Quantitative GLC Determination of *cis*- and *trans*-Isomers of Doxepin and Desmethyldoxepin

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Abstract □ A GLC method for the simultaneous quantitative determination of the *cis*- and *trans*-isomers of doxepin and desmethyldoxepin in human plasma was developed. The method involves the use of a capillary column for efficient separation of the four compounds and the internal standards, amitriptyline and nortriptyline. A high sensitivity is obtained with a nitrogen detector, enabling quantitation of the compounds in plasma of humans treated chronically with doxepin. Confirmation of the identity of the *cis*- and *trans*-isomers of doxepin and desmethyldoxepin in biological samples was carried out by selected ion monitoring.

Keyphrases □ Doxepin, *cis* and *trans*—simultaneous GLC analyses in human plasma □ Desmethyldoxepin, *cis* and *trans*—simultaneous GLC analyses in human plasma □ GLC—simultaneous analyses, *cis*- and *trans*-doxepin and desmethyldoxepin in human plasma □ Antidepressants—*cis*- and *trans*-doxepin, simultaneous GLC analyses in human plasma

Doxepin, 11-(3-dimethylaminopropylidene)-6*H*-dibenz[*b*,*e*]oxepin, is structurally related to imipramine and amitriptyline. A 15:85% mixture of the *cis*- and *trans*-isomers of doxepin is used as an antidepressant agent.

The determination of the plasma levels of a drug such as doxepin would help optimize therapy, but only limited data are available (1). The problem is complicated by the fact that the *N*-demethylated metabolite, desmethyldoxepin, has pharmacological activity. Furthermore, for the metabolite and the unchanged product, *in vivo* conversion of the *cis*-isomer into the less active *trans*-isomer has been suggested (1).

Several GLC methods (2–4) and a GLC selected ion monitoring method (5) for simultaneous measurement of doxepin and desmethyldoxepin were published, but none permits differentiation between the isomeric forms for both products.

The present paper describes a method for the separate determination of nanogram amounts of the *cis*- and *trans*-isomers of both doxepin and desmethyldoxepin in plasma, using capillary GLC with nitrogen detection; in some instances, a packed column was used. The identity of the peaks on the chromatograms was confirmed by chemical-ionization mass spectrometry.

EXPERIMENTAL

Materials—Doxepin hydrochloride (specified as 83.2% *trans* and 16.8% *cis*) and desmethyldoxepin hydrochloride (no specifications) were available as powders[1]. By using capillary GLC and assuming equal detector responses for the *cis*- and *trans*-isomers, values of 82% *trans* and 18% *cis* were found for doxepin and values of 96.7% *trans* and 3.3% *cis* were calculated for desmethyldoxepin; these ratios were used for quantitation. The internal standards, amitriptyline[2] and nortriptyline[3], were purchased as 1% solutions. The derivatizing reagent was trifluoroacetic anhydride[4]. All other solvents and reagents were analytical grade.

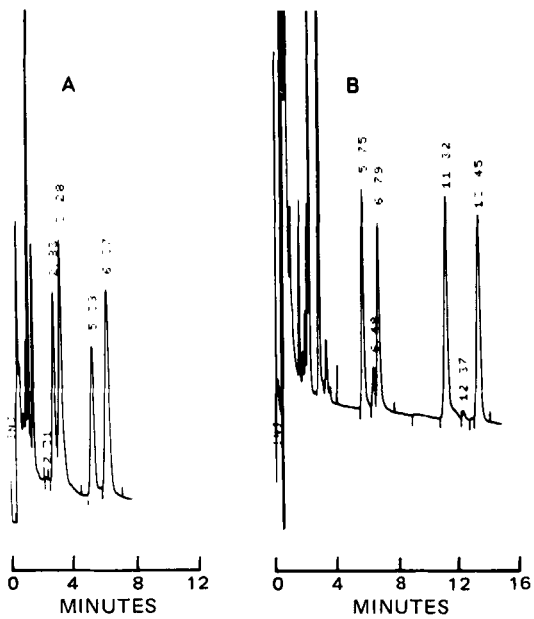

Figure 1—*Gas chromatograms of 1 ml of extracted human plasma. Retention time, R_t, data and the amounts of the drugs added to 1 ml of plasma are given. A (packed column): R_t 2.83, amitriptyline (100 ng); R_t 3.28, cis-doxepin (27 ng) and trans-doxepin (123 ng); R_t 5.38, N-trifluoroacetylnortriptyline (100 ng); and R_t 6.37, trans-(N-trifluoroacetyl)desmethyldoxepin (145 ng). B (capillary column): R_t 5.75, amitriptyline (100 ng); R_t 6.48, cis-doxepin (18 ng); R_t 6.79, trans-doxepin (82 ng); R_t 11.32, N-trifluoroacetylnortriptyline (100 ng); R_t 12.37, cis-(N-trifluoroacetyl)desmethyldoxepin (3.3 ng); and R_t 13.45, trans-(N-trifluoroacetyl)desmethyldoxepin (96.7 ng).*

Glassware—All glassware was silanized by soaking for 1 hr in a 5% solution of trimethylchlorosilane in benzene, rinsed with benzene and methanol, dried for 1 hr at 100°, and rinsed with ethyl acetate immediately before use.

Instrumental Conditions—A gas chromatograph[5], equipped with a dual nitrogen–phosphorus flame-ionization detector, was used. GLC was performed on a 20-m × 0.5-mm i.d. glass capillary column[6], coated with OV-17, with a hydrogen flow of 4 ml/min or on a 1.80-m × 2-mm i.d. glass column containing 3% OV-17[7] on 80–100-mesh Gas Chrom Q[8] with a helium flow of 30 ml/min. Temperatures were: injector, 300°; column, 230°; and detector, 300°. For capillary GLC, direct on-column injection of 0.2-μl samples without stream splitting was used. Detector conditions were set on the highest response. The peak areas were recorded on a recording integrator[9].

A chemical-ionization gas chromatograph–mass spectrometer[10], equipped with an all-glass chromatographic inlet system and interfaced

[1] Pfizer.
[2] Tryptizol, Merck Sharp & Dohme.
[3] Nortrilen, Lundbeck.
[4] Pierce Chemical Co.
[5] Hewlett-Packard 5730 A series.
[6] RSL (Research Specialties for Laboratories), Belgium.
[7] Applied Science Laboratories.
[8] Supelco.
[9] Hewlett-Packard 3380 A.
[10] Finnigan 3200.

Table I—Abundances for Ions in the Methane Chemical-Ionization Spectra of Doxepin, Amitriptyline, and the *N*-Trifluoroacetyl Derivatives of Desmethyldoxepin and Nortriptyline [a]

Compound	*m/e* (Relative Intensities)
cis-Doxepin	235 (6.9), 278 (30.4), 279 (5.2), 280 (100), 281 (28.4), 282 (2.8), 308 (16.1), 309 (6.3), 320 (2.9)
trans-Doxepin	235 (2.0), 278 (13.3), 279 (3.1), 280 (100), 281 (21.9), 308 (13.6), 309 (3.7), 320 (2.5)
Amitriptyline	91 (5.4), 233 (3.2), 276 (47.4), 277 (13.1), 278 (100), 279 (22.0), 280 (3.3), 306 (18.2), 307 (4.3), 318 (2.5)
cis-(*N*-Trifluoroacetyl)-desmethyldoxepin	107 (12.3), 135 (2.3), 140 (4.3), 168 (10.5), 195 (2.3), 234 (9.6), 235 (100), 236 (11.7), 263 (15.5), 264 (2.0), 268 (12.6), 269 (2.0), 275 (4.5), 342 (5.4), 361 (2.4), 362 (68.2), 363 (12.9), 390 (9.6), 402 (2.8)
trans-(*N*-Trifluoroacetyl)-desmethyldoxepin	91 (2.1), 107 (18.2), 128 (5.2), 135 (2.9), 140 (6.5), 154 (2.4), 168 (31.9), 195 (3.0), 213 (3.0), 221 (3.8), 234 (17.6), 235 (100), 236 (12.7), 237 (2.5), 263 (13.0), 264 (3.0), 268 (15.2), 269 (2.9), 275 (2.7), 342 (7.9), 360 (2.7), 361 (5.2), 362 (78.1), 363 (16.2), 390 (13.6), 391 (2.2), 402 (5.2)
N-Trifluoroacetyl-nortriptyline	130 (2.0), 211 (9.2), 212 (2.0), 231 (4.1), 232 (20.1), 233 (47.8), 234 (8.8), 261 (10.5), 262 (2.0), 273 (2.0), 359 (7.2), 360 (100), 361 (23.5), 362 (2.6), 388 (14.2), 389 (3.7), 400 (3.9), 401 (2.0)

[a] Due to the high abundance of reagent gas ions in the low mass region, the abundances, expressed as relative intensities, are presented only for ions above *m/e* 70; relative intensity values are only given for ions with a relative intensity ≥2%.

with a data system[11], was used[12]. GLC was carried out on a 50-m × 0.5-mm i.d. glass capillary column, coated with SE-30, with a helium flow of 10 ml/min; for the working conditions used, this flow gave the best results. Temperatures were: injector, 250°; column, 235°; and interface, 250°. The reagent gas, methane, was added as makeup gas until the source pressure reached 1 torr. The mass spectrometric conditions were: electron energy, 150 ev; emission current, 0.5 mamp; continuous dynode electron multiplier voltage, 2.2 kv; and preamp range, 10^{-8} amp/v for analysis of plasma samples and 10^{-7} amp/v for recording of reference spectra.

Extraction and Derivatization—A 100-μl aliquot of an internal standard solution containing 100 ng of amitriptyline and 100 ng of nortriptyline, 50 μl of 0.0005 *M* triethylamine, and 0.1 ml of 5 *N* NaOH were added to 1 ml of plasma in a 10-ml glass-stoppered centrifuge tube. The mixture was extracted with 4 ml of *n*-hexane by shaking for 20 min horizontally. After centrifugation for 10 min at 6000 rpm, the organic phase was transferred with a Pasteur pipet to a 6-ml glass-stoppered conical tube, and the organic phase was removed under a gentle stream of nitrogen at room temperature until about 100 μl was left.

Another 4 ml of *n*-hexane was added to the aqueous phase, which was shaken and centrifuged as before. This 4 ml was then transferred to the same conical tube, and the solvent was removed under nitrogen. Before evaporation was complete, the conical tube wall was washed down with 300 μl of *n*-hexane, and the hexane was evaporated to about 200 μl; 40 μl of trifluoroacetic anhydride was added.

After the tube was stoppered, the mixture was left to react for 10 min at 50°. The sample was taken to dryness under nitrogen. The residue was stored at −18° and used within 6 hr. For GLC analysis, the residues were dissolved in 10 μl of ethyl acetate; aliquots of 0.2 μl were injected on the capillary column, whereas aliquots of 2 μl were used on the packed column.

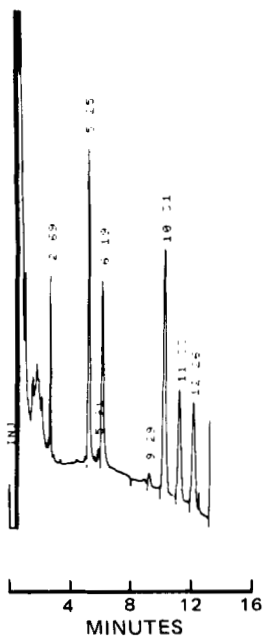

Figure 2—*Gas chromatogram of extracted human plasma. Aliquots of 100 ng of amitriptyline (R_t 5.25) and 100 ng of nortriptyline (R_t 10.31) were added to 1 ml of plasma of Subject WM receiving a daily dose of 200 mg of doxepin. Key:* R_t *5.91,* cis-*doxepin;* R_t *6.19,* trans-*doxepin;* R_t *11.33,* cis-*(N - trifluoroacetyl)desmethyldoxepin; and* R_t *12.26,* trans-*(N-trifluoroacetyl)desmethyldoxepin.*

Quantitation—Standard solutions (0.01%) of doxepin and desmethyldoxepin were prepared. From these solutions, 1:100 dilutions were made before use. Standards for the calibration curve were prepared by adding 10–200 ng of the doxepin isomeric mixture and 10–200 ng of the desmethyldoxepin isomeric mixture to drug-free 1-ml plasma samples and by carrying these samples through the extraction and derivatization procedures, including the addition of 100 ng of the internal standards. Amitriptyline and nortriptyline were selected as internal standards for doxepin and desmethyldoxepin, respectively, because of their close structural relationship.

Quantitation was based on GLC peak area ratios of the compounds *versus* their respective internal standards. Area ratios for the standard mixtures were plotted against the concentration of the product under study, and an unweighted least-squares linear regression analysis was performed. Unknown drug concentrations were estimated from the calibration curve.

Human Samples—Blood samples were taken from two female patients, 38 (WM) and 65 (SM) years old, taking 75 and 200 mg of doxepin/day, respectively, for 2 weeks. The last dose was administered at 8 pm; blood was drawn at 8 am. Plasma was obtained by centrifugation and was stored frozen (−20°) until analyzed.

RESULTS AND DISCUSSION

While doxepin and its internal standard amitriptyline gave rise to symmetrically shaped peaks, the more polar secondary amines, desmethyldoxepin and its internal standard nortriptyline, tailed when analyzed as such. To improve peak shape, volatile *N*-trifluoroacetyl derivatives were formed using trifluoroacetic anhydride. The identity of the GLC peaks was checked by chemical-ionization mass spectrometry. The abundances for ions in the methane chemical-ionization spectra of doxepin, amitriptyline, and the *N*-trifluoroacetyl derivatives of desmethyldoxepin and nortriptyline are summarized in Table I.

The methane chemical-ionization spectra of the tertiary amines I and III were characterized by an intense protonated molecular ion (MH^+). The spectra of the *N*-trifluoroacetyl derivatives of the secondary amines

Table II—Accuracy, Reproducibility, and Recovery of the Procedure for Extraction and GLC Determination on a Capillary Column of Doxepin (*cis*- and *trans*-Isomers) and Desmethyldoxepin Added to 1 ml of Blank Plasma [a]

Compound	Quantity Added to 1 ml of Plasma, ng	Mean Assay Results, % (± *RSD*)	Mean Recovery[b], % (± *RSD*)
Doxepin			
trans	41.0	97.8 (±7.1)	88.1 (±7.1)
cis	9.0	107.8 (±6.8)	93.6 (±8.2)
trans	82.0	99.3 (±3.8)	96.0 (±6.1)
cis	18.0	109.4 (±7.6)	97.5 (±7.9)
Desmethyldoxepin			
trans	48.3	96.7 (±2.9)	88.1 (±4.9)
trans	96.7	100.1 (±3.8)	82.0 (±3.8)
cis	3.3	106.1 (±6.1)	84.9 (±7.8)

[a] All determinations were done five times. [b] Recovery was calculated by comparing the area ratios obtained by adding the substances before and after extraction, while the internal standard was always added before extraction.

[11] Finnigan 6000.

[12] All selected ion monitoring was performed by using this data system and the revision H software.

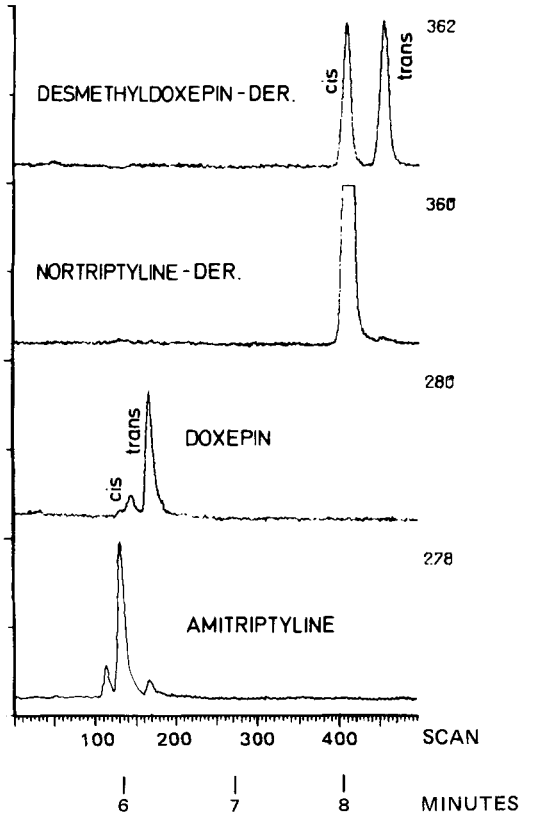

Figure 3—*Selected ion monitoring analysis of a plasma extract in which the [MH]$^+$ ions of doxepin, amitriptyline, and* N*-trifluoroacetyl derivatives of desmethyldoxepin and nortriptyline were monitored. The amitriptyline signal on the* m/e *278 trace is plotted as the 100% peak.*

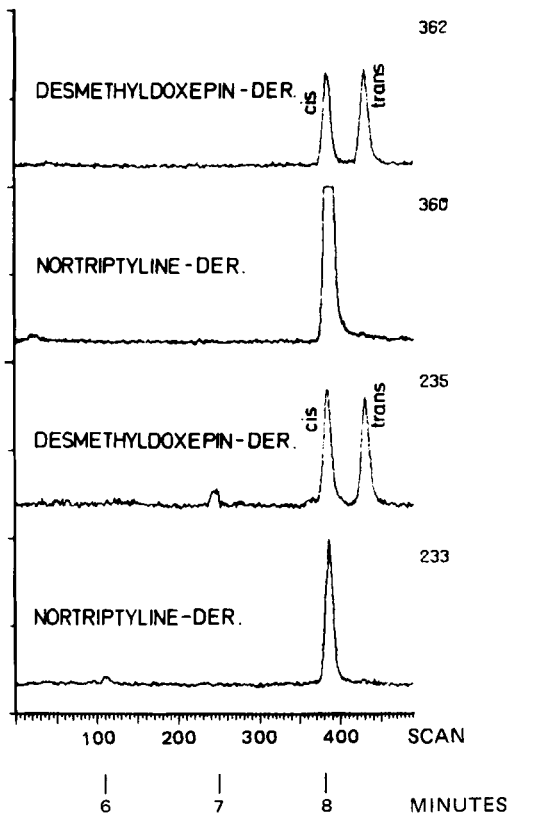

Figure 4—*Selected ion monitoring analysis of a plasma extract in which the [MH]$^+$ ion and [MH−$CF_3CONHCH_3$]$^+$ fragment ion of the* N*-trifluoroacetyl derivatives of desmethyldoxepin and nortriptyline were monitored. The* N*-trifluoroacetylnortriptyline signal on the* m/e *233 trace is plotted as the 100% peak.*

II and IV showed two prominent ions, the MH^+ ion and a fragment ion, corresponding with loss of a $CF_3CONHCH_3$ moiety from the side chain. These ions were selected for confirming the identity of the GLC peaks obtained for the tricyclic drugs in biological samples.

Representative gas chromatograms of extracted human plasma samples spiked with doxepin and desmethyldoxepin are shown in Fig. 1 for the capillary column; for comparison, the results on a packed column are also shown. The use of a short capillary column had the advantage of improved resolution (the peaks of *cis*- and *trans*-doxepin overlapped on the packed column), resulting in higher specificity. The capillary column also had the advantage that an interfering peak of unknown origin, occurring in some plasma samples, was separated from the doxepin peak whereas it completely coeluted on the packed column.

The use of a capillary column also resulted in greater absolute sensitivity compared to a packed column, but it had the disadvantage of decreased sample loading capacity (0.2 μl instead of 2 μl on the packed column). Therefore, the overall sensitivity was similar for both columns (2 ng/ml of plasma for doxepin and 1 ng/ml for desmethyldoxepin). The accuracy and reproducibility of the assay for doxepin and desmethyldoxepin, as well as the extraction recovery, are given in Table II. Calibration curves were made on 4 separate days over 2 weeks. The least-squares linear regression analysis of the calibration curve for *trans*-doxepin gave a slope of 0.0114 ± 0.0021 and a y-intercept of 0.0155 ± 0.0132 (r 0.995–0.998). The curve for *trans*-desmethyldoxepin had a slope 0.0099 ± 0.0006 and a y-intercept of −0.0300 ± 0.0064 (r 0.994–0.999). With each set of unknown samples, a four-point standard curve was made. The calibration curves for the *trans*-isomers were also used for estimation of the corresponding *cis*-isomers, extracted in a similar way as the *trans*-isomers, and can be expected to yield equal detector responses.

An example of a typical GLC analysis of a plasma sample from a patient receiving doxepin chronically is given in Fig. 2. Both doxepin and desmethyldoxepin were present in the two isomeric forms.

The identity of the GLC peaks was confirmed by selected ion monitoring of specific ions, formed upon chemical ionization with methane as a reagent gas. Figure 3 shows a representative analysis, in which the protonated molecular ions, $[MH]^+$, of doxepin, amitriptyline, N-triflu-

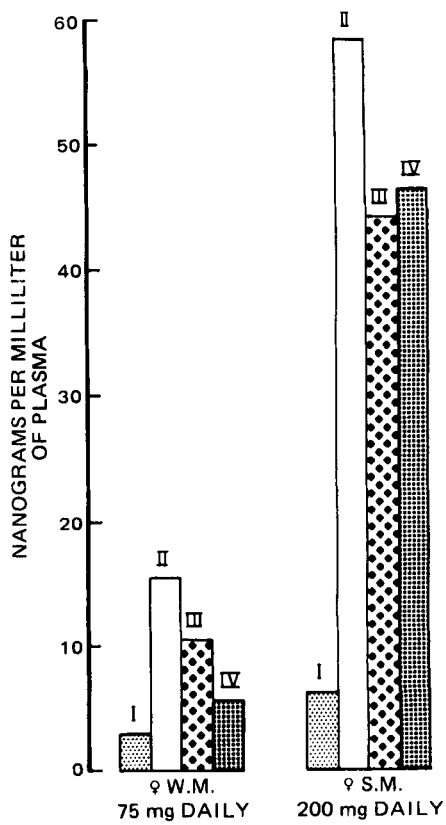

Figure 5—*Plasma levels of* cis*-doxepin (I),* trans*-doxepin (II),* cis*-desmethyldoxepin (III), and* trans*-desmethyldoxepin (IV) in two patients receiving doxepin for 2 weeks.*

oroacetyldesmethyldoxepin, and *N*-trifluoroacetylnortriptyline at *m/e* 280, 278, 362, and 360, respectively, were monitored.

Figure 4 shows the analysis in which the $[MH]^+$ ion and the $[MH - CF_3CONHCH_3]^+$ fragment ion of *N*-trifluoroacetyldesmethyldoxepin and *N*-trifluoroacetylnortriptyline at *m/e* 362, 360, 235, and 233 were monitored. The ratios of the $[MH]^+$ and $[MH - CF_3CONHCH_3]^+$ selected ion traces obtained from the analysis of a biological sample (Fig. 4) were 0.82 and 0.87 for the *N*-trifluoroacetyl derivatives of *cis*- and *trans*-desmethyldoxepin, respectively. These values are in close agreement with the theoretical values of 0.68 and 0.78 obtained from the mass spectra, which were recorded under the same experimental conditions (Table I), confirming as such the presence of the *N*-trifluoroacetyl derivatives of *cis*- and *trans*-desmethyldoxepin. Since *N*-trifluoroacetylnortriptyline overlapped with *cis*-(*N*-trifluoroacetyl)desmethyldoxepin on the SE-30 capillary column used in the selected ion monitoring experiments, the ^{13}C-isotope contributions of the nortriptyline signals were taken into account for calculating the true ratio of the $[MH]^+$ and $[MH - CF_3CONHCH_3]^+$ signals of *cis*-(*N*-trifluoroacetyl)desmethyldoxepin.

Preliminary results of the plasma levels obtained in two patients receiving chronic doxepin therapy are given in Fig. 5. The *trans*-isomer of doxepin was more prominent than its *cis*-form. For desmethyldoxepin, however, a considerable fraction occurred as the *cis*-form, although the product given to the patients contained only approximately 15% *cis*- *versus* 85% *trans*-isomer.

More patients are now being studied to evaluate if the levels of *cis*- and *trans*-doxepin and desmethyldoxepin can be correlated with clinical outcome.

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Simple, Rapid, and Micro High-Pressure Liquid Chromatographic Determination of Plasma Griseofulvin Levels

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Received July 25, 1977, from the *Clinical Pharmacokinetics Laboratory and the Department of Pharmacy, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612.* Accepted for publication September 29, 1977.

Abstract □ A rapid high-pressure liquid chromatographic assay is described for the quantitative determination of griseofulvin in plasma. An aliquot (25–100 μl) of plasma was deproteinized by a simple procedure involving the addition of 2.5 volumes of acetonitrile, vortex mixing for a few seconds, and centrifugation for 1 min. The clear supernate, 50 μl, was injected into the high-pressure liquid chromatograph. A reversed-phase column was used with a mobile phase of distilled water–acetonitrile (1:1) at a flow rate of 2 ml/min and was operated at ambient temperature. A fluorescent detector with an excitation wavelength of 260 nm was employed to monitor the column effluent. Griseofulvin had a retention time of 3.8 min. This procedure yields reproducible results with high sensitivity; plasma concentrations as low as 50 ng/ml can be measured. Several commonly used drugs do not interfere. Analysis of plasma samples collected from a rabbit injected with griseofulvin indicated that the procedure is suitable for pharmacokinetic studies and clinical monitoring of plasma concentrations in patients. Assay turnaround time is less than 6 min. For clinical monitoring of plasma griseofulvin concentrations, a sample volume as small as 10 μl can be used.

Keyphrases □ Griseofulvin—high-pressure liquid chromatographic analysis in plasma □ High-pressure liquid chromatography—analysis, griseofulvin in plasma □ Antifungal agents—griseofulvin, high-pressure liquid chromatographic analysis in plasma

Griseofulvin is administered orally for treatment of fungal infections of the skin, hair, and nails in humans; this drug treatment is sometimes required for many months. The incidence of relatively minor untoward effects associated with the use of griseofulvin may be as high as 15%, but more serious reactions occur less frequently (1).

BACKGROUND

A number of factors could possibly influence the time course of blood griseofulvin concentrations following multiple oral doses. For example, a reduction of the extent of systemic biological availability was demonstrated in human subjects administered single doses of certain griseofulvin formulations (2, 3). Repeated single oral doses of the same batch of some griseofulvin products resulted in extremely variable extents of absorption in some subjects (2). Additionally, the concurrent administration of phenobarbital decreased blood levels of griseofulvin by impairing its absorption (4, 5). The metabolic clearance of griseofulvin is subject to wide interindividual variability (2), and this factor alone may result in pronounced differences (probably severalfold) in blood levels of the drug among individuals receiving the same dosage regimen. In view of the chronic nature of griseofulvin therapy and the factors influencing its pharmacokinetic profile, it may be beneficial to patient care to monitor plasma drug concentrations, particularly if toxicity or therapeutic failure is suspected (6).

Spectrofluorometric and GLC methods were described previously for the quantitative determination of griseofulvin in plasma. The spectrofluorometric assay (2) and modifications of it (7, 8) require 0.5–1 ml of plasma, time-consuming extraction and evaporation steps, and the reading of fluorescence intensity of the final aqueous solution both before and after the addition of sulfuric acid. Moreover, griseofulvin metabolites interfere with the method (8). The lower detection limit of the spectrofluorometric procedures is approximately 0.1 μg of griseofulvin/ml of plasma.

GLC with an electron-capture detector was used for the estimation of griseofulvin in plasma (8). The preparation of plasma samples for analysis involved extraction and evaporation, dissolution of the residue in benzene, and injection into the gas chromatograph. Because of the nonlinear response of the detector, the standard curve was nonlinear above about