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COMMUNICATION

QUANTITATION OF FLUOXETINE HYDROCHLORIDE IN CAPSULES USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Yashoda Pramar, V. Das Gupta, and Charlie Bethea*

Department of Pharmaceutics, University of Houston
1441 Moursund St., Houston, TX 77030 and

*Ben Taub General Hospital Pharmacy
Houston, TX 77030

ABSTRACT

A stability-indicating high-performance liquid chromatographic method for the quantitation of fluoxetine hydrochloride in capsules (the only dosage form available) has been developed. The method is accurate and precise with a percent relative standard deviation of 1.04 based on 6 readings. An excellent separation of fluoxetine from methyltestosterone (the internal standard) was achieved, and sharp peaks were obtained by adding acetic acid to the mobile phase. The inactive ingredients present in the capsule powder did not interfere with the assay procedure. The recovery of fluoxetine from the synthetic mixtures was quantitative. The drug appears to be very stable in the acidic medium and highly susceptible to degradation in the basic medium.

INTRODUCTION

Fluoxetine hydrochloride (Figure 1) is extensively used as an antidepressant for oral administration. It is a relatively newer

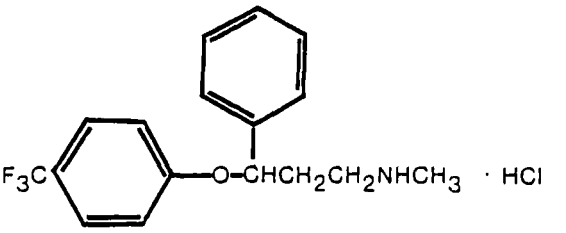

Figure 1 Structure of Fluoxetine Hydrochloride.

compound and unrelated to the tricyclic, tetracyclic or other available antidepressants. The quantitation of fluoxetine from capsules has not been reported.

The purpose of these investigations was to develop a stability-indicating assay method for the quantitation of fluoxetine hydrochloride using HPLC.

METHODOLOGY

Chemicals and Reagents: All the chemicals and reagents were USP-NF or ACS quality and used without further purification. Fluoxetine hydrochloride powder was generously supplied by Eli Lilly & Co. and used as such. All the capsules were of commercial lots.

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 C_{18} column (Microbondapak, 30 cm x 3.9 mm i.d., Waters Associates) was the stationary phase.

Chromatographic Conditions: The mobile phase contained 45% V/V of acetonitrile in 0.01M KH_2PO_4 aqueous buffer solution, with or without 0.2% glacial acetic acid(V/V). The flow rate was 2.0 ml/min,

the sensitivity was 0.1 AUFS (at 234 nm), the chart speed was 30.5 cm/hr and the temperature was ambient.

Preparation of Solutions: A 0.08% stock solution of fluoxetine hydrochloride in water was prepared fresh daily. A 0.025% stock solution of methyltestosterone (the internal standard) in methanol was prepared by dissolving 25.0 mg of the drug in enough methanol to make 100.0 ml. These stock solutions were mixed and diluted further with water as needed. The most commonly used standard solution contained 160 µg/ml of fluoxetine hydrochloride and 50 µg/ml of methyltestosterone. A 0.1 ml quantity of ∿0.1NHCl was added to 25 ml of the final volume.

Extraction Procedure From The Capsules: The contents of 10 capsules were weighed accurately and a quantity of the powder representing 20 mg of fluoxetine hydrochloride was mixed with 0.5 ml of ∿0.1NHCl and 20 ml of water and stirred for ∿3 minutes using a stirring rod. The mixture was brought to volume (25.0 ml) with water, filtered (Fisher's 9-803-5-E filter paper), the first 5 ml of the filtrate was rejected and then some was collected for further dilution. A 5.0 ml quantity of the clear filtrate was mixed with 5.0 ml quantity of the stock solution of methyltestosterone and brought to 25.0 ml with water.

Extraction Procedure From The Synthetic Mixtures: The extraction procedure from the synthetic mixtures was the same as for the capsules, except that no filtration was required.

Decomposition of Fluoxetine Hydrochloride: A 5.0 ml quantity of the stock solution was mixed with either 0.1 ml of ∿1N H_2SO_4 or 1 ml of ∿ 0.1 N NaoH solution in a 150 ml beaker. A 10 ml quantity of water was added and the mixture heated to boiling for 15 minutes using a hot plate (more water was added as needed, to prevent splashing). The

solution was cooled to room temperature, acidified (pH~2), using 1N H_2SO_4 solution, brought to volume (25.0 ml) with water, and assayed. The internal standard was not added, in order to detect new peaks in the chromatograms.

Assay Procedure: A 20.0 µl quantity of the assay solution was injected into the chromatograph using the conditions described above. For purpose of comparison, an identical volume of the standard solution was injected after the assay sample eluted. The standard solution contained identical concentrations of fluoxetine hydrochloride (based on the label claim) and the internal standard.

Calculations: Since preliminary investigations indicated that the ratios of peak heights (drug/internal standard) were directly related to the concentrations of fluoxetine hydrochloride, 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 $(R_{ph})_a$ is the ratio of the peak heights of the assay sample and $(R_{ph})_s$ that of the standard solution. In the case of the decomposed solutions, the results were estimated by direct comparison of peak heights (assay/standard) of fluoxetine hydrochloride, since no internal standard was added. It should be pointed out that the above equation can be used only if the concentrations of drug in the assay and standard were similar ($\pm$ 3%), otherwise, a standard curve should be constructed. The standard curve (Figure 2) did show an unexpectedly high Y-intercept value which the authors cannot explain. However, the correlation coefficient value for the readings was 0.999.

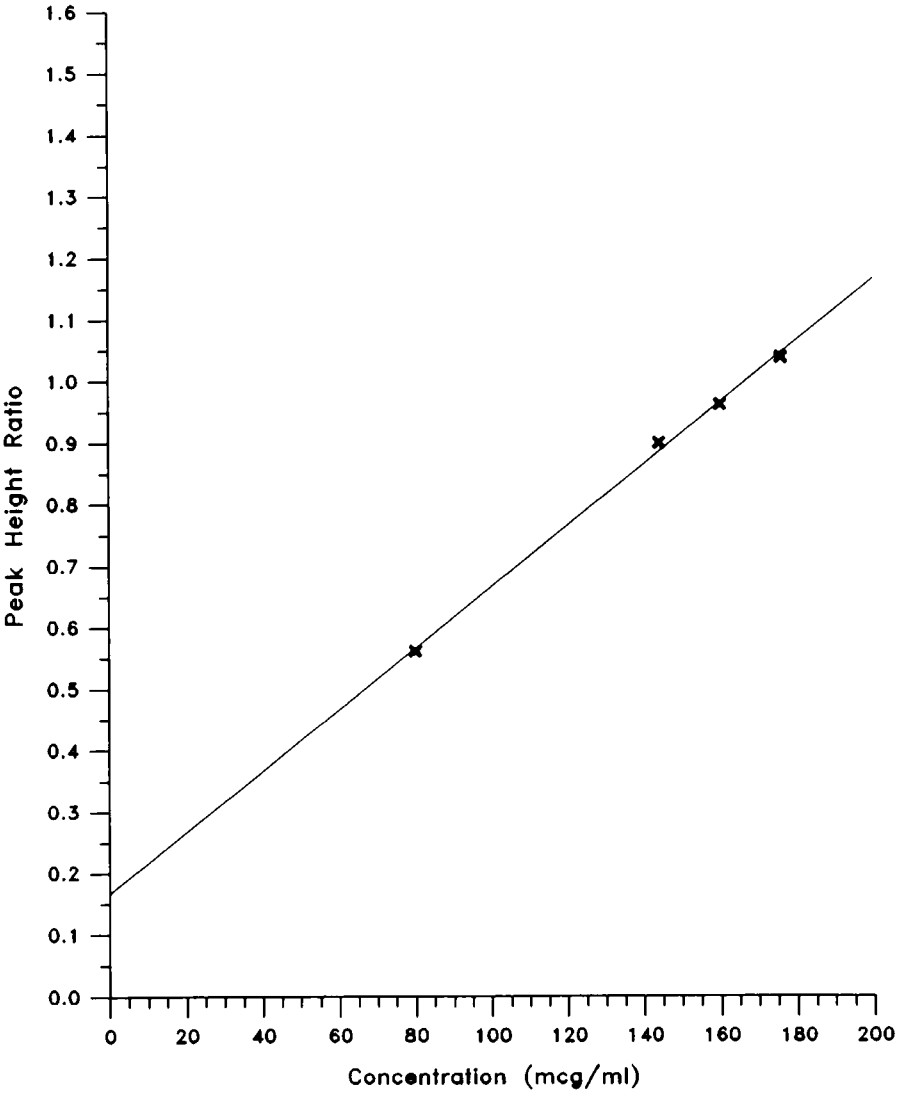

Figure 2 Standard curve of the ratios of the peak heights (drug/internal standard) versus fluoxetine hydrochloride concentrations.

TABLE 1

ASSAY RESULTS FROM THE CAPSULES AND SYNTHETIC MIXTURES

Dosage Form or Synthetic Mixture	Claim per Capsule	Percent of the Label Claim Found
1. Capsules	20 mg of Fluoxetine Base	99.1
2. Capsules (different lot)	as above	98.3
3. Capsules (different lot)	as above	96.3
4. Synthetic Mixture 1	20 mg of Fluoxetine HCl in 200 mg of dextrose	100.9
5. Synthetic Mixture 2	20 mg of Fluoxetine HCl in 200 mg of Lactose	100.6

RESULTS AND DISCUSSION

The results indicate (Table 1) that the developed method can be used to quantify fluoxetine hydrochloride in capsules (the only dosage form available). The method is accurate and precise with percent relative standard deviation of 1.04 based on 6 readings. The separation of methyltestosterone (peak 2 in Figure 3B) from the drug (peak 1 in Figure 3B) was complete. It should be pointed out that Chromatogram A in Figure 3 was developed using a mobile phase without glacial acetic acid. In this chromatogram the separation was not complete and the peaks were not sharp. However, the addition of 0.2% (V/V) of acetic acid gave excellent separation and sharp peaks (Figure

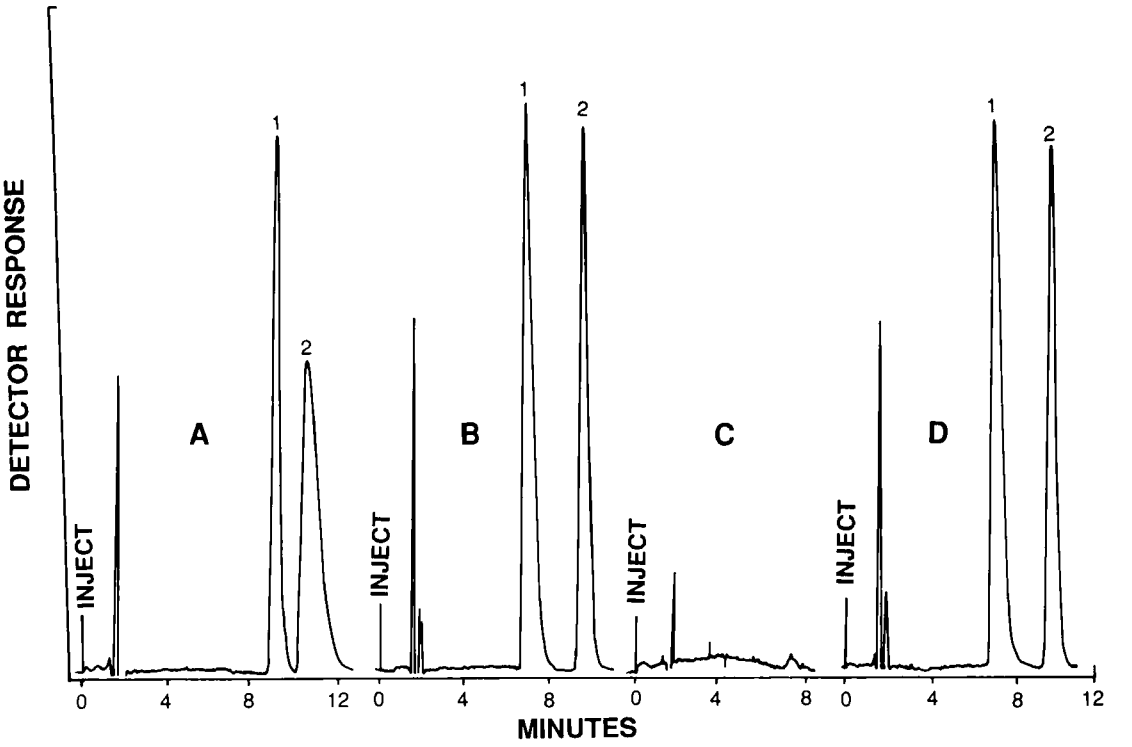

Figure 3 Sample chromatograms. Peaks 1-2 are from fluoxetine and methyltestosterone, respectively. Chromatogram A is from a standard solution when the mobile phase did not contain acetic acid; B from a standard solution, with acetic acid in the mobile phase; C from a base catalyzed sample; and D from a capsule (#1 in Table 1). For chromatographic conditions, see text.

3B). Therefore, all the studies were conducted using a mobile phase with acetic acid. Fluoxetine hydrochloride did not decompose in an acidic solution. However, in an alkaline solution, the decomposition was almost total (Figure 3C). On the acidic side, the drug appears to

be stable. The decomposed solution did not show any peaks which could interfere with the drug or the internal standard.

Extraction Procedure from Capsules and the Synthetic Mixtures: The extraction procedure is very simple, and there was no interference (Figure 3D) from the excipients present, which included FD&C Blue #1, gelatin, iron oxide, starch and titanium dioxide.