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Note

Determination of metronidazole and tinidazole in human plasma using high-performance liquid chromatography

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Metronidazole (1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole) and tinidazole (1-(2-(ethylsulphonyl)ethyl)-2-methyl-5-nitroimidazole) are two chemically related drugs used in the treatment of trichomoniasis. Several procedures have been described for the detection and quantitation of metronidazole in serum. These methods are based on polarography[1], microbiological assay[2], gas chromatography[3,4], and recently, high-performance liquid chromatography (HPLC)[5,6]. The method described in this paper uses a simple protein precipitation technique from 100 μl of plasma and the estimation of the drugs by HPLC. It is suitable for the analysis of metronidazole and tinidazole in plasma, down to levels of less than 0.5 and 2.0 μg/ml respectively.

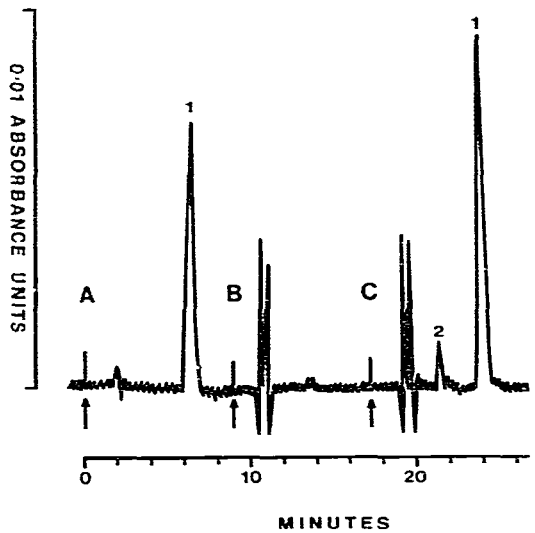
MATERIALS AND METHODS

Apparatus

A Waters Assoc. (Milford, Mass., U.S.A.) high-performance liquid chromatograph equipped with a Waters 450 variable-wavelength detector operated at 320 nm was used throughout the determination. The column (30 cm × 4 mm) was packed with μBondapak C_{18} (Waters Assoç.). Samples were introduced by means of a variable-loop injector (Waters Model U6K). The eluent was 7% acetonitrile in 20 m*M* acetate buffer adjusted to pH 4.0 with acetic acid, used at a flow-rate of 1.5 ml/min for metronidazole and 2.0 ml/min for tinidazole. Under these conditions the elution times of metronidazole and tinidazole were 6.4 and 9.2 min respectively (Figs. 1 and 2).

Extraction procedure

To 100 μl of plasma in a pointed tube 1.0 ml acetonitrile was added carefully down the side of the tube. This ensured that two layers were produced, as the rapid addition of acetonitrile can cause a lumpy precipitate which may lead to decreased recoveries. The tube was stoppered and shaken vigorously by hand for 1 min. The mixture was centrifuged and 1.0 ml of the supernatant was transferred to a second tube and dried at 50° under an air stream. The residue was redissolved in 100 μl



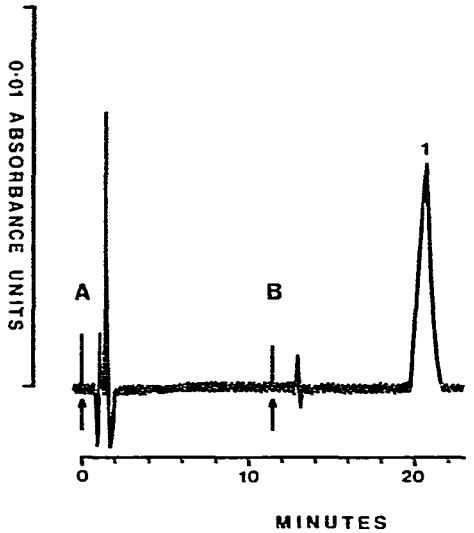


Fig. 1. Analyses of metronidazole by HPLC. (A): Chromatogram of 80 ng metronidazole; (B): chromatogram of an extract from a blank serum; (C): chromatogram of a patient receiving metronidazole (6.4 μg/ml). Peaks: 1 = metronidazole; 2 = unknown, possible the hydroxy metabolite. The arrows mark the times of injection and the vertical bar indicates 0.01 a.u.

Fig. 2. Analyses of tinidazole by HPLC. (A): Chromatogram of an extract from a blank serum; (B): chromatogram of 300 ng tinidazole. Peak: 1 = tinidazole. The arrows mark the times of injection and the vertical bar indicates 0.01 a.u.

of the elution solvent and 10–20 μl injected onto the HPLC. The peak height obtained was compared with those of a series of standards.

RESULTS AND DISCUSSION

Recovery studies

Amounts of metronidazole were added to blank plasma to give a concentration in the range 0.5–40 μg/ml. Each sample was then examined by the stated procedure. The mean recovery of ten spiked samples was 98 ± 3%. Similarly, amounts of tinidazole were added to blank plasma to give a concentration range of 5–80 μg/ml. The mean recovery of ten spiked samples was 102 ± 4%.

Discussion

Since the recoveries obtained were consistent and almost theoretical, an internal standard was not incorporated in the assay.

In the plasma samples which were examined, the chromatograms were free from interfering peaks. It is known that using the described method, paracetamol and theophylline will also be extracted; both have a retention time similar to that of metronidazole. These two compounds, however, show no significant absorption at the wavelength used (320 nm) and therefore cause no interference. In the estimation of metronidazole in patients a peak has been found which elutes prior to that of metronidazole and which may be due to the hydroxy metabolite[5,6].

The method described has been found to be rapid and reproducible, and allows metronidazole and tinidazole to be estimated down to levels of less than 0.5 and 2.0 μg/ml respectively.

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