The analyses of the penicillins in 5% dextrose solutions are summarized in Table II.

In most cases, the apparent molar absorptivities of the ferric–hydroxamate complexes were slightly lower in the 5% dextrose samples. Replicate determinations of 1-mg of methicillin sodium/ml samples gave good precision and accuracy.

Only ampicillin in 5% dextrose showed any appreciable change in concentration over 48 hr at room temperature (Fig. 1). Samples were obtained at zero time and at 48 hr and were analyzed by TLC. A large spot at R_f 0.65, indicative of ampicillin degradation, appeared in the 48-hr sample.

Although cephalothin was previously determined by this method (16), a study was undertaken to determine if the method is applicable to cephalothin analysis in dextrose solutions. Replicate analyses of a solution containing cephalothin sodium (1.00 mg/ml) gave an average value of 1.04 mg/ml with a coefficient of variation of 2.7%. In this study, the absorbance was measured at 490 nm and the complex was stable for at least 15 min.

Oxacillin was analyzed in several other intravenous fluids with good precision and accuracy (Table III).

The nickel-catalyzed method also was applied to a chloramphenicol succinate sodium solution. In this case, it was necessary to heat the reaction for 2 hr at 80° to obtain an adequate color yield. Under these conditions, as little as 25 μg/ml could be detected. A typical calibration curve can be described by:

$$\text{absorbance} = 0.00273 \text{ concentration } (\mu g/ml) + 0.00 \quad \text{(Eq. 2)}$$

In the 25–250-μg/ml range, correlation coefficients for this equation were typically 0.999.

Considering the extensive use of β-lactam antibiotics in intravenous admixtures, the nickel(II)-catalyzed hydroxylaminolysis method offers a rapid and reliable means of assessing antibiotic stability in numerous intravenous fluids.

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COMMUNICATIONS

Identification of Griseofulvic Acid as a Urine Metabolite of Griseofulvin in Humans

Keyphrases □ Griseofulvin—human urinary metabolite, identification, griseofulvic acid □ Antifungal agents—griseofulvin, human urinary metabolite, identification, griseofulvic acid □ Metabolite—of griseofulvin, human urine, identification, griseofulvic acid □ Griseofulvic acid—metabolite of griseofulvin, human urine, identification

To the Editor:

Griseofulvin was introduced in 1959 as an effective oral antifungal agent for humans and animals. Many studies of griseofulvin metabolism have been reported since then. Investigators of griseofulvin metabolism in humans reported that almost all of a daily griseofulvin dose was excreted as 6-desmethylgriseofulvin in the urine in 24 hr (1). Studies using ^{14}C-griseofulvin and GLC showed that the major metabolite was 6-desmethylgriseofulvin but that the drug also was excreted partially as 4-desmethylgriseofulvin and as several other unidentified metabolites (2). Although these unidentified compounds represent only a small portion of the total drug, their identities are important in understanding griseofulvin metabolism.

A simple and rapid determination of griseofulvin and its derivatives using reversed-phase high-pressure liquid chromatography was developed in this laboratory. Analysis of human urine samples by this method revealed that, in addition to 6-desmethylgriseofulvin, griseofulvic acid (7-chloro-4,6-dimethoxy-6′-methylgrisan-3,2′,4′-trione) also is a urinary griseofulvin metabolite. However, the reported 4-desmethylgriseofulvin was not observed in this study. The urinary concentration ratio of griseofulvic acid to 6-desmethylgriseofulvin was ~1:15.

Figure 1 shows chromatograms of urine samples obtained from a normal subject 6 hr after a 250-mg oral griseofulvin dose. The griseofulvin metabolites in the urine sample were extracted into cyclohexane–ethylene dichloride (1:1) (3), and the mixture then was evaporated to dryness and reconstituted with the chromatographic mobile phase solvent. Figure 1A represents the direct extraction of the urine sample. Figure 1B represents the same sample after incubation overnight at 37° with β-glucuronidase to hydrolyze the glucuronide conjugate prior to the extraction.

The chromatograms were obtained with a reversed-phase column[1] at a 1.0-ml/min flow rate. The mobile phase was 45% acetonitrile in water (v/v), with the pH adjusted to 3.4 using acetic acid. The upper chromatograms represent the response of a fluorescence detector excited at 280 nm. The lower chromatograms represent the response of a UV detector at 290 nm. The advantage of using both

[1] μBondapak C_{18}, Waters Associates, Milford, Mass.

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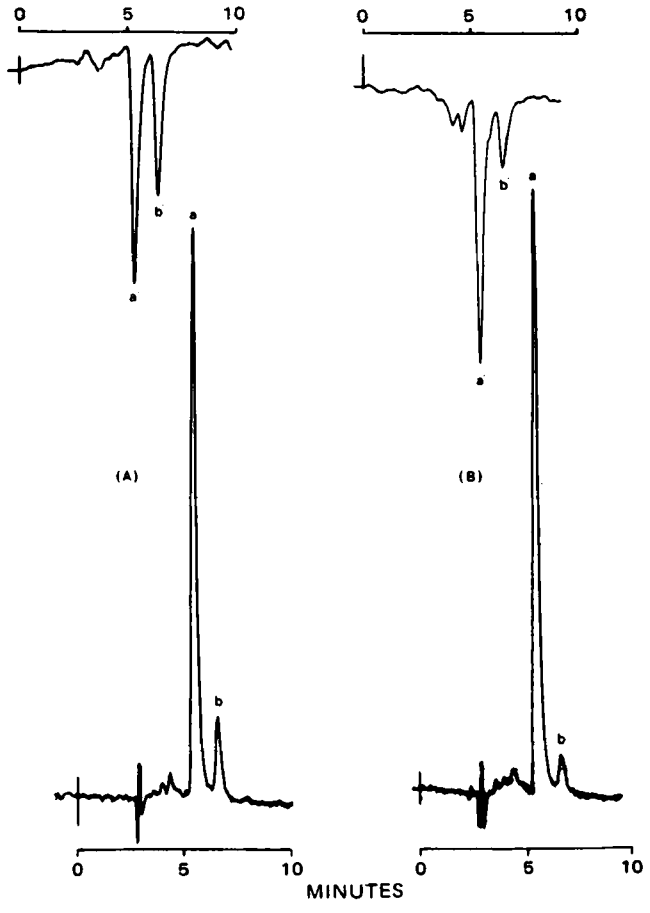

Figure 1—*Chromatograms of human urinary griseofulvin metabolites. Key: A, untreated sample; and B, the same sample after incubation overnight with β-glucuronidase at 37°. Peak response in A represents the original urine concentration; in B, the urine concentration was diluted to one-half. The top and bottom chromatograms were obtained with fluorescence and UV detection, respectively.*

detectors is that the purity of a separated component in a chromatogram may be evaluated with the peak response ratio of the two detections; if the peak response ratio of a component is different from that of the standard, then either the peak response represents a different species or some impurities may have eluted with the same retention time.

Peaks a and b, which were not observed in the blank urine sample, had retention times identical to those of standards of 6-desmethylgriseofulvin and griseofulvic acid, respectively. Furthermore, the UV–fluorescence peak response ratios of peaks a and b also were the same with respect to their standards. It is concluded that peak a represents 6-desmethylgriseofulvin and that peak b represents griseofulvic acid. The presence of griseofulvic acid in urine samples was evidenced further in TLC studies. With a silica gel plate[2] and chloroform–methanol (10:1 v/v), two urinary metabolites were observed and identified by comparison with 6-desmethylgriseofulvin (R_f 0.18) and griseofulvic acid (R_f 0.05) standards. 4-Desmethylgriseofulvin (R_f 0.36) was not found in the urine samples.

The 6-desmethylgriseofulvin standard was isolated from dog urine according to a literature method (3). The purified product was positively identified as 6-desmethylgriseofulvin by a melting-point measurement (4) and by its mass spectrum, which was identical with that reported previously (5). The griseofulvic acid standard was synthesized by reacting griseofulvin with boron tribromide at −60°. The purified product showed a melting point and a mass spectrum identical to those reported for griseofulvic acid (4, 5). The 4-desmethylgriseofulvin standard was prepared according to a literature method (4), and it also was identified by its melting point and its mass spectrum.

The griseofulvic acid formation is speculated to occur *via* microsomal demethylation at the 2′-position and subsequent tautomerization of the 2′-enol to the 2′,4′-dione. The pharmacokinetics of this metabolite and its toxicological effect are under investigation.

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Hossein Zia
School of Pharmacy
University of Isfahan
Isfahan, Iran
John P. O'Donnell
Joseph K. H. Ma ˣ
School of Pharmacy
West Virginia University
Morgantown, WV 26506

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Effect of Instrumental Vibration Levels on Dissolution

Keyphrases □ Dissolution tests—effects of instrumental vibration, tablets □ Vibration—effect on dissolution tests, tablets

To the Editor:

In the process of investigating the dissolution of two commercial enteric-coated aspirin tablets, large variations in dissolution profiles were observed between two dissolution apparatus (1). The dissolution profiles were followed in simulated intestinal juice for 10 hr using the dissolution procedure described in USP XIX (2). The apparatus[1], designated left (L) and right (R), were operated at a stirring speed of 50 ± 1.5 rpm throughout the study. The tablets in the L apparatus dissolved more slowly, leaving partial tablet residues after 10 hr of dissolution. Additionally, the dissolution profiles were low, indicating poor dissolution characteristics. However, in the R apparatus, tablets from the same batch dissolved completely in the same time period, and dissolution profiles were substan-

[2] Silica gel 60F, 0.05 mm thick, E. Merck, Darmstadt, West Germany.

[1] Hanson Research Corp., Northridge, Calif.

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