DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 13(12), 2231-2238 (1987)

QUANTITATION OF CEPHALEXIN IN PHARMACEUTICAL DOSAGE FORMS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A high-performance liquid chromatography method has been developed to quantify cephalexin in pharmaceutical dosage forms, capsules, pediatric drops and suspensions. The method is accurate and precise with a percent relative standard deviation of 0.8 based on 6 readings. There is no interference from a variety of excipients present in the dosage forms. The procedure for the extraction of cephalexin from the dosage forms is very simple. The method is stability indicating since a sample decomposed using sodium hydroxide showed very little potency and new peaks in the chromatogram. In the powder form cephalexin appears to be very stable.

BACKGROUND

Cephalexin (Figure 1) is a semisynthetic cephalosporin antibiotic. It is extensively used in medicine for its antibacterial properties. The USP-NF method[1] for the quantitation of cephalexin

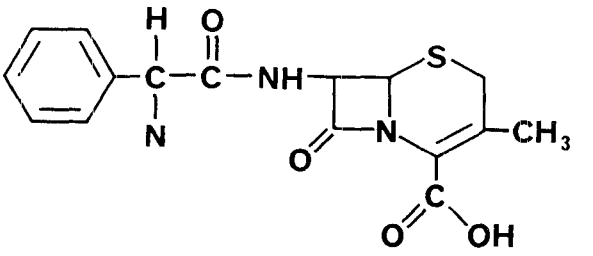

FIGURE 1

Structure of cephalexin.

in dosage forms is based on the microbiological assay procedure which is cumbersome and the results can vary widely (± 15%).

The purpose of these investigations was to develop a stability-indicating high-performance liquid chromatography assay method for the quantitation of cephalexin in pharmaceutical dosage forms.

MATERIALS AND METHODS

Reagents and Chemicals: All the reagents and chemicals were USP-NF or ACS quality and used without further purification. The cephalexin powder (Eli Lilly & Co.) was used as received. All the dosage forms were from the commercial lots.

Apparatus: A high-pressure liquid chromatograph (Waters ALC 202 equipped with U6K Universal Injector, Waters Associates) was connected to a multiple wavelength detector (Schoeffel's SF770, Kratos Inc.) and a recorder (Omniscribe 5213-12, Houston Instruments). A microphenyl column (Microbondapak, 30 cm x 3.9 mm i.d., Waters Associates) was used.

Chromatographic Conditions: The mobile phase contained 9% (V/V) of acetonitrile in 0.01M aqueous solution of ammonium acetate. The flow rate was 2.0 ml/min, the chart speed 30.5 cm/hr and the temperature was ambient. The sensitivity was set at 0.04 at 260 nm (the wavelength of maximum absorption).

Preparation of Solutions: The 0.025% stock solutions of cephalexin and cefazolin sodium (the internal standard) in water were prepared fresh daily. All the other solutions were prepared by diluting the appropriate quantities of the stock solutions with water. The most commonly used standard solution contained 40.0 µg/ml of cephalexin and 60.0 µg/ml of cefazolin sodium.

Extraction Procedures - From Capsules: An appropriate quantity of the powder representing 50.0 mg of cephalexin was weighed and mixed with 100 ml of water. The mixture was stirred occasionally for about 5 minutes or until no lumps were present and brought to volume (200.0 ml) with water using a volumetric flask. It was then filtered (Fisher's 9-801E filter paper), first 20 ml of the filtrate was rejected and then some collected for further dilution. A 4.0 ml quantity of the clear filtrate was mixed with 6.0 ml quantity of the stock solution of the internal standard (cefazolin sodium) and brought to volume (25.0 ml) with water.

From Powders for Suspensions: The suspensions were prepared according to the directions on the label. Then a portion of the suspension representing 125.0 mg of cephalexin was diluted with water to 500.0 ml. After a thorough mixing, a 4.0 ml quantity of the clear solution was mixed with 6.0 ml quantity of the stock

solution of cefazolin sodium and brought to volume (25.0 ml) with water.

From Powder for Pediatric Drops: A proecedure similar to extraction from powders for suspension was used except that a portion of the drops representing 250.0 mg of cephalexin was diluted to 1000.0 ml with water.

Assay Procedure: A 20.0 µl of the assay solution was injected into the chromatograph using the conditions described above. For comparison, an identical quantity of the standard solution was injected after the assay eluted. The standard solution contained identical concentrations of drug and the internal standard as in the assay solution based on the label claim.

Calculations: Preliminary investigations indicated that the ratio of peak heights (cephalexin/cefazolin) were directly related to concentrations of cephalexin (range tested, 0.6 and 1.0 µg). Therefore, the results were calculated using a simple equation:

$$\frac{(R_{ph})a}{(R_{Ph})s} \times 100 = \text{Percent of the label claim found}$$

where $(R_{Ph})_s$ is the ratio of peak heights of the assay solution and $(R_{Ph})_s$ that of the standard solution.

Assay of an Old Powder of Cephalexin: An assay solution was prepared from an old lot of cephalexin powder with an expiry date of January, 81 using the procedure described above.

Decomposed Solutions: A 4.0 ml portion of the stock solution of cephalexin was mixed with 10 ml of water and either with 0.15 ml of $\sim$1N H_2SO_4 or with $\sim$1N NaOH solution. The mixture was heated to

boiling on a hot plate (5 minutes) and cooled. The mixture was then neutralized using sulfuric acid/sodium hydroxide solution and brought to volume (25.0 ml) with water. Each mixture was then assayed according to the procedure described above except that no internal standard was added to the standard or the assay solution to avoid interference from the product(s) of decomposition. The results were calculated by comparing the peak heights of the assay and the standard solutions as described above.

RESULTS AND DISCUSSION

The results indicate (Table 1) that the developed HPLC assay method can be used to quantify cephalexin in pharmaceutical dosage forms. The method is accurate and precise with a percent relative standard deviation of 0.8 based on six readings. The separation of internal standard (cefazolin) from the drug was complete and excellent (Figures 2-3).

The method appears to be stability-indicating since a sample decomposed by using sodium hydroxide (see text) showed less than 5% potency and new peaks (Figure 2B) in the chromatogram. Cephalexin appears to be a lot more stable in the acidic medium since the solution which was treated with sulfuric acid (see text) lost about 17% of the potency. This is not unusual.

The assay results using the developed method compared excellently with the results provided by the manufacturer (Table 1). There was no interference from the various excipients (Figure 3) present in the dosage forms such as D & C yellow #10, F D & C blue #2, F D & C yellow #6, gelatin, magnesium stearate, silicone, and

TABLE 1
ASSAY RESULTS OF VARIOUS DOSAGE FORMS

Dosage Form	Claim per Capsule or per ml	Percent of Label Claim Found	Manufacturer's Results[a]
Capsules	250 mg	100.4	101.6
Capsules (different lot)	25 mg	103.4	102.0
Capsules	500 mg	100.8	100.0
Capsules (different lot)	500 mg	100.2	98.8
Suspension	25 mg	105.8	106.6
Suspension (different lot)	25 mg	104.2	105.8
Suspension	50 mg	102.9	105.5
Suspension (different lot)	50 mg	105.8	108.0
Pediatric Drops	100 mg	106.4	112.4

[a]These are the results of assays performed by manufacturer at the time of manufacture. The assay method was not disclosed to us.

titanium dioxide etc., which were present in the capsules. The suspensions and drops contained flavors, methylcellulose, silicone, sodium lauryl sulfate, sucrose and colors. Cephalexin appears to be a very stable compound when in powder form. For example, an old powder whose expiry date was January 81 had lost very little potency in 6 years. There was no new peak in the chromatogram and

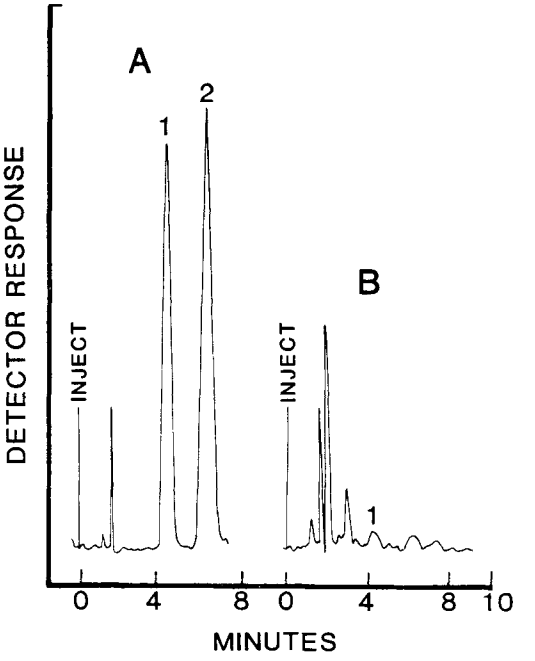

FIGURE 2

Sample chromatograms. Peaks 1-2 are from cephalexin and cefazolin (the internal standard), respectively. Chromatogram A is from a standard solution and B from a solution decomposed using sodium hydroxide (see text). For chromatographic conditions, see text.

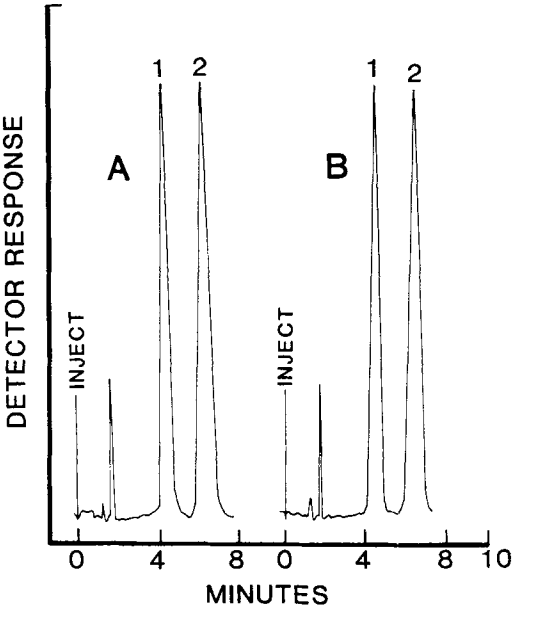

FIGURE 3

Sample chromatograms. Peaks 1-2 are from cephalaxin and cefazolin (the internal standard), respectively. Chromatogram A is from 500 mg capsules and B from a suspension (50 mg/ml). For chromatographic conditions, see text.

the shape of the peak was exactly the same as from the fresh reference powder. The HPLC methods for the quantitation of cephalosporin may be better since the results can be reproduced within about ± 2% versus microbiological assay methods whose results can very up to ± 15%. Furthermore, when the cephalosporin decomposes only at the side chain (that is β-lactam ring is intact), it will still show some activity in a microbiological assay method and none by HPLC method.

REFERENCES

1. The United States Pharmacopeia, 21st Rev. The National Formulary, 15th Rev., 1985; The United States Pharmacopeial Convention, Rockville, MD., pp. 179-80.