DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 19(14), 1723-1729 (1993)

QUANTITATION OF CEFACLOR IN PHARMACEUTICAL DOSAGE FORMS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A stability-indicating high-performance liquid chromatography for the quantitation of cefaclor in pharmaceutical dosage forms has been developed. The method is accurate and precise with a percent relative standard deviation of 1.2 based on 5 readings. A number of inactive ingredients present in the capsules and suspensions did not interfere with the assay procedure. The extraction procedure from the dosage forms is very simple. The recovery from the synthetic mixtures was quantitative. The capsules which had expired 3 years ago lost only 3% of the potency. The drug appears to be very sensitive to strong acids or bases since a 5 minute boiling caused 100% degradation of drug in both the solutions.

BACKGROUND

Cefaclor (Figure 1) is extensively used in medicine for the treatment of ear, respiratory and urinary tract infections. It is available in the form of capsules and powders for oral suspensions at the time of dispensing. The USP-NF method (1) for the analysis of

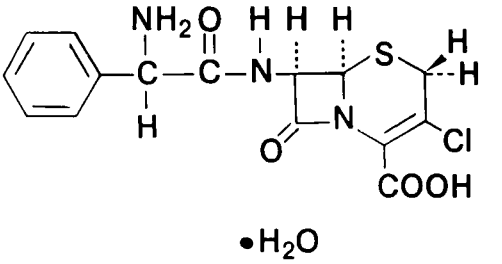

Figure 1 - Structure of Cefaclor monohydrate

cefaclor is based on reaction with hydroxylamine. The purpose of these investigations was to develop a stability-indicating high performance liquid chromatography assay method for the quantitation of cefaclor in pharmaceutical products.

MATERIALS AND METHODS

Chemical Reagents: All the chemicals and reagents were USP-NF or ACS quality and were used without further purification. Cefaclor Powder was supplied by Eli Lilly & Co. and used as received. All the dosage forms and cefazolin sodium powder (Marsam Pharmaceuticals) were from the commercial lots.

Apparatus: A high-pressure liquid chromatograph (Waters ALC 202) equipped with a universal injector (Rheodyne Model 7125), a multiple wavelength detector (Schoeffel's SF 770, Kratos, Inc.) and a recorder (Omniscribe 5213-12, Houston Instruments, Austin) was used. A micro C_{18} column (Waters Associates, 30 cm x 3.9 mm i.d.) was the stationary phase.

Chromatographic Conditions: The mobile phase contained acetonitrile 12% (v/v) and 0.2% glacial acetic acid in water(PH ~ 2.85). The flow rate was 1.5 ml/min., the wavelength was 262 nm (sensitivity 0.1 AUFS), the chart speed was 30.5 cm/hr and the temperature was ambient.

Preparation of Stock and Standard Solutions: A stock solution of cefaclor monohydrate was prepared fresh every day by dissolving 50.0 mg of the powder (based on anhydrous cefaclor) in enough water to make 50.0 ml of the solution. A stock solution of cefazolin sodium (the internal standard) was prepared by dissolving 200.0 mg of the powder in enough water to make 100.0 ml of the solution. A most commonly used standard solution was prepared by mixing 2.4 ml of the stock solution of drug with 3.0 ml of the stock solution of the internal standard, and bringing to volume (50.0 ml) with water. The solutions of other concentrations were prepared as needed.

Extraction from the Capsules: A quantity of the powder representing 50.0 mg of cefaclor was accurately weighed and mixed with 40 ml of water. The mixture was stirred for 5 minutes and brought to volume(50.0 ml) with water. The mixture was filtered (Fisher's 9-803-SE filter paper), the first 10 ml of filtrate was rejected, and then collected for analysis. A 2.4 ml quantity of the clear filtrate was mixed with 3.0 ml of the stock solution of cefazolin sodium and brought to volume (50.0 ml) with water.

Preparation of Assay Solutions from Powders for Suspensions: The Suspensions were prepared according to directions on the label. A quantity of suspension representing 250 mg of cefaclor was mixed with enough water to make 250 ml of the mixture. A 2.4 ml quantity of this solution was mixed with 3.0 ml quantity of the stock solution of cefazolin sodium and brought to volume (50.0 ml) with water.

Decomposition of Cefaclor: A 2.4 ml of the stock solution of cefaclor was mixed with 10 ml of water and either ~ 1 ml of ~ 1N H_2SO_4 or NaOH in a 150 ml beaker. The mixture was heated to boiling (~ 5 minutes), cooled and brought to volume (50.0 ml) with water. Before bringing to

volume, the pH was adjusted to approximately 6.0 using either 0.1N solution of H_2SO_4 or NaOH. The mixtures were analyzed without the addition of an internal standard in order to detect new peaks (if any) in the chromatogram.

Assay Procedure: A 20 μl quantity of the assay solution was injected into the chromatograph using the conditions described. For comparison, an identical volume of the standard solution was injected after the sample eluted. The standard solution contained identical concentrations of the drug (based on the label claim) and the internal standard.

Calculations: Preliminary investigations indicated that the ratio of peak heights were related to the concentrations of the drug (range tested ± 50% of the Standard Concentration). The results were calculated using a simple equation:

$$\frac{(Rph)_a}{(Rph)_s} \times 100 = \text{percent of the label claim found,}$$

where $(Rph)_a$ is the ratio of the peak heights of drug to internal standard of the assay solution and $(Rph)_s$, that of the standard solution.

RESULTS AND DISCUSSION

The results (Table 1) indicate that the developed method can be used to quantify cefaclor in the capsules and powders of suspensions. The method is accurate and precise with percent relative standard deviation of 1.2 based on 5 readings. The ratio of peak heights were related to the drug concentrations (range tested 24-72 μg/ml of cefaclor). The correlation factor, r was 0.9997. The recovery from the synthetic mixtures was quantitative (Table 1) and there was no interference (Figure 2C) from the excipients present in capsules such as FD & C blue No. 1, FD & C red No.3, corn starch, gelatin, iron oxide (in

TABLE 1

ASSAY RESULTS

Name of the Sample	Percent of the Label Claim Found	Other Ingredients if any
1. Capsules 250 mg	98.2	The capsules contained FD&C blue No.1, FD&C red No.3,corn starch,gelatin magnesium stearate, and titanium dioxide. 500 mg capsules also contained iron oxide.
2. Capsules 250 mg (different lot)	100.1	
3. Capsules 250 mg (expired May 1, 89)	97.1	
4. Capsules 500 mg	103.3	
5. Suspension 25mg/ml	104.0	The suspensions contained cellulose, FD & C red No.40, flavors, silicone, sodium lauryl sulfate, sucrose, and xanthan gum.
6. As above after 34 day storage at 5°	100.3	
7. Suspension 50 mg/ml	102.4	
8. As above after 34 day storage at 5°	101.3	
9. Suspension 75mg/ml	102.9	
10. As above after 34 day storage at 5°	102.8	
11. Synthetic Mixture 1	103.2	Glucose
12. Synthetic Mixture 2	101.5	Lactose

500 mg capsules), magnesium stearate and titanium dioxide. The procedure for the extraction of drug from capsules is very simple. The inactive ingredients in the powders for suspensions such as cellulose, FD & C Red No. 40, flavors, silicone, sodium lauryl sulfate, sucrose and xanthan gum did not interfere with the assay procedure (Figure 2D). The directions on the label recommend storage of suspension in the

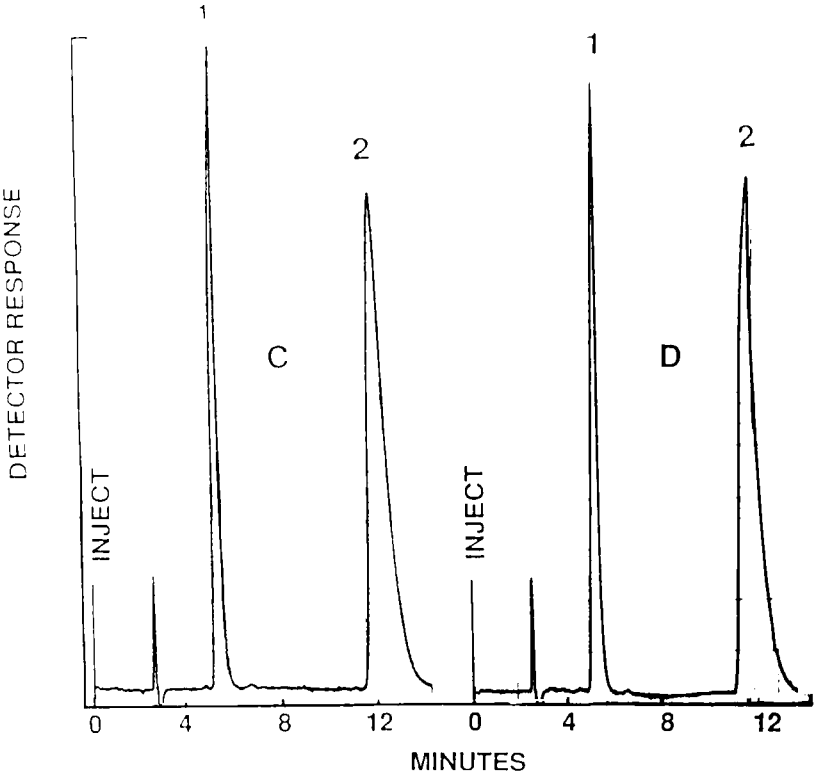

Figure 2 - Sample Chromatograms, Peaks 1-2 are from Cefaclor and Cefazolin sodium (the internal standard), respectively. Chromatogram C is from a suspension (75 mg/ml and D from capsules (number 2 in Table 1). For Chromatographic conditions, see text.

refrigerator for up to 14 days without significant loss in the activity of cefaclor. After 34 days of storage in the refrigerator (5°), our studies determined the loss in potency of cefaclor to be less than 4% (Table 1). The capsules which had expired more than 3 years ago lost only 3% of the potency (Table 1).

There were no new peaks in the chromatograms from the solution decomposed by using a base (Figure 3B) or an acid (not shown in the figure). In both the solutions, the potency of the drug remaining was

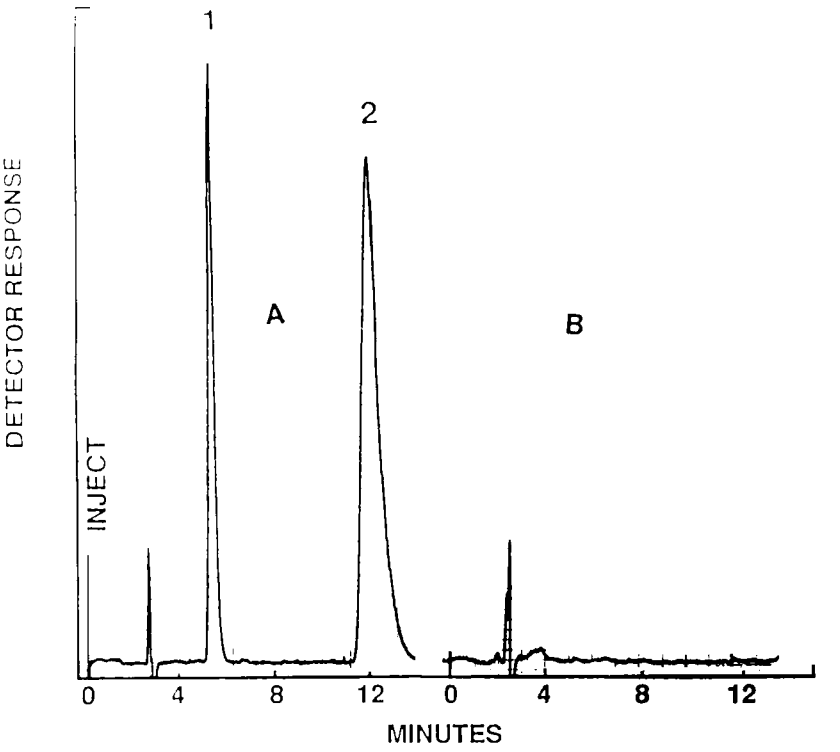

Figure 3 - Sample Chromatograms. Peaks 1-2 are from Cefaclor and Cefazolin Sodium (the internal standard), respectively. Chromatogram A is from a standard solution and B from a base decomposed sample. For Chromotographic conditions, see text.

almost zero. The mobile phase and the wavelength used for the developed method have been mentioned in the analytical profiles of drug substances (2) without any details or references. No internal standard was suggested (2).

REFERENCES

1. Anonymous, United States Pharmacopeia 22nd ed. and National Formulary 17th ed. U.S. Pharmacopeial Convention, Rockville, MD, 1990, p.239.

2. L.J. Lorenz in Analytical Profiles of drug substances, Vol. 9, K. Florey ed., Academic Press, New York, 1980, p.119.