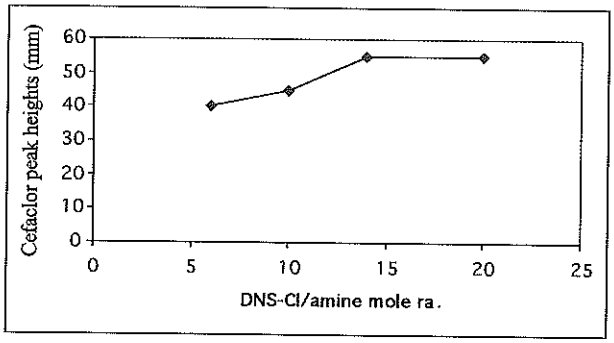

Figure 2. Effect of DNS-Cl concentration on the peak height of dansylated cefaclor

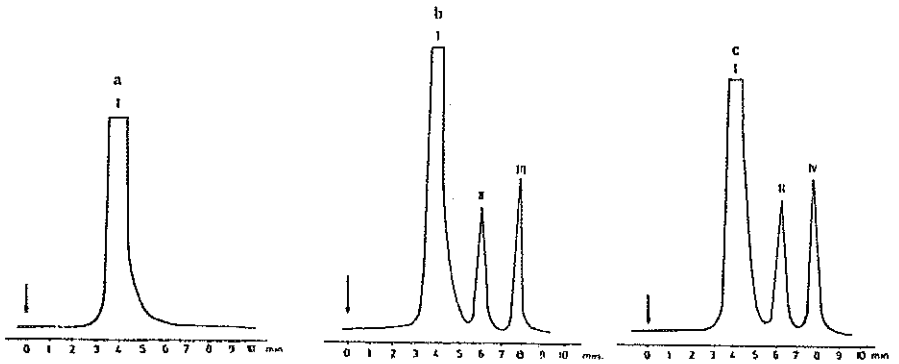

Figure 3. a) Chromatograms of 0.05 M $NaHCO_3$+DNS-Cl b and c) Chromatograms of dansyl derivatives (I-DNS-Cl, II-Dansyl derivative of phenylgylcine, III-Dansyl derivative of cephalexin, IV- Dansyl derivative of cefaclor)

EXPERIMENTAL

Chemicals: Cefaclor hydrate (Mustafa Nevzat), cephalexin hydrate (Fako), dansyl chloride (Aldrich), potassium dihydrogen phosphate (Merck), sodium bicarbonate (Merck), HPLC grade acetonitrile (Merck), acetone (Merck).

Reagent solution: 2.5 mg of DNS-Cl were dissolved in 5 ml of acetone (the solution was prepared daily).

Apparatus: Model 6000 A pump with U6K injection valve, Model 420 AC Fluorescence Detector (Waters Associates, Milford, MA), Yew Model 3020 Pen Recorder, μBondopak C_{18} reversed phase column (30 x 0.39 cm ID, particle size 10 μm).

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Method

Assay Procedure: A stock solution of the antibiotic (A) was prepared (cefaclor 0.10 mg ml^{-1}; cephalexin 0.15 mg ml^{-1} /water). Aliquots of the stock solution (cefaclor 20-60 µl; ceplalexin 30-70 µl) were transferred to centrifuge tubes and made up to 100 µl with glass distilled water. After addition of 50 µl of internal standard phenylglycine (IS) (0.01 mg ml^{-1}/water), 250 µl of 0.05 M $NaHCO_3$ and 250 µl of DNS-Cl (0.5 mg ml^{-1}/acetone), the mixture was vortexed and left aside for 1 h and 5 µl of this solution were chromatographed on a µBondopak C_{18} column with a mobile phase of acetonitrile: 0.01 M KH_2PO_4 (35 : 65) (pH=4.5) and a flow rate of 1 ml min^{-1}. The working curve was constructed by plotting peak height ratio (A/IS) versus concentration (µg ml^{-1}).

Sample Preparation: Commercially available dosage forms were assayed as follows:

Cafaclor Capsules: The contents of 10 capsules were mixed with water to obtain a concentration of about 1 mg of anhydrous cefaclor per ml, sonicated for 25 min and filtered. This solution was accurately diluted to yield a test dilution having a concentration assumed to be equal to the median concentration of the standard. The method described under the assay procedure was followed.

Cephalexin Tablet: 20 Tablets were accurately weighed and powdered. An amount equivalent to 100 mg of anhydrous cephalexine was accurately weighed, mixed with water made up to 100 ml, sonicated for 25 min and filtered. This solution was accurately diluted to yield a test dilution having a concentration assumed to be equal to the median concentration of the standard. The method described under the assay procedure was followed.

Oral Suspensions *(cefaclor and cephalexin):* An accurately weighed amount of the dry mixture for oral suspension (equivalent to 100 mg of anhydrous cefaclor or ceplalexin) was dissoved in water, made up to 100 ml, sonicated for 25 min and filtered. This solution was accurately diluted to yield a test dilution having a concentration assumed to be equal to the median concentration of the standard. The method desribed under the assay procedure was followed.

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