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QUANTITATION OF 5-FLUCYTOSINE IN CAPSULES USING HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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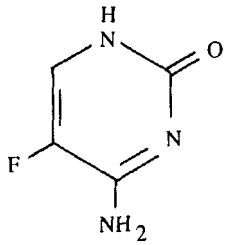
ABSTRACT

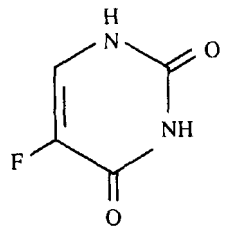
A stability-indicating reversed phase HPLC method for the quantitation of 5-flucytosine in capsules (the only dosage form available) has been developed. The method requires the use of a mobile phase without any counterion and the samples can be assayed at room temperature. The method is simple, reproducible, precise and accurate with percent relative standard deviation of 0.77 based on 6 readings. There was no interference from the excipients present in capsules and from fluorouracil (the major product of decomposition of 5-flucytosine). The recovery of 5-flucytosine from the synthetic mixtures was quantitative. A simple extraction procedure for 5-flucytosine from the capsules has been developed.

INTRODUCTION

5-Flucytosine (Figure 1) is used as an antifungal agent. It is often given orally in the form of capsules. No other dosage form is available commercially. The USP-NF method[1] for the quantitation of flucytosine is based on UV spectroscopy at 285 nm. The major product of decomposition of flucytosine, fluorouracil, interferes with this



5 - Flucytosine.



Fluorouracil.

Figure 1 Structures of 5-flucytosine and fluorouracil.

method. Another method[2] for the quantitation of flucytosine has been reported. This method requires the use of a pellicular cation-exchange resin at 78°. Biondi et al[3] have reported a method based on HPLC with a mobile phase containing two counterions, 0.0025 M each of pentane sulfonic acid sodium and heptane sulfonic acid sodium. The counterions usually affect the costly columns adversely and can shorten their lives. The purpose of these investigations was to develop a stability-indicating high performance liquid chromatography method for the quantitation of 5-flucytosine using a mobile phase without a counterion and at room temperature.

METHODOLOGY

Materials: All the chemicals and reagents were USP, NF or ACS quality and used without further purification. 5-Flucytosine and 5-fluorouracil powders (Roche Laboratories) were used as received. 5-Aminouracil powder for the internal standard was supplied by Sigma Chemical Co.

Equipment: A high-pressure liquid chromatograph (ALC 202, Waters Associates) equipped with an injector (Rheodyne Model 7125), a multiple wavelength detector (Schoeffel's SF 770, Applied Biosystems) and a recorder (Omniscribe 5213-12, Houston Instruments) was used. A Med. Pharmex C_{18} column (Part No. MP 002, 25 cm x 4.6 mm i.d.) was used.

Chromatographic Conditions: The mobile phase was 0.05 M KH_2PO_4 buffer solution in water (pH $\sim$4.5). The flow rate was 1.0 ml/min. The detector was set at 300 nm (AUFS 0.1), the chart speed was 30.5 cm/hr and the temperature was ambient.

Preparation of Solutions: The stock solutions of 5-flucytosine and 5-fluorouracil were prepared by dissolving 100.0 mg of the powder (0.1% each) in enough water to make 100.0 ml of the solution. A stock solution of 5-aminouracil (the internal standard) was prepared by dissolving 20 mg of powder in enough water to make 500 ml of the solution. All the stock solutions were prepared fresh daily. These stock solutions were diluted further with water as needed. The most commonly used standard solution contained 100.0 µg/ml of 5-flucytosine and 12 µg/ml of the internal standard.

Extraction of 5-Flucytosine from Capsules: After an accurate weight of 10 capsules, a quantity of the powder representing 100.0 mg of 5 flucytosine was mixed with about 80 ml of water. The mixture was stirred occasionally for 3-4 minutes, brought to volume (100.0 ml) with water and filtered (Fisher's 9-803-5E filter paper). First 20 ml of the

filtrate was rejected and then some collected for further dilution. A 2.5 ml quantity of the clear filtrate was mixed with a 7.5 ml quantity of the stock solution of 5-aminouracil, the mixture was brought to volume (25.0 ml) with water and assayed using the procedure described below.

Standard Solution with 5-Fluorouracil: The most commonly used standard solution containing varying quantities of 5-fluorouracil (20, 40 and 60 μg/ml) were also prepared to determine the interference in the assay procedure.

Assay Procedure: A 25 μl quantity of the assay solution was injected into the chromatograph using the described conditions. For comparison, an identical quantity of the standard solution was injected after the assay 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 the peak heights (drug/internal standard) were directly related to the concentrations of the drug (range tested ± 40% of the standard concentration), therefore, the results were calculated using a simple equation:

$$\frac{(R_{ph})_a}{(R_{ph})_s} \times 100 = \text{Percentage of the label claim found}$$

where $(R_{ph})_a$ is the peak height ratio of the assay sample and $(R_{ph})_s$ that of the standard solution.

RESULTS AND DISCUSSION

The results indicate (Table 1) that 5-flucytosine can be quantified in capsules using an HPLC method without the addition of counter-

TABLE 1

ASSAY RESULTS OF THE DOSAGE FORMS, SYNTHETIC MIXTURES AND SOLUTIONS WITH 5-FLUOROURACIL

Sample Tested	Claim (5-Flucytosine)	Other Ingredients (if any)	Percent of the Label Claim Found
Capsules	250 mg/capsule	Excipients	100.6
Capsules	500 mg/capsule	Excipients	102.8
Solution 1	100 μg/ml	20 μg/ml of fluorouracil	99.8
Solution 2	100 μg/ml	40 μg/ml of fluorouracil	99.4
Solution 3	100 μg/ml	60 μg/ml of fluorouracil	100.4
Synthetic Mixture 1	20 mg/100 mg	10 mg fluorouracil and 70 mg dextrose	100.4
Synthetic Mixture 2	20 mg/100 mg	10 mg fluorouracil and 70 mg of lactose	99.8

ions and at room temperature. The method developed is accurate and precise with a relative percent standard deviation of only 0.77 based on 6 readings. The recovery of 5-flucytosine from the synthetic mixtures was quantitative (Table 1). The extraction procedure for 5-flucytosine from capsules is very simple.

There was no interference from the major product of decomposition, fluorouracil, in the assay method (Figure 2B). Actually the wavelength of maximum absorption for 5-flucytosine is 285 nm, however, at this wavelength, fluorouracil also had strong absorption, hence the separation of 5-flucytosine from fluorouracil was not complete. By using 300

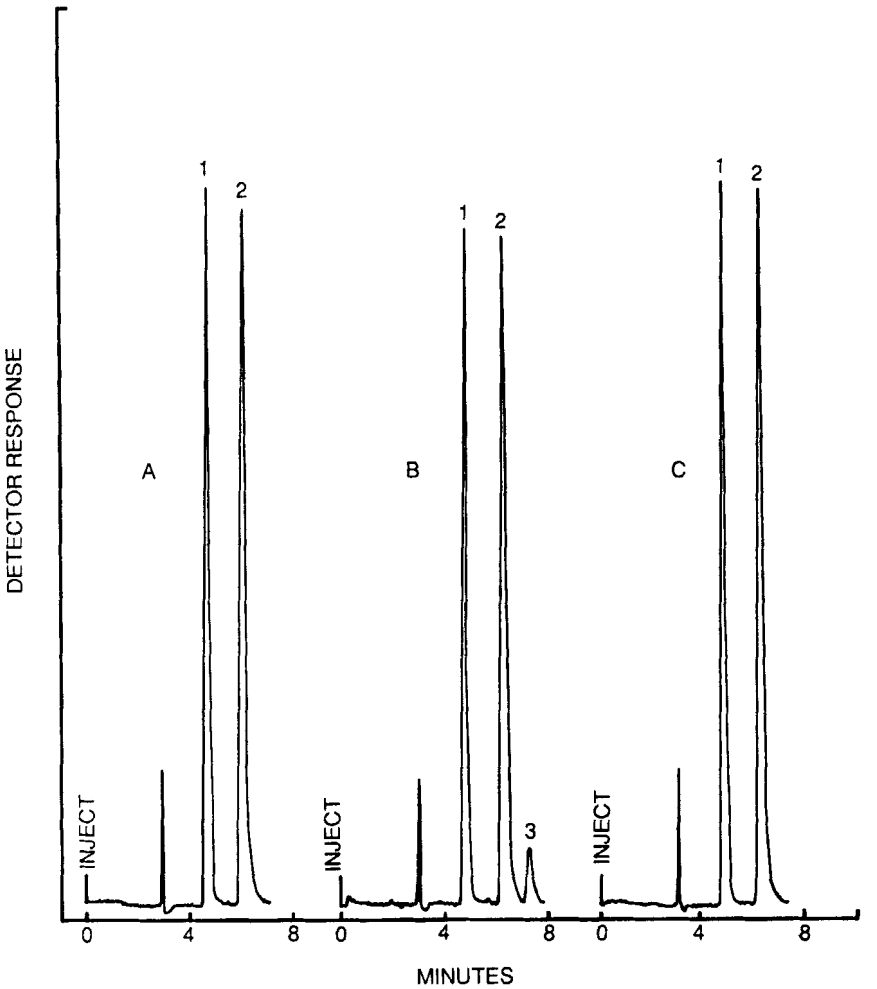


Figure 2 Sample chromatograms. Peaks 1-3 are from aminouracil (the internal standard), 5-flucytosine and fluorouracil, respectively. Chromatogram A is from a standard solution; B from a standard solution with 60 μg/ml of fluorouracil; and C from 250 mg capsules. For chromatographic conditions, see text.

nm as the wavelength, it was possible to separate both of them completely (peaks 2-3, Figure 2). The chromatogram in Figure 2B was developed from a solution containing 60 µg/ml of fluorouracil (peak 3) and 100 µg/ml of 5-flucytosine (peak 2). More than 35% of flucytosine had to decompose for the solution to contain this much fluorouracil. Also, there was no interference in the assay method from the excipients present in the capsules.

The internal standard, 5-aminouracil, had very strong absorption at 300 nm (∿8 times more on weight/weight basis than 5-flucytosine) and it separated completely from 5-flucytosine (peaks 1-2, Figure 2).

It is interesting to point out that to establish an equilibrium between the mobile phase and the column it required about 30 minutes versus the usual time of 15-20 minutes.

In conclusion, it can be stated that the developed HPLC method is stability-indicating, reproducible, precise and accurate.

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