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Note

Determination of griseofulvin in human serum using high-performance liquid chromatography

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Griseofulvin [7-chloro-2′,4,6-trimethoxy-6′-β-methylspiro-benzofuran-2(3H)-1′-(2)-cyclohexene)-3,4′-dione] is an antifungal antibiotic produced by *Penicillium* griseofulvin and other species of *Penicillia*. It is used for the systemic treatment of infections caused by fungi of the dermatophyte group. Several methods have been described for its detection and quantitation in serum, and fermentation media. These methods are based on microbiologic[1], spectrophotometric[2], spectrofluorimetric[3,4] or gas–liquid chromatographic[5–7] procedures. The method described in this paper uses a single extraction from 1 ml of plasma and the estimation of the drug by high-performance liquid chromatography (HPLC). It is suitable for the analysis of the drug in serum down to levels of less than 0.2 μg/ml.

MATERIALS AND METHODS

Apparatus

A Waters Assoc. (Milford, Mass., U.S.A.) high-performance liquid chromatograph equipped with a Waters 450 variable-wavelength UV detector operated at 295 nm was used throughout the determination. The column (30 cm × 4 mm) was packed with μBondapak C_{18} (Waters Assoc.). Samples were introduced by means of a variable-loop injector (Waters Model U6K). The eluant was 45% acetonitrile in 45 m*M* KH_2PO_4 adjusted to pH 3.0 with phosphoric acid used at a flow-rate of 2.5 ml/min. Under these conditions the elution time of griseofulvin and diazepam were 4.8 and 6.3 min, respectively (Fig. 1).

Extraction procedure

To 1.0 ml of serum in a tube was added 20.0 ml dichloromethane and 5 μg of diazepam as internal standard. The mixture was shaken well and centrifuged. The aqueous supernatant was aspirated off and 10.0 ml of the organic phase transferred to a second tube and taken to dryness at 50° under a stream of nitrogen. The residue was dissolved in 100 μl of the solvent used for elution and 10–20 μl injected on the high-performance liquid chromatograph.

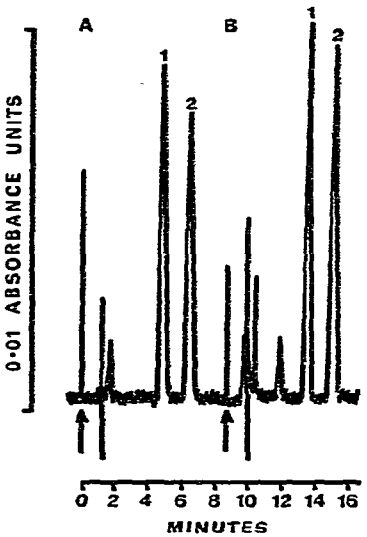

Fig. 1. HPLC analyses. (A) Chromatogram of a standard mixture of griseofulvin and diazepam (amounts injected: griseofulvin 125 ng, diazepam 500 ng); (B) chromatogram of an extract of plasma from a patient (contains 1.15 μg/ml griseofulvin). Peaks: 1 = griseofulvin; 2 = diazepam. The arrows mark the time of sample injection and the vertical bar indicates 0.01 a.u.

RESULTS AND DISCUSSION

Quantitation

To known amounts of griseofulvin in pointed glass tubes was added 5 μg of diazepam and 100 μl of the elution solvent. Each was then examined by HPLC using the stated conditions.

Over a range of 0.2 to 5.0 μg of the drug, the ratio of peak height of griseofulvin to that of internal standard was linear.

Recovery studies

Amounts of griseofulvin ranging from 0.2 to 6.0 μg were added to 1 ml of blank plasma in order to examine the efficiency of the extraction procedure. The mean recovery of fourteen spiked samples (0.2–6 μg/ml) was 94 ± 4%.

Discussion

In all the plasma samples examined, the chromatograms have been free from interfering peaks. It was also determined that none of the other benzodiazepines, tricyclics, acetaminophen, antiepileptics or antiarrythmic drugs have retention times similar to griseofulvin or diazepam. Patients receiving long term diazepam therapy have an average drug concentration of 0.2 μg/ml (ref. 8). If it is known that the patient uses this drug, the amount of internal standard added could be doubled, which would decrease its interference to a level of 2%. The method has been found to be rapid and reproducible, and allows the drug to be estimated down to a level of less than 0.2 μg/ml.

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