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A Sensitive and Simplified HPLC Analysis for the Determination of Fluconazol in Human Plasma

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Abstract □ A sensitive and simplified HPLC assay of fluconazol is described. The calibration curve of fluconazol in plasma ranging 0-10 μg/m*l* was linear with the correlation coefficients of 0.9900. The limit of detection was 0.3 μg/m*l*. The average recovery of the drug was 89.1 ± 9.05%. After oral administration of single dose(150mg) of fluconazol in man, C_{max} and T_{max} were 3 μg/m*l* and 4hr., respectively.

Keywords □ HPLC, fluconazol, pharmacokinetic studies in man.

Fluconazol, developed from Pfizer central-Research, England, is a new orally active antifungal drug. It was reported that the fluconazol has higher activity than any other orally active azoles, such as itraconazol and ketoconazol[1]. The pharmacokinetic profile of fluconazol is markedly different from that of imidazol antifungal drugs and this contributes to the excellent efficacy of fluconazol, *in vivo*[2]. This drug will be an important advance in the antifungal treatments.

The analysis of fluconazol in human plasma had been developed[3] but practically the procedure is rather complex: The drug must be extracted from basified plasma into ethylacetate, followed by back extraction into acid, and followed by basification and re-extraction into ethylacetate. The newly modified method described in this paper has the great advantage that the drug need only single extraction without acid-base treatment.

EXPERIMENTAL METHODS

Materials

Fluconazol was obtained from Pfizer Laboratories. Temed (N,N,N,N-tetramethyl-ethylenediamine) was guaranteed grade (E. Merck). Acetonitrile was HPLC grade (E. Merck) and the water used was deionized in the laboratory with NanopureII (Barnstead).

Apparatus

The chromatographic equipment consisted of a HPLC pump (LKB. Broma, model 2150), a valco injector with 100 μ*l* loop, a superisorb S_5 C_8 (25 cm × 4.6 mm i,d, 5 μm particle size) column, a UVIDEC-100-VI variable wavelength UV detector (Jasco) and a two channel recorder (LKB Broma, model 2210). For the sample mixing, tube Mixer (International, model-S-T, RPM-50) was used.

Chromatographic conditions

The eluent was a 0.2 M temed buffer (pH 7.0, adjusted with phosphoric acid) containing 25% acetonitrile. The flowrate was 1.0 m*l*/min. The temperature was ambient and the wavelength of the detector was 261 nm at a sensitivity of 0.01 AUFS.

Sample preparation

Heparinized human plasma (1 m*l*) was placed in a PTFE-lined and screwcapped culture tube, and 4 m*l* acetonitrile was added. The samples were rotated at 60 rpm for 4 min. After centrifugation at 3000 rpm (4 °C) for 15 min, the supernatant was decanted and evaporated under the stream of nitrogen gas at 45 °C. The residue was dissolved in 300 μ*l* of mobile phase, and 10 μ*l* were injected to the chromatograph.

Analysis

Calibration curve was constructed by analyzing plasma spiked with various amounts of fluconazol and plotting the peak heights of fluconazol against the amount of fluconazol added. To calculate the recoveries, the peak heights obtained from the plasma spiked with known amounts of fluconazol were compared with the respective peak heights by

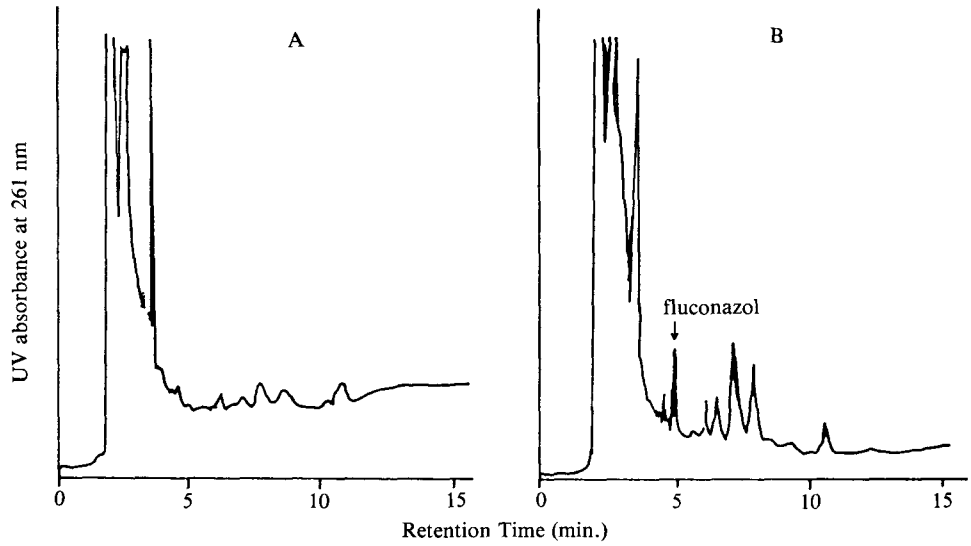

Fig. 1. HPLC Chromatograms of Human Plasma Samples.
(A) Control plasma
(B) Plasma sample obtained from a human volunteer
Column: Superisorb S-5 C_8 (5 μm, 250 mm × 4.6 mm i,d.) mobile phase: Acetonitrile/0.2 M temed buffer (pH 7.0) (25:75, v/v) flow rate: 1.0 ml/min Detection: UV at 261 nm at a sensitivity of 0.01 AUFS. sample size: 10 μl.

Table I. Recoveries and accuracy of fluconazol from plasma

Amount Added(μg)	Amount Found(μg)	Coefficient of Variation(%)
1	0.88 ± 0.09	10.2
2	1.65 ± 0.15	9.1
5	4.7 ± 0.5	10.6
7	6.6 ± 0.5	7.6
10	8.5 ± 1.6	18.8
mean ± S.D.		11.3 ± 3.9

injecting equal amounts directly into the chromatograph.

Human study

After overnight fasting, a healthy adult male (50 kg, 27 old of age) received 150 mg of fluconazol in capsule form. Blood samples were obtained from the cubital vein at 0.5, 1, 2, 4, 8, 24, 48 hr after dosing. Heparinized blood sample were centrifuged at 3,000 rpm for 15 min. The plasma sample were prepared and assayed immediately with the methods described above.

RESULTS AND DISCUSSION

Selectivity of the method

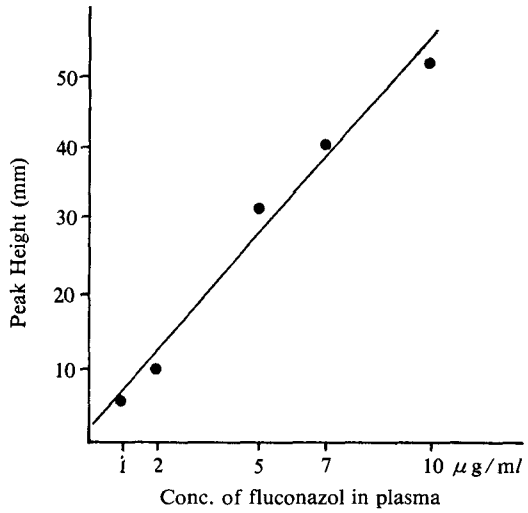

Fig. 2. Calibration Curve for the Analysis of Fluconazol in Human Plasma.
Each point represents the mean from four experiments

Fig. 1 shows the representative chromatograms of plasma samples obtained from a human volunteer after oral administration of fluconazol. Fluconazol is well separated and no interfering peaks were observed in the blank control plasma. Retention time for fluconazol was 5.05 min.

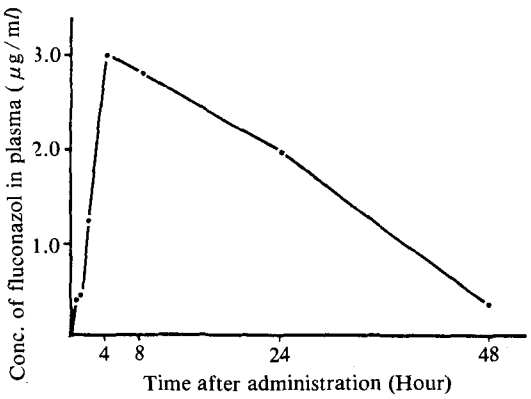

Fig. 3. Plasma Concentrations of Fluconazol after Oral Administration of 150 mg Fluconazol to a Human Volunteer.

Accuracy, precision and linearity

Table 1. shows that the present method is accurate and reproducible. The present method allowed the quantitative assay of fluconazol down to 0.3 μg/ml. The detection limit reported by previous workers[3] was 0.8μg/ml. The extraction recoveries of fluconazol from plasma are 89.1 ± 9.05%, which is not significantly different from the result of the previous workers (~95%).

As shown in Fig. 2, a calibration curve obtained from the analysis of human plasma samples containing various amounts of fluconazol gives a straight line over the concentration range of 1-10 μg/ml: the regressional equation for fluconazol is y = 5.3772x + 0.7989 [X: plasma concentration of fluconazol (ug/ml), Y: peak heights (mm)] and correlation coefficient is 0.9900.

Human study

The present method was applied to the quantitative assay of fluconazol in the plasma of a normal subject. Fig. 3. shows the time course fluconazol concentrations in plasma obtained from a healthy subject after oral administration. After a single dose (150 mg) of fluconazol, C_{max} was calculated to be 3.0 μg/ml with T_{max} of 4 hr. This results are comparable to the values determined by other workers[2]: When they gave orally 50 mg fluconazol (capsule) to man, the peak plasma concentration (C_{max}) was 1,2 μg/ml (T_{max}~4 hr.)

This rapid and simplified HPLC assay could be applied to determining the fluconazol concentration in human plasma for the pharmacokinetic studies as well as for the drug monitoring.

LITERATURE CITED

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