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Note

Sensitive gas chromatographic assay of tinidazole in tissue and plasma

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Tinidazole (1-[2-(ethylsulfonyl)-ethyl]-2-methyl-5-nitroimidazole) is a synthetic antimicrobial agent used in the therapy of trichomoniasis in man. For the in vivo assay of tinidazole, methods have been described based on microbiological [1] and polarographic [2] measurements as well as thin-layer chromatography (TLC) with fluorescence detection [3] and high-performance liquid chromatography (HPLC) with spectrophotometric detection [4]. The sensitivity of these methods is about 0.2—0.5 μg/ml, which has proven to be satisfactory for the determination of therapeutic plasma levels of tinidazole in animal and in man [1—4].

Pharmacokinetic studies on the absorption and distribution of tinidazole at the sites of potential action, e.g. muscle and organ tissue, however, require a more sensitive method. This paper describes a sensitive and selective gas chromatographic (GC) assay which allows the measurement of concentrations of tinidazole in tissue to a lower limit of 50 ng/g. The method is also suitable for determination of tinidazole plasma concentrations.

EXPERIMENTAL

Standards and reagents

Tinidazole was supplied by Pfizer (Karlsruhe, G.F.R.), and 4-nitrodiphenylamine by Aldrich (Milwaukee, Wisc., U.S.A.). Dichloromethane, methanol, acetone, sodium sulphate and sodium hydroxide were obtained from Merck (Darmstadt, G.F.R.). All reagents were of analytical grade.

Extraction procedure

To 0.5 g of tissue homogenate in a 20-ml round-bottom centrifuge tube 50 μl of 6 N sodium hydroxide solution and 10 ml of a dichloromethane—metha-

nol (98:2) mixture were added. The organic solvent had been previously spiked with 10 μl of a solution of 4-nitrodiphenylamine in dichloromethane corresponding to 1 μg internal standard. The tube was shaken vigorously for 7 min and centrifuged at 2000 g for 5 min. The organic layer was transferred to a second centrifuge tube containing 500 mg powdered sodium sulphate, the mixture was then shaken for 5 min. After centrifugation, the organic phase was decanted into a tapered tube and reduced to approx. 1 ml under a stream of purified nitrogen. The cooled solution was kept at 4° for approx. 10 min. The liquid phase was then separated from the precipitates by pipetting it into a small reaction vessel and evaporating to dryness. The residue, dissolved in 50 μl acetone was shaken on a vibrator for 5 sec.

For the extraction of tinidazole from plasma the procedure was slightly modified: The internal standard was added directly to the plasma and the step of cooling the extracts was omitted, because no precipitate was formed.

Gas chromatographic assay

The gas chromatograph used was a Becker Packard Model 419 equipped with a PN detector (Perking Elmer). Integration of the chromatographic signals was performed by a digital integrator (Spectra Physics Model SP 4000).

Using a 5-μl Hamilton microsyringe, 0.2—1 μl of the acetone solution was injected under GC conditions: analytical glass column (1 m × 2 mm I.D.) packed with 3% OV-11 on Gas-Chrom Q (80—100 mesh) (WGA, Düsseldorf, G.F.R.); carrier gas, nitrogen at a flow-rate of 20 ml/min; air and hydrogen flow-rates, 200 ml/min and 1.5 ml/min, respectively; column temperature, 215°; injection port and detector temperature 245°.

RESULTS AND DISCUSSION

Under the conditions described, integrable peaks of tinidazole (retention time 225 sec) and 4-nitrodiphenylamine (retention time 305 sec) were obtained. Chromatograms from tinidazole assays in tissue and plasma are shown in Fig. 1. Calibration curves were plotted after adding suitable amounts of tinidazole to human plasma or rat tissues. The linearity of the method was established for ranges of 0.1—50 μg tinidazole per ml plasma and 0.05—0.5 μg tinidazole per g tissue.

The repoducibility of six consecutive analyses of plasma to which 0.5 μg/ml tinidazole had been added, was ± 4.1% (coefficient of variation), corresponding to an analytical value of 0.5 ± 0.021 μg/ml. The lower limit of quantitation was 50 ng tinidazole per g tissue homogenate. Although tinidazole in plasma could be detected to a lower limit of 20 ng/ml, there was no need to confirm the feasibility of this method for quantification of plasma in the lower nanogram range, because therapeutic tinidazole concentrations in plasma are in a far higher range. The yield of the assay was determined at concentrations of 500 ng/ml plasma by successive injections of plasma extract containing tinidazole and the respective standard solution. For tinidazole, a yield of 76.5 ± 3.2% was obtained. The yield of the internal standard was 53.9 ± 3.1%. These yields cover the entire analytical procedure up to the injection of the sample into the gas chromatograph.

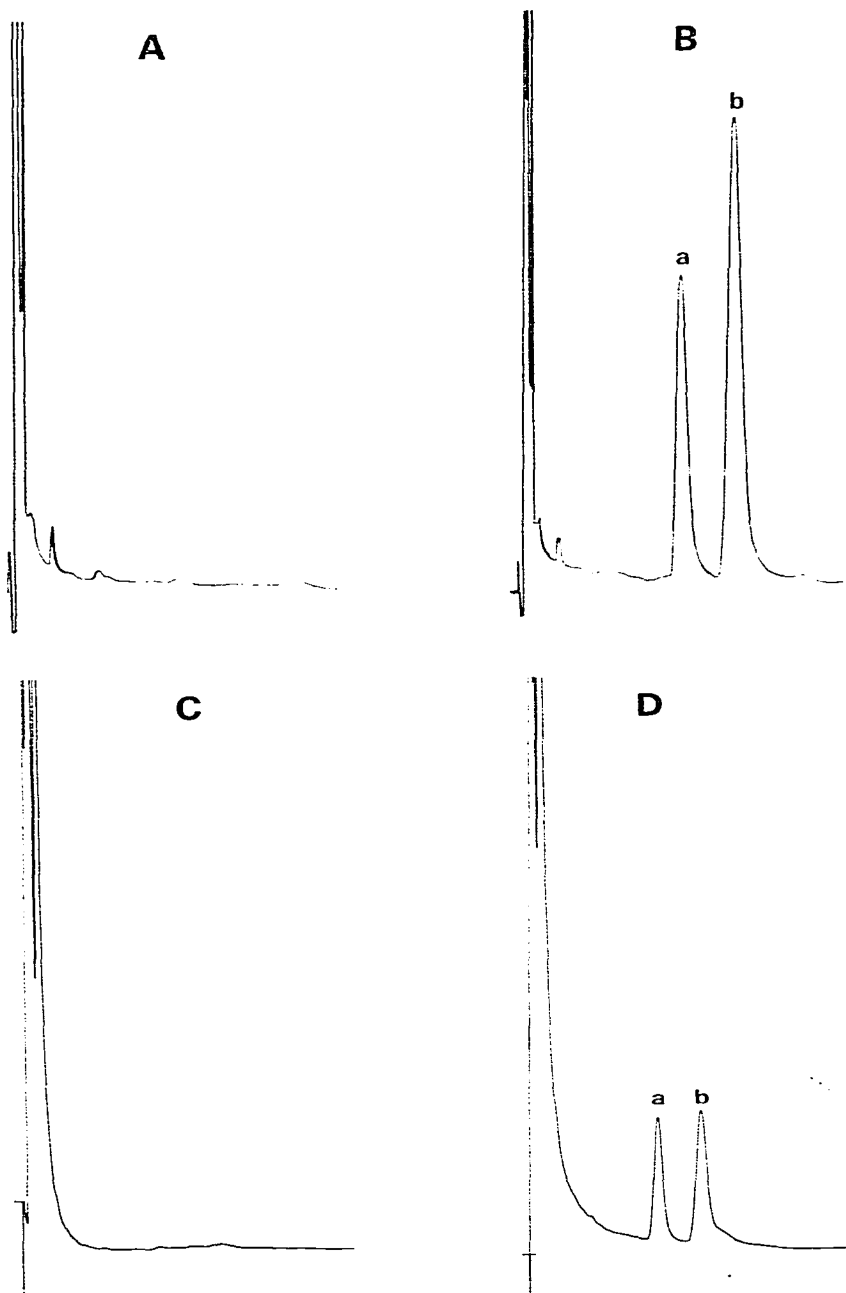

Fig. 1. Chromatograms of plasma and tissue extracts. (A) Blank plasma; (B) plasma spiked with tinidazole (a) and 4-nitrodiphenylamine (b); (C) intestinal tissue blank; (D) intestinal tissue spiked with tinidazole (a) and 4-nitrodiphenylamine (b).

This method has been applied to the quantitative analysis of tinidazole in tissue and plasma of humans and rats. The application of the same method of assay to several biological materials with a widespread range of tinidazole concentrations enhances the use of this analytical procedure.

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