Table VI—Comparison of the Predicted and Input Data

t, hr	Input[a]	Predicted by Spline[b]	Predicted by LEASQ[b]	Theoretical (Expected)[b]
0.1	18.68	19.7470 (−4.26)	18.7512 (−0.28)	17.8285 (3.40)
0.2	15.92	16.9891 (−5.01)	16.6838 (−3.58)	15.9506 (−0.14)
0.5	10.82	11.3095 (−3.37)	12.0142 (−8.23)	11.7020 (−6.08)
1.0	8.38	6.8147 (13.92)	7.5799 (7.12)	7.6489 (6.50)
1.5	5.57	5.0242 (7.30)	5.3858 (2.47)	5.6179 (−0.64)
2.0	4.83	4.2152 (9.49)	4.2544 (8.89)	4.5397 (4.48)
3.0	3.51	3.4274 (1.75)	3.2208 (6.14)	3.4777 (0.69)
4.0	2.59	2.8980 (−8.86)	2.6901 (−2.88)	2.8726 (−8.13)
6.0	1.92	2.0464 (−4.91)	1.9381 (−0.70)	1.9997 (−3.10)
8.0	1.29	1.4026 (−6.51)	1.3608 (−4.09)	1.3617 (−4.15)
10.0	0.92	0.9385 (−1.50)	0.9294 (−0.76)	0.9083 (0.95)
12.0	0.63	0.6176 (1.47)	0.6228 (0.85)	0.5977 (3.83)
14.0	0.39	0.4020 (−2.30)	0.4128 (−4.36)	0.3900 (−0.01)
16.0	0.27	0.2600 (2.77)	0.2721 (−0.59)	0.2533 (4.60)

[a] Simulated plasma levels containing 10% random noise. [b] Weighted residuals are given in parentheses ($\times 10^2$).

ability of the estimated parameters and of the efficiency of the estimation procedure. As can be seen in Table III, the errors associated with either the spline or the LEASQ results are in the same range as the expected error. Compared to the errors of the LEASQ solutions, the expected *ASE* values were greater but the expected *WSS* was smaller. The first observation suggests that the true parameters probably are irretrievable from corrupted input data. The second observation indicates that further improvement of the parameter estimates can be made by applying superior numerical algorithms. This conclusion is manifested in Table VI where the predicted and the observed (input) data are compared.

Conclusions—Two numerical examples have been described to illustrate the proposed method in the estimation of pharmacokinetic parameters. For a given model and weighting scheme, the reliability of the estimates is dependent on data accuracy as well as on the numerical algorithms employed. Obviously, when experimental errors are large, meaningful estimates are difficult, irrespective of algorithmic sophistication.

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Impurities in Drugs III: Trihexyphenidyl

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Received January 17, 1979, from the *Drug Research Laboratories, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, K1A 0L2, Canada.* Accepted for publication March 16, 1979.

Abstract □ Two lots of trihexyphenidyl hydrochloride raw material, one lot of elixir, and 10 lots of tablets were examined for impurities by TLC. Impurities found were 1-phenyl-2-propenone, 3-piperidinopropiophenone, and 3-aminopropiophenone. Not all impurities were present in all lots, and none exceeded 1.9% of the label drug claim. Impurities were identified by mass spectrometry and by comparison of TLC R_f values and GLC retention times to those of synthesized specimens of the impurities.

Keyphrases □ Trihexyphenidyl—analysis, TLC, impurities in tablets and elixir □ Trihexyphenidyl, derivatives—1-phenyl-2-propenone, 3-piperidinopropiophenone, 3-aminopropiophenone, TLC analysis, impurities in tablets and elixir □ Drug impurities—trihexyphenidyl, tablets and elixir, TLC analysis

Impurities in drug raw materials and formulations may be intermediates or by-products of the synthetic process, products of degradation or drug–excipient interaction, or the result of contamination. The nature of impurities may depend on the synthetic route, the reagent purity, and the excipient quality. To obtain a good perspective of potential impurities, raw materials and formulations from as many sources as possible should be examined (1–4). This paper describes the impurities found in trihexyphenidyl (I) raw material and tablet and elixir products.

Trihexyphenidyl was synthesized first (5) by the addition of cyclohexylmagnesium bromide to 3-piperidinopropiophenone (II), obtained by the Mannich reaction with acetophenone (III), formaldehyde, and piperidine hydrochloride in acidic medium. Trihexyphenidyl hydrochloride raw material and tablets are official in the USP (6) and BP as benzhexol (7). An elixir is official in the USP only. The only impurity specification is that in the BP for 3-piperidinopropiophenone in drug raw material.

EXPERIMENTAL

Materials—All drugs and formulations were obtained from the

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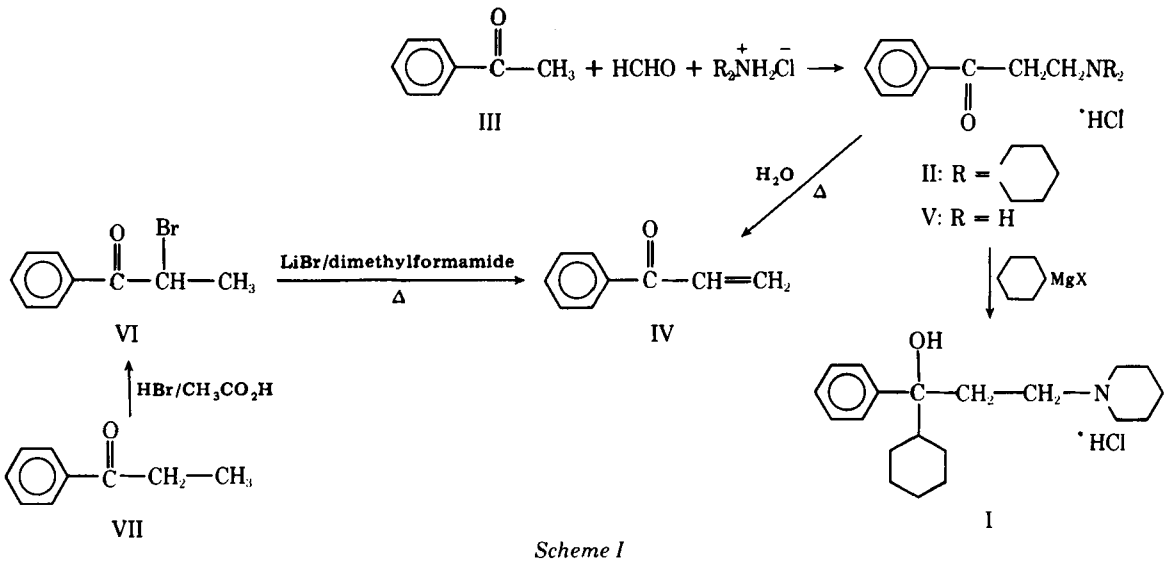


Scheme I

manufacturer. Acetophenone[1], 3-piperidinopropiophenone[1], formaldehyde 37% solution[2], ammonium chloride[2], lithium carbonate[3], lithium bromide[3], acetic acid[4], dimethylformamide[4], bromine[5], and hydrogen chloride gas[6] were obtained commercially. All solvents were analytical grade.

TLC plates were precoated with silica gel GF[7] (20 × 20 cm, 0.25 mm). The gas chromatograph[8] was equipped with flame-ionization detectors and 1.8-m × 0.4 cm i.d., U-shaped glass columns, packed with 3% OV-210 coated on acid-washed, dimethylchlorosilane-treated, high-performance flux-calcined diatomite support[9] (100–120 mesh).

Standard Solutions—For TLC, chloroform solutions contained 5 mg of trihexyphenidyl hydrochloride/ml and either 0.01 mg of 1-phenyl-2-propenone (IV)/ml or 0.04 mg of 3-aminopropiophenone (V)/ml. For GLC, a solution of 5.2 mg of 3-piperidinopropiophenone in 50 ml of chloroform was prepared.

TLC Systems—A neutral system of chloroform–methanol–ethyl acetate–acetone (20:20:20:40) and a basic system of chloroform–methanol–concentrated ammonium hydroxide (100:25:1) were used. Spots were visualized with UV light at 254 nm and with Dragendorff spray.

Extraction from Tablet Formulations—An amount of powdered tablet equivalent to 10 mg of trihexyphenidyl hydrochloride was weighed into a 10-ml screw-capped culture tube[5] and extracted by shaking[10] for 15 min with 2 ml of chloroform. After centrifuging, the solutions were filtered, and aliquots were applied to the TLC plates and injected into the gas chromatograph.

Extraction from Elixir Formulation—An amount (25 ml) equivalent to 10 mg of trihexyphenidyl hydrochloride in 15 ml of water was partitioned three times with 20 ml of chloroform. The chloroform portions were concentrated, and the volume was adjusted to 2 ml with chloroform. Aliquots of this solution were applied to the TLC plates or injected into the gas chromatograph.

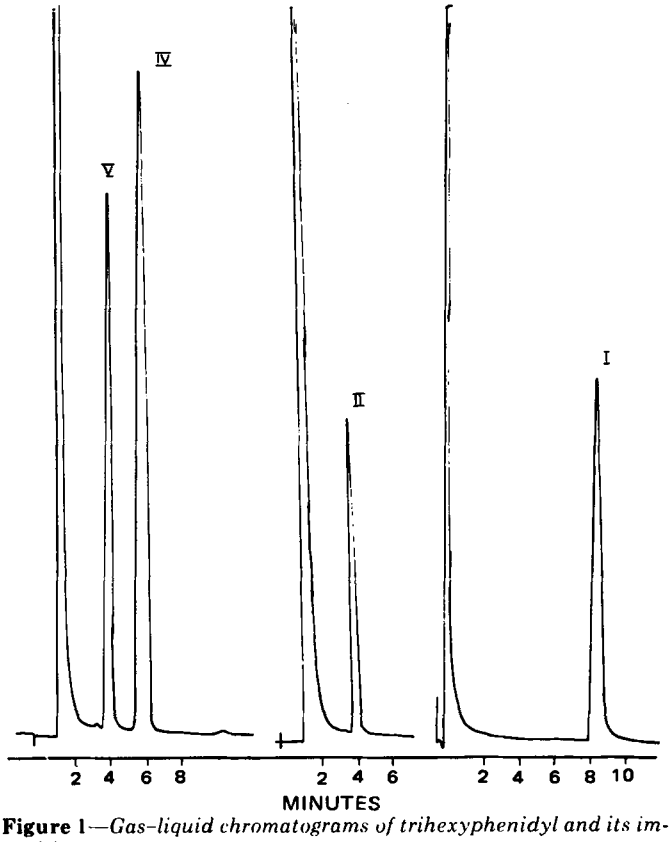


Figure 1—*Gas-liquid chromatograms of trihexyphenidyl and its impurities.*

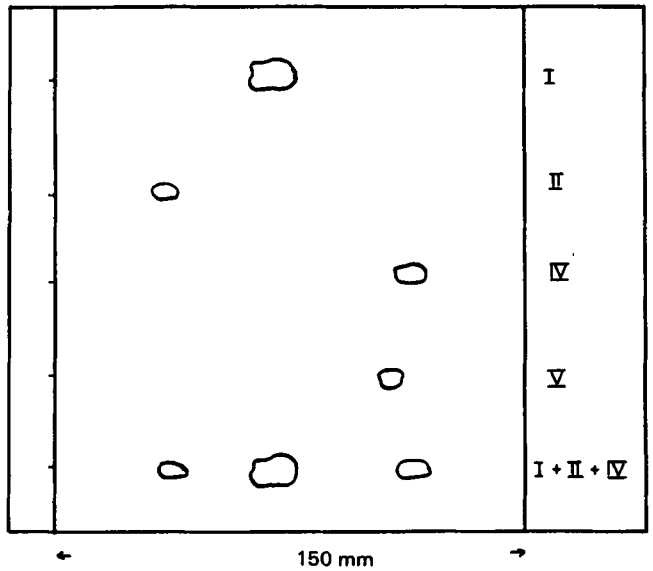
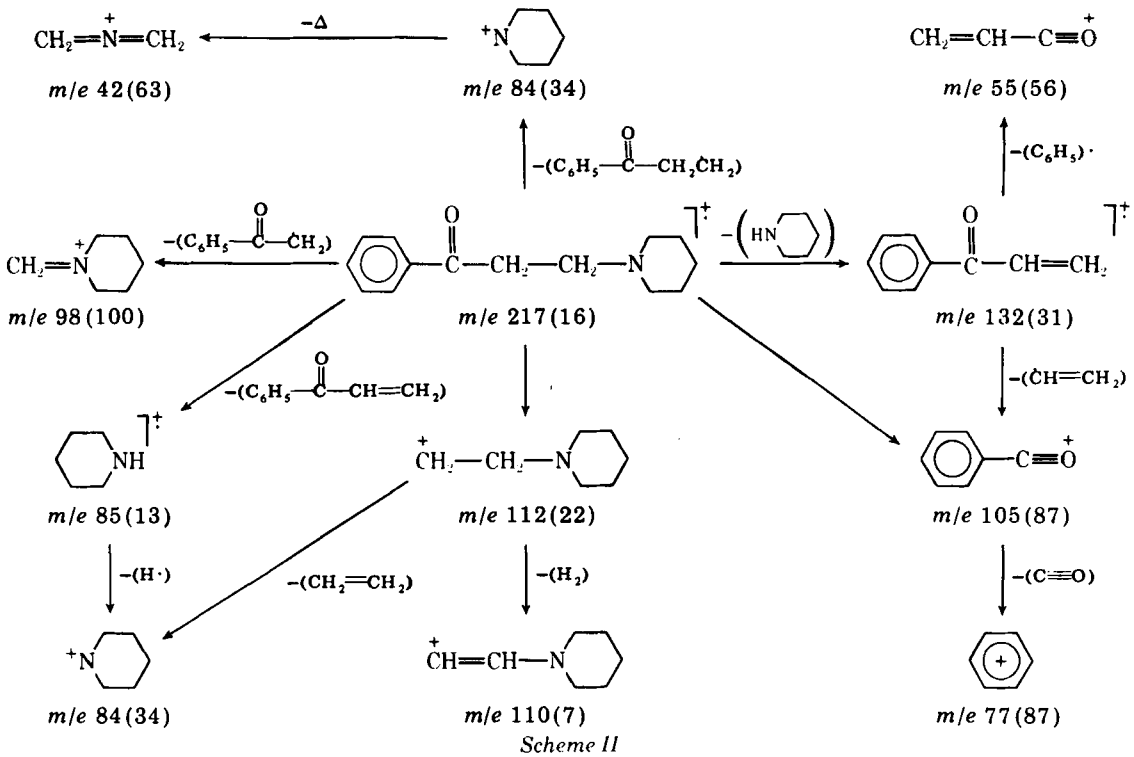


Figure 2—*TLC separations of trihexyphenidyl and its impurities using the neutral system.*

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] Anachemia Chemicals Ltd., Montreal, Canada.
[3] BDH Chemicals Ltd., Poole, England.
[4] J. T. Baker Chemical Co., Phillipsburg, N.J.
[5] Canlab Laboratories, Ottawa, Canada.
[6] Matheson, Toronto, Canada.
[7] Brinkmann Instruments, Toronto, Canada.
[8] Bendix 2500, Aviation Electric, Montreal, Canada.
[9] Chromosorb W, Chromatographic Specialties, Brockville, Canada.
[10] Horizontal shaker, Eberbach Corp., Ann Arbor, Mich.



Scheme II

Screening for Impurities—The R_f values of the drug and of each impurity were determined in both TLC systems, and the lower detectability limit was established by serial dilutions of the stock solutions. Aliquots of 50 μl (250 μg) of the tablet extracts in chloroform were applied to the TLC plates. The concentration of impurities was approximated by comparison of the spot diameters and intensities to those of the corresponding spots from the standard solutions.

Aliquots, 5 μl, of the appropriate solution were injected into the gas chromatograph for quantification of the 3-piperidinopropiophenone. Quantification was done by peak height. To check for possible decomposition on the plate, each compound was subjected to two-dimensional TLC.

Isolation of Impurities—Impurities IV and V were isolated from Tablet Formulation B and Raw Material M, respectively. A chloroform extract containing 200 mg of the drug in ~6 ml of the solvent was applied in a narrow band to a 20 × 20-cm × 2-mm TLC plate[7] and developed in the neutral system. Impurities were visualized by UV light at 254 nm and by spraying a 1-cm band along each side of the plate with Dragendorff[1] spray. Impurity bands were scraped from the plate, stirred with 30 ml of methanol for 30 min, filtered, evaporated to dryness, redissolved in 20 ml of chloroform, and evaporated to dryness.

The purity of the isolated compound was checked by TLC and GLC. The injection port, detector block, and column temperatures were 250, 250, and 140°, respectively, and the flow rates of nitrogen, hydrogen, and air were 13, 50, and 400 ml/min, respectively. The impurities were subjected to GLC–mass spectral analysis[11].

Syntheses—Scheme I illustrates the syntheses of the following compounds.

Table I—Chromatographic Characteristics

Compound	TLC R_f[a]	TLC R_f[b]	TLC Detectability Limit[c], μg	GLC Retention Time, min
I	0.46	0.78	2.5	8.4[d]
II	0.24	0.74	2.5	4.2[e]
IV	0.75	0.84	0.25	6.0[e]
V	0.73	0.86	2.5	4.0[e]

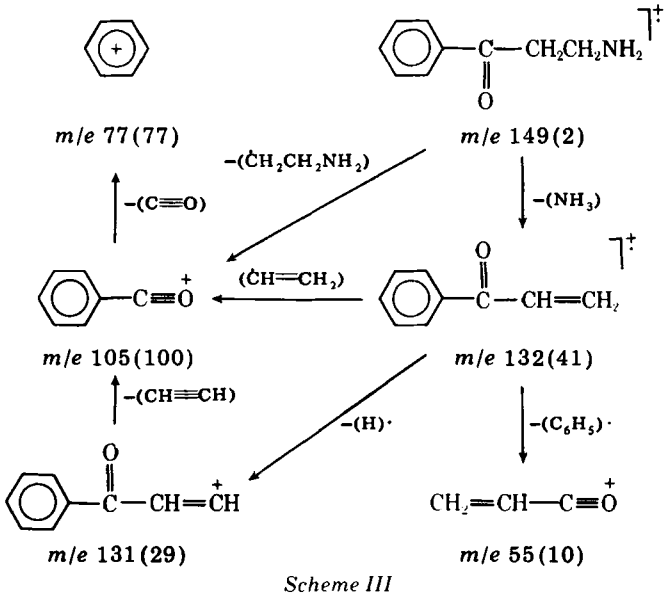
[a] In the neutral system. [b] In the basic system. [c] Applied with 250 μg of the drug. [d] 3% OV-210, Chromosorb W (high performance), 100–120 mesh, 170°, nitrogen flow of 38 ml/min. [e] Same as footnote *d* except 140° and nitrogen flow of 13 ml/min.

[11] Hewlett-Packard model 5985 and Varian MAT 311A mass spectrometers with electron energy of 70 ev.

2-Bromopropiophenone (VI)—In a 250-ml, three-necked round-bottom flask, 17.4 g (0.13 mole) of propiophenone (VII) (8) was dissolved in 100 ml of glacial acetic acid. A 135-ml normal solution of bromine in glacial acetic acid was added at 0° by a dropping funnel over 30 min. The agitation was maintained for an additional 2 hr, and the mixture was diluted with water and extracted with ether.

The organic layer was washed, neutralized with sodium bicarbonate and dried over anhydrous sodium sulfate. The mixture was filtered and evaporated[12] at 30° to yield 25.3 g (92%) of VI, bp_{10} 125–128° [lit. (9) bp_{12} 130–132°]; IR (film): 1690 (C=O), 710, and 685 (C–Br) cm^{-1}.

1-Phenyl-2-propenone (IV)—In a 500-ml, round-bottom flask equipped with a condenser, 21.2 g (0.1 mole) of VI (10) was refluxed for 1 hr in 180 ml of dimethylformamide containing 10.7 g of lithium carbonate and 1.2 g of lithium bromide. The reaction mixture was diluted with water and extracted with ether, and the organic phase was washed with a saturated sodium carbonate solution. The ether phase was dried over anhydrous sodium carbonate, filtered, evaporated, and distilled to



Scheme III

[12] Rotavapor-R, Büchi, Switzerland.

Table II—Impurities in Trihexyphenidyl Tablets

Lot	Dose, mg	Impurities[a], % II	IV	V
A	2	ND[b]	0.4	ND
B	5	Tr[c]	1.9	ND
C	2	1.0	0.2	ND
D	5	1.6	0.2	ND
E	2	ND	0.2	ND
F	5	0.1	ND	ND
G	2	0.1	ND	ND
H	5	0.1	0.1	ND
I	2	ND	ND	ND
J	5	ND	0.1	ND
K (elixir)	2/5 ml	ND	ND	1.0
Raw material	—	ND	ND	ND
Raw material	—	ND	ND	1.6

[a] Expressed as percentage of the label claim of drug in the hydrochloride form. [b] None detected. [c] Trace.

yield 5.3 g (40%) of crude IV, bp_3 75–79° [lit. (11) $bp_{2.5}$ 74–76°]; IR (film): 1680 (C=O) cm^{-1}.

3-Aminopropiophenone (V)—In a 300-ml, round-bottom flask, 5.3 g (0.1 mole) of ammonium chloride, 4.5 g (0.15 mole) of formaldehyde 37% solution, and 0.2 ml of concentrated hydrochloric acid were dissolved in 40 ml of ethanol (5). To this solution, 12.0 g (0.1 mole) of acetophenone (III) was added. The mixture was refluxed for 1.5 hr, another 9 ml of formaldehyde (37% solution) was added, and the mixture was refluxed for another 1.5 hr.

Compound V was extracted into ether as the free base. The ether phase was dried over sodium sulfate and filtered, and hydrogen chloride gas was passed through the solution. The yield of the hydrochloride salt was 5.0 g (33%) of V, mp (hydrochloride) 125–127° [lit. (11) mp 128°]; IR ($CHCl_3$): 1685 (C=O) and 3440 (NH_2 vibration) cm^{-1}.

RESULTS AND DISCUSSION

The identity of 3-piperidinopropiophenone (II), 1-phenyl-2-propenone (IV), and 3-aminopropiophenone (V) found in raw material and tablet formulations was established by comparison of TLC R_f values, GLC retention times, and mass spectral fragmentation patterns to those of synthetic samples. The authenticity of the synthetic samples was demonstrated by TLC, GLC, and mass spectra.

The structure postulated for the impurities is supported by the mass spectral results, which conform to the fragmentation diagrams presented in Schemes II and III. Detectability limits, R_f values, and GLC retention times for trihexyphenidyl, 3-piperidinopropiophenone, 1-phenyl-2-propenone, and 3-aminopropiophenone are listed in Table I (Figs. 1 and 2). Two lots of trihexyphenidyl hydrochloride, 10 lots of tablets, and one lot of elixir preparation from five manufacturers were screened for impurities (Table II). Five lots contained II at levels from 0.1 to 1.6%, and half of the lots contained IV at levels from 0.1 to 1.9%. Compound V was found in two lots from the same manufacturer at levels of 1.0 to 1.6%.

The presence of V may be the result of ammonium chloride in the piperidine hydrochloride used as a starting material in one synthetic process (5) (Scheme I). Compound II is an intermediate in the synthesis of trihexyphenidyl (5). The presence of IV, which was observed in low levels, may be due, according to previous investigators (12), to the hydrolysis of II or to an elimination reaction in the step involving the Grignard reagent (11).

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High-Pressure Liquid Chromatographic Assay for Griseofulvin in Plasma

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Received January 17, 1979, from the *Division of Biopharmaceutics and Pharmacokinetics, Department of Pharmaceutics, College of Pharmacy, University of Tennessee Center for the Health Sciences, Memphis, TN 38163.* Accepted for publication March 14, 1979.

Abstract □ A high-pressure liquid chromatographic procedure was developed for griseofulvin assay in human plasma. The method utilized warfarin as an internal standard and easily quantitated griseofulvin plasma levels as low as 0.10 μg/ml. The method was compared to two fluorometric assay methods and was more specific for griseofulvin. Assay of 6-demethylgriseofulvin isolated from human urine demonstrated that this material was not responsible for the interferences apparent in the fluorometric procedures.

Keyphrases □ Griseofulvin—analysis, high-pressure liquid chromatography, human plasma, compared to fluorometric assays □ Antifungal agents—griseofulvin, high-pressure liquid chromatographic analysis, human plasma, compared to fluorometric assays □ Fluorometry—analysis, griseofulvin in human plasma, compared to high-pressure liquid chromatography

Griseofulvin is a poorly water-soluble, antifungal agent. This orally administered drug may be subject to reduced bioavailability, and particle-size reduction and preparation of polyethylene glycol dispersions have been employed to improve absorption from the GI tract (1, 2). In view of the potential for griseofulvin dosage forms to exhibit poor bioavailability, a convenient and specific assay method for intact drug measurement in the plasma of patients or volunteer subjects receiving griseofulvin is needed.

The most widely utilized analytical methods have em-

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