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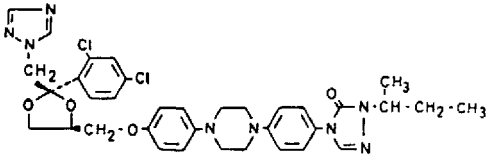
Letter to the Editor

Determination of itraconazole in serum with high-performance liquid chromatography and fluorescence detection

Sir,

Itraconazole is a lipophilic, weakly basic, triazole antifungal agent, which is finding increasing use as an oral drug against systemic mycotic infections [1,2]. We have developed a simple method for therapeutic monitoring of itraconazole in serum. Itraconazole and the internal standard R51012 (Fig. 1) are isolated using solid-phase extraction and separated on a reversed-phase high-performance liquid chromatographic (HPLC) column; the native fluorescence is detected. The use of HPLC with UV detection for the determination of itraconazole in serum has been previously reported [3–5]. In our method the fluorescence detection is specific, and the sensitivity makes an evaporation step unnecessary.



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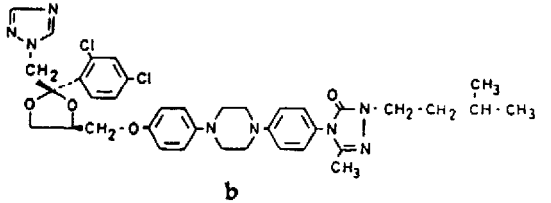

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Fig. 1 Structures of (a) itraconazole and (b) the internal standard

EXPERIMENTAL

The chromatographic system consisted of an LKB 2150 HPLC pump (Pharmacia LKB Biotechnology, Uppsala, Sweden), a CMA200 refrigerated microsampler (Carnegie Medicin, Stockholm, Sweden), an RF-535 fluorescence detec-

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tor and a C-R6A integrator (Shimadzu, Kyoto, Japan). The separations were performed on a 100 mm × 4.6 mm I.D. Brownlee Labs. MPLC RP-18 Spheri-5 column cartridge (Applied Biosystems, Santa Clara, CA, U.S.A.). The mobile phase was 60% (v/v) acetonitrile in water with triethylamine (TEA) added to a final concentration of 20 m*M*. The pH was adjusted to 2.3 with phosphoric acid.

Reagents

Working standard solutions of itraconazole in serum were prepared with concentrations ranging from 10 to 250 ng/ml. A working solution of the internal standard R51012 (Janssen Life Sciences, Beerse, Belgium) was prepared in methanol (1 μg/ml).

Procedure

To 1 ml of standard solution or sample were added 100 μl of working internal standard solution with mixing. A solid-phase extraction column, Bond Elut® No. 607101 (Analytichem International, Harbor City, CA, U.S.A.) was activated with 1 ml of methanol and rinsed with 1 ml of water. The serum sample was applied to the column and drawn through the bed under vacuum. The column was consecutively rinsed with 1–2 ml of water and 1–2 ml of 50% (v/v) methanol in water, and eluted with 0.4 ml of methanol with 0.3% (v/v) TEA and concentrated orthophosphoric acid added. Part of the effluent (20 μl) was injected into the chromatographic system. The flow-rate was 1 ml/min. The detector excitation wavelength was set to 260 nm and the emission wavelength to to 365 nm.

RESULTS AND DISCUSSION

A typical chromatogram from a patient receiving itraconazole per os is shown in Fig. 2a. No interference was encountered when serum samples from healthy subjects were taken through the procedure (Fig. 2b).

We tested the selectivity by taking amphotericin B, cefaclor, imipenem, flucytocin, gentamycin, ketoconazole, netilmicin, salicylic acid, sulphamethoxazole, tienamycin, tobramycin and trimethoprim through the procedure. None was detected with the present method.

The concentration of itraconazole to the peak-area ratio of itraconazole and the internal standard was tested for linearity up to 250 ng/ml ($r = 0.999$), which is adequate for therapeutic monitoring. The absolute recovery ranged between 95 and 100%. The within-run and between-run coefficients of variation (C.V.) were 2.10 and 2.08%, respectively, at a mean concentration of 55.3 ng/ml ($n = 20$). The limit of quantitation was 4 ng/ml.

Both the wavelength of the emission maximum and the fluorescence intensity were found to vary with pH. A shift of the emission maximum from 390 to 365 nm takes place when the pH is changed from 5 to 3 (Fig. 3a). The shift in wavelength is accompanied by an intensity increase, which is four-fold when the

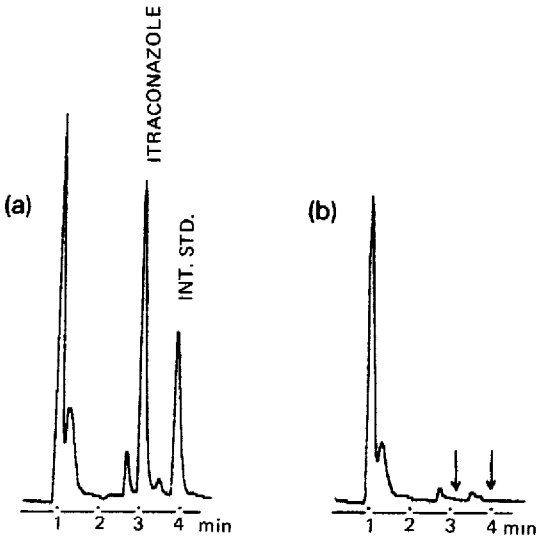

Fig. 2. Chromatograms of (a) itraconazole (retention time = 3 1 min) and the internal standard (retention time = 4.0 min) in serum from a patient receiving itraconazole per os and (b) a blank serum. The serum concentration was 107 ng/ml.

pH is changed from 7 to 2.3 (Fig. 3b). The effect is most likely caused by protonation of the N-aryl-substituted triazole ring system responsible for the fluorescence of the molecule. Hence, although it is essential to control the pH of the mobile phase, variations will not change the peak-area ratio of itraconazole and the internal standard.

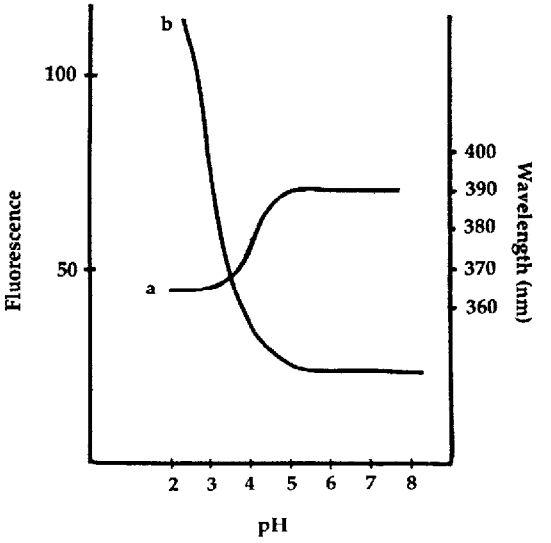

Fig 3 Influence of pH on (a) the emission maximum wavelength of itraconazole and (b) the fluorescence signal at emission maximum wavelength. Excitation at 260 nm

A previously reported method [3] based on UV detection is said to give a lower limit of quantitation (1 ng/ml; C.V. = 14%). However, that method requires 2 ml of serum, which is concentrated twenty times before analysis, and also a larger injection volume.

We conclude that the proposed method is rapid, specific and sensitive enough for use in therapeutic monitoring of itraconazole in serum.

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