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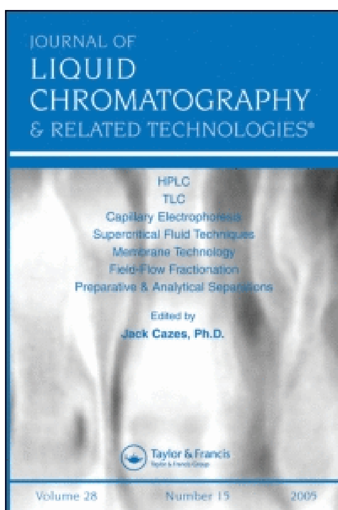

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High Performance Liquid Chromatography Assay of Itraconazole in Human Plasma

S. A. Babhair[a]
[a] Department of Pharmaceutics, College of Pharmacy King Saud University, Riyadh, Saudi Arabia

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ASSAY OF ITRACONAZOLE IN HUMAN PLASMA

SALIM A. BABHAIR
Department of Pharmaceutics
College of Pharmacy
King Saud University
Riyadh, Saudi Arabia

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ABSTRACT

A high performance chromatographic method, using internal standard quantification, for the analysis of Itraconazole in human plasma is described. The standard curve was linear over the concentration range tested. The detection limit of the method was 250 ng. An authentic sample of both Itraconazole and the internal standard (ketoconazole) were used to establish the calibration curve.

INTRODUCTION

Itraconazole is a new, orally active, azole antifungal drug. It has been shown that the drug is about five times more potent than ketoconazole in treatment of some diseases (vaginal candidosis).

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The pharmacokinetic profile has shown that Itraconazole has a preference for tissues which will result in tissue level several times higher than plasma (1). The drug was shown to be effective against a broad range of deep fungal pathogens (2). Unlike other azole the drug is free of hepatotoxic effect (3). It is well absorbed after oral administration due to its lipophilic characteristics. Its biological half-life (t ½) is 11-15 hours (4-5). Several methods for the determination of the drug in plasma and serum has been described, including agar diffusion and HPLC (6,7). In this report a simple HPLC method for the determination of Itraconazole in plasma is described.

EXPERIMENTAL

Apparatus

The apparatus used was a high pressure liquid chromatographic system equipped with basic unit model 6000 A, automatic injector model 710 B, Variable wavelength UV detector model 481 operating at 261 nm, and data module M 730 all of waters associates (Milford, Mass.). A 10 cm. x 8 mm. - ID C_{18} U. Bondapak Radial - PAK with Z - module system (waters associate) column was used for separation. The mobile phase consisted of methanol-water glacial acetic acid (80:20:1 V/V), which was filtered through a 0.45 millipore filter and degassed or 5 minutes. The flow rate was 2 ml/min.

Reagents and Chemicals

An authentic sample of Itraconazole and the internal standard (Ketoconazole) were obtained from Janssen Pharmaceutical (Beerse, Belgium), methanol, acetonitrile and acetic acid, spectral grade, were obtained from Merck (Darmstadt, Germany). Sodium chloride and

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citric acid were analytical grade and were obtained from BDH (Poole, England). A stock solution of 0.2 mg/ml Itraconazole in 5% citric acid was prepared. Solutions containing 1-20 ug/ml of the drug were made from the stock solution. For the internal standard, a stock solution containing 40 ug/ml was used.

Standard Curve in Solution

A series of dilutions from the stock solution to cover a range of 1 to 20 ug/ml were made. To 1 ml of each of these solutions 50 ul of the internal standard solution was added and 20 ul of the mixed resultant solution was injected onto the column in triplicate.

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Standard Curve in Plasma

One ml of plasma and aliquot stock solution of Itraconazole to cover a range of 1 to 10 ug/ml and 50 ul of the internal standard solution were mixed in a 20 x 150 mm. centrifuge tube. One gm. of sodium chloride and 2 ml of acetonitrile were added, and the mixture was vortexed for two mins. and then centrifuged at 2500 rpm for 5 mins. 20 ul from the acetonitrile layer was injected onto the column in triplicate.

Characteristics of the Assay

The assay was calibrated by analyzing 1 ml aliquots of plasma to which 1-10 ug of Itraconazole and 1 ug of the internal standard. Three replicates were determined for each concentration. Five concentrations were used for each calibration curve. In each sample the ratio of the area under the peak of the drug to the area under the peak of the internal standard was measured and plotted against the concentration of the drug. Linear regression was applied to determine the intercepts and the variation coeffecient.

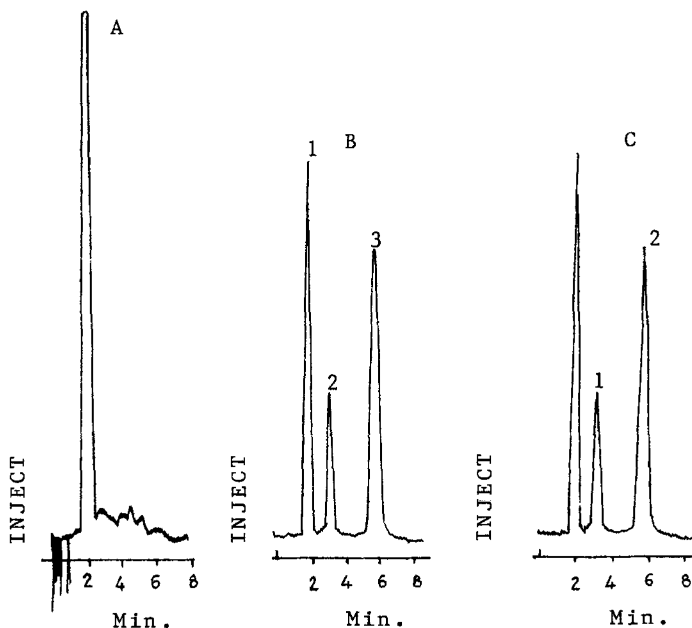

Figure 1 - High performance liquid chromatograms of in panel A plasma; in panel B, I = unassigned peak, 2 = internal standard, 3 = drug from aqueous solution; in panel C, I = internal standard, 2 = drug extracted from plasma.

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Recovery from Extraction

One milliliter aliquot of water and plasma were spiked with known quantities of the drug and the internal standard (10 ug drug and 1 ug internal standard), after plasma was extracted and chromatographed as previously described. The area under the peak for the drug and internal standard were compared with that obtained when the same amount of the drug and internal standard was injected directly onto the column. The triplicate determinations at each concentration were performed.

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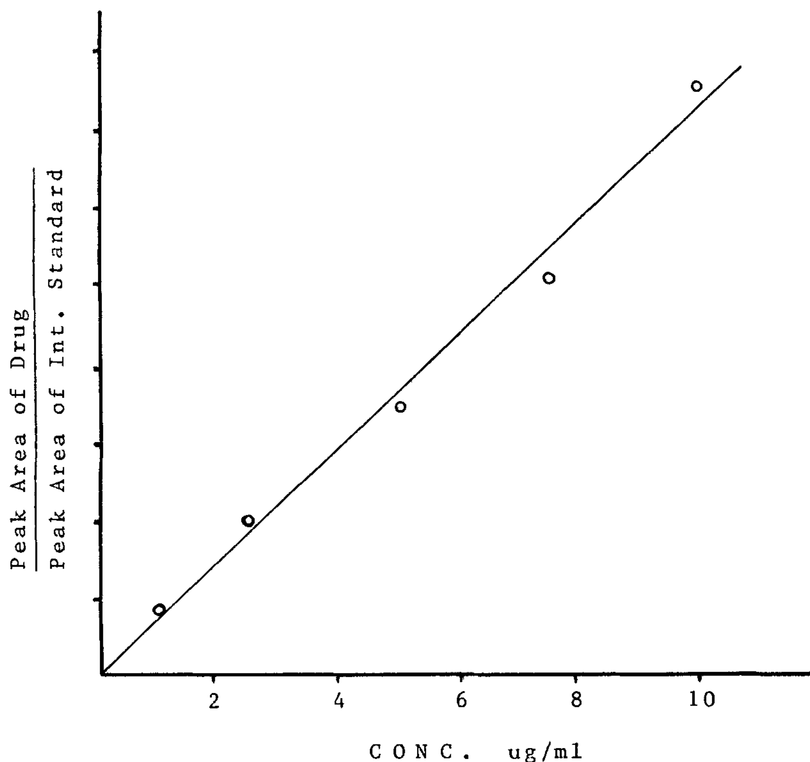

Figure 2 - Standard curve of itraconazole in solution.

RESULTS AND DISCUSSION

A simple sensitive method for the determination of Itraconazole by high pressure liquid chromatography was developed. The method depends on adding sodium chloride to the plasma which is then extracted by small volume of acetonitrile, from which the appropriate volume is injected directly onto the column. The retention time of the drug and the internal standard is (5.9) and (3.3) minutes respectively giving complete separation with minimum inter-

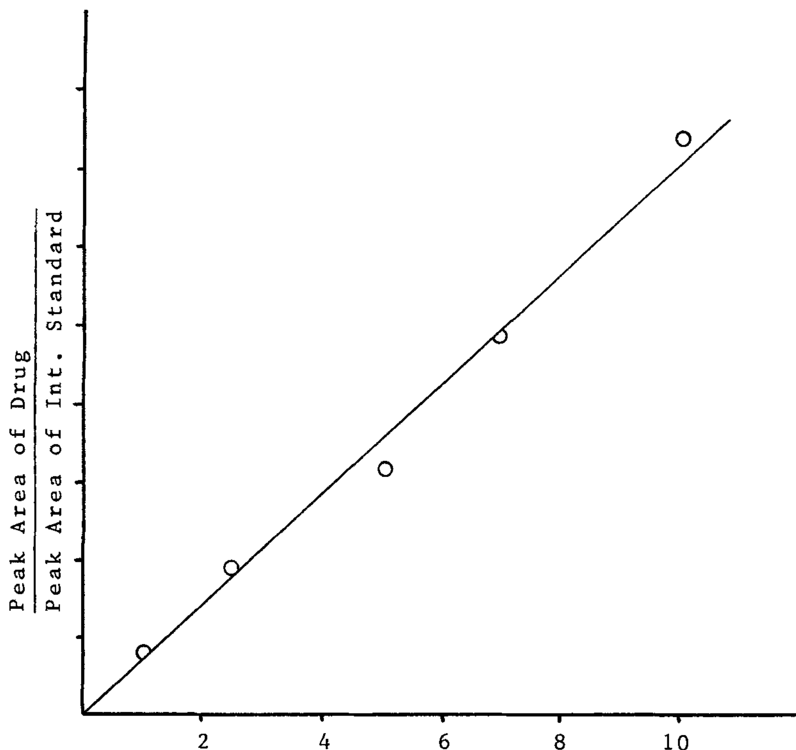

Figure 3 - Standard curve of itraconazole in plasma.

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ference from other plasma constituents. Chromatograms of typical aqueous injection of the drug, blank plasma and plasma sample are shown in Figure 1. The area under the peak of drug divided by the area under the peak of the internal standard from both the aqueous solution and that resulted from extracting the drug from the plasma correlated linearly with concentrations in the range of the concentrations tested. Correlation coefficients of the lines were 0.9998 and 0.998. Figures 2 and 3 show the standard curve of drug form solution and that extracted from plasma. The limit of detection, defined as four times the baseline noise, was 250 ng/ml

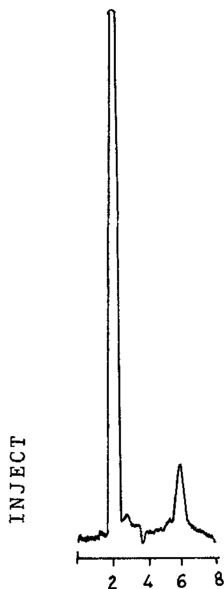

Figure 4 - High performance chromatogram of the lowest detectable amount of the drug.

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TABLE 1 - Total Recovery from the Extraction of Itraconazole

Conc. ug/ml	% Recovery
1	97.9
2.5	98.3
5	97.0
7.5	97.5
10.0	98.8

for the drug in plasma, Fig. 4. There was little variation in the observed peak heights when plasma samples from different individuals were used to construct the calibration curves. The method is reproducible with variation coefficient of less than 12% at each concentration. The method of extraction is very efficient and Table 1 shows the total recovery for the extraction of the drug from plasma.

The limit of detection could be increased by evaporating one ml of the organic phase and reconstituting the residue in 100 ul and injecting 20 to 30 ul onto the column.

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