vidual radii. The equations describing the radii are obtained from Eq. 6:

$$-\frac{dr_{ASA}}{dt} = \frac{D_{ASA}C^*_{ASA} + D_{ASA\text{-}CAF}M_{ASA}\tilde{C}^*_{ASA\text{-}CAF}}{(h + r_{PH} - r_{ASA})X_{ASA}\rho} \quad \text{(Eq. A13)}$$

$$-\frac{dr_{CAF}}{dt} = \frac{D_{CAF}C^*_{CAF} + D_{ASA\text{-}CAF}M_{CAF}\tilde{C}^*_{ASA\text{-}CAF}}{(h + r_{PH} - r_{CAF})X_{CAF}\rho} \quad \text{(Eq. A14)}$$

$$-\frac{dr_{PH}}{dt} = \frac{D_{PH}C^*_{PH}}{hX_{PH}\rho} \quad \text{(Eq. A15)}$$

where:

$$X_{PH} + X_{ASA} + X_{CAF} = 1 \quad \text{(Eq. A16)}$$

At 25°, the equations can be written as:

$$-\frac{dr_{ASA}}{dt} = \frac{9.76 \times 10^{-8}}{(30 \times 10^{-4} + r_{PH} - r_{ASA})X_{ASA}\rho} \text{ cm/sec} \quad \text{(Eq. A17)}$$

$$-\frac{dr_{CAF}}{dt} = \frac{2.22 \times 10^{-7}}{(30 \times 10^{-4} + r_{PH} - r_{CAF})X_{CAF}\rho} \text{ cm/sec} \quad \text{(Eq. A18)}$$

$$-\frac{dr_{PH}}{dt} = \frac{3.33 \times 10^{-6}}{X_{PH}\rho} \text{ cm/sec} \quad \text{(Eq. A19)}$$

These equations are coupled (r_{ASA} is a function of r_{PH}, *etc.*). For given values of X_i and ρ, these equations can be solved simultaneously to yield r_{ASA}, r_{CAF}, and r_{PH} as a function of time. A fourth-order Runge–Kutta procedure (8) was used in this study to solve these equations. When the radii are evaluated, the instantaneous fluxes may be calculated from Eqs. A10–A12. The average fluxes may be calculated using Eq. 12.

The equations describing the cases in which either aspirin or caffeine is the outer layer can be obtained similarly. The equations describing the hypothetical case in which aspirin and caffeine do not interact are obtained by setting $\tilde{C}^*_{ASA\text{-}CAF}$ to zero in these equations.

REFERENCES

(1) E. Nelson, *J. Am. Pharm. Assoc., Sci., Ed.*, **46**, 607 (1957).

(2) M. Gibaldi and H. Weintraub, *J. Pharm. Sci.*, **57**, 832 (1968).

(3) W. I. Higuchi, N. A. Mir, and S. J. Desai, *ibid.*, **54**, 1405 (1965).

(4) S. A. Shah and E. L. Parrott, *ibid.*, **65**, 1784 (1976).

(5) E. L. Parrott, D. E. Wurster, and T. Higuchi, *J Am. Pharm. Assoc., Sci. Ed.*, **44**, 269 (1955).

(6) R. J. Braun and E. L. Parrott, *ibid.*, **61**, 175 (1972).

(7) S. A. Shah, Ph.D. thesis, University of Iowa, Iowa City, Iowa, 1975.

(8) B. Carnahan, H. A. Luther, and J. O. Wilkens, "Applied Numerical Methods," Wiley, New York, N.Y. 1969, pp. 361–365.

(9) T. Higuchi and D. A. Zuck, *J. Am. Pharm. Assoc., Sci. Ed.*, **42**, 138 (1953).

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Reversed-Phase High-Pressure Liquid Chromatographic Analysis of Sulconazole in Plasma

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Received June 2, 1980, from *Syntex Research, Palo Alto, CA 94304.* Accepted for publication May 12, 1981.

Abstract □ A sensitive and specific analytical method for the measurement of sulconazole in plasma is described. The compound was extracted from plasma at pH 10 with hexane–methylene chloride. Samples were subjected to high-pressure liquid chromatography (HPLC) using an acetonitrile–phosphate buffer mixture as the mobile phase. The components of interest were measured using a variable-wavelength detector at 229 nm. Sulconazole concentrations of ≥0.5 μg/ml can be measured with confidence using this method. Linear calibration curves were constructed over the concentration range of 0.5–5 μg/ml for sulconazole from dog plasma. A dog was administered a single oral 1000-mg dose of tritiated sulconazole nitrate; total plasma radioactivity and sulconazole plasma levels determined by HPLC are reported.

Keyphrases □ Sulconazole—reversed-phase high-pressure liquid chromatographic analysis in plasma □ High-pressure liquid chromatography—analysis of sulconazole in plasma □ Antifungal agents—sulconazole, reversed-phase high-pressure liquid chromatographic analysis.

Sulconazole nitrate, 1-[β-(4″-chlorobenzylthio)-2′, 4′-dichlorophenethyl]imidazole mononitrate (I), is a potential new antimycotic agent[1] developed for human use. Since it is necessary to conduct animal toxicity studies supported by plasma level data for new drugs, a sensitive and specific analytical method was developed.

A high-pressure liquid chromatographic (HPLC) method for the determination of econazole, a structural analog of sulconazole, was described previously (1). Direct application of this extraction–injection method to sulconazole analysis provided unsatisfactory chromatography. Interfering peaks at the retention time of the compounds of interest were observed, and laborious distillation of ether prior to extraction to remove peroxides did not prevent random oxidative degradation of sulconazole.

This paper describes a reversed-phase HPLC method for the measurement of sulconazole in plasma using a structurally related analog as the internal standard. The method was applied to the measurement of sulconazole in the plasma of a dog given a single oral dose of 1000 mg of tritiated sulconazole nitrate.

EXPERIMENTAL

Reagents and Materials—All solvents[2] were distilled-in-glass and liquid chromatography grade. All chemicals[1], available in-house, were analytical grade and were used without further purification. Inorganic reagents were prepared in purified water[3]. Standard solutions of sulconazole were prepared in methanol at the following concentrations: 40 μg/ml (stock solution) and 20, 16, and 4 μg/ml (spiking solutions). The internal standard solution was prepared in methanol at a concentration of 100 μg/ml. Solutions were stored at 4°. Standard solutions were prepared every 2 weeks.

[1] Syntex, Palo Alto, Calif.

[2] Burdick & Jackson Laboratories, Muskegon, Mich.

[3] Milli Q System, Millipore Corp., Bedford, Mass.

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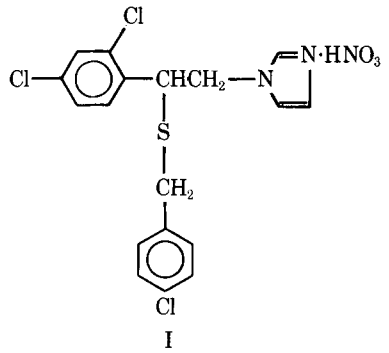
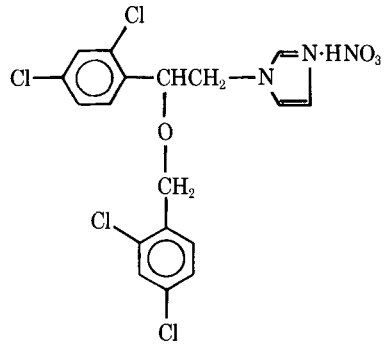


I



II

Extraction—Plasma (400 μl) was added to 400 μl of purified water in a 15-ml test tube. Samples were then fortified with the internal standard at the 12.5-μg/ml level (50 μl of 100 μg/ml of spiking solution) and made alkaline by the addition of 100 μl of 1 *N* potassium hydroxide. This mixture was extracted with 6 ml of hexane–methylene chloride (1:1). Tubes were shaken on a tilter for 3 min, and samples were then centrifuged[4] at 4000 rpm for 6 min. The upper organic layer was transferred to a 15-ml conical test tube and was blown to dryness under nitrogen. Samples were reconstituted in 100 μl of methanol, and 25 μl was injected onto the chromatograph.

Preparation of Standard Curve—Samples of control plasma were spiked with sulconazole at concentrations of 0.5, 1.0, 2.5, 4.0, and 5.0 μg/ml and with the internal standard at a fixed concentration of 12.5 μg/ml. Concentrations of sulconazole were calculated from calibration curves constructed by plotting the ratio of the peak height of sulconazole to the peak height of the internal standard *versus* the concentration of sulconazole spiked.

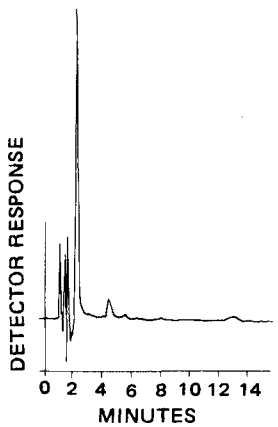


Figure 1—*Chromatogram of unfortified plasma. Conditions were a μBondapak C_{18} column, acetonitrile–0.01 M NaH_2PO_4 buffer (pH 8.0) as the mobile phase, and a 2-ml/min flow rate.*

[4] Model HNS. IEC, Needham, Mass.

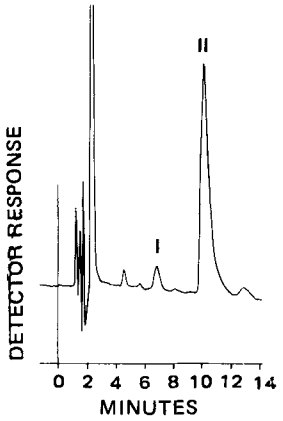


Figure 2—*Chromatogram of plasma fortified at the 0.5-μg/ml level with I and at the 12.5-μg/ml level with II.*

HPLC and Chromatography Conditions—A high-pressure liquid chromatograph[5] was equipped with a solvent programmer, an injector, and a spectrophotometric variable-wavelength detector[6] set at 229 nm. Samples were analyzed on a 4-mm × 30-cm reversed-phase column[7] fitted with a 4-mm × 8-cm precolumn[8]. The mobile phase was a solvent system of acetonitrile–0.01 *M* NaH_2PO_4 buffer (pH 8.0, 66:34), and the flow rate was 2 ml/min.

Each day, prior to sample injection, the column was equilibrated with mobile phase for ~20 min or until a level baseline was achieved. To check column suitability, a 25-μl aliquot of standard solution of sulconazole (20 μg/ml) and the internal standard (50 μg/ml) in methanol was injected onto the chromatograph under the described conditions. The suitability of the column was established by following three chromatographic parameters:

1. Resolution (R). The column was considered acceptable if the resolution of sulconazole and the internal standard was equal to or better than baseline separation or an *R* value of 1.5 as determined by the resolution equation (2).
2. Peak height ratio. A maximum variation of 5% for peak height ratio for injection on the same day was acceptable before major changes in the solvent system or column were implemented.
3. Retention time. The retention times for sulconazole and the internal standard were ~7 and 10 min, respectively. Retention times for replicate injections on a single day were not expected to vary by more than 5%.

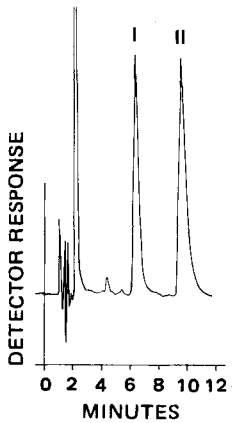


Figure 3—*Chromatogram of plasma fortified at the 5-μg/ml level with I and at the 12.5-μg/ml level with II.*

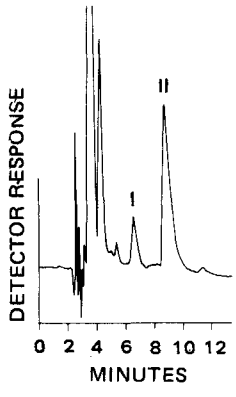


Figure 4—*Chromatogram of plasma sample from a dog 6 hr after dosing with 1000 mg of tritiated sulconazole nitrate.*

[5] Model ALC/GPC-204 with U6K injector and model 660 solvent programmer, Waters Associates, Milford, Mass.
[6] Model 770, Schoeffel, Westwood, N.J.
[7] μBondapak C_{18}, Waters Associates, Milford, Mass.
[8] CoPell ODS, Whatman Inc., Clifton, N.J.

Table I—Plasma Levels of Total Sulconazole Nitrate Radioequivalents and Concentration of Unchanged Drug in a Dog Given Tritium-Labeled Sulconazole Nitrate (1000 mg)

Hours after Dosing	Sulconazole Radioequivalents in Plasma, μg/ml	Unchanged Sulconazole, μg/ml
0.5	1.150	BDL[a]
1.0	4.343	1.27
2.0	12.356	4.81
4.0	14.755	2.64
6.0	15.929	1.45
8.0	14.772	0.90
24.0	6.864	BDL
48.0	4.623	BDL
72.0	3.645	BDL
96.0	3.331	BDL
168.0	2.379	BDL

[a] Below detectable level.

Dog Experiment—A female beagle received 1000 mg (four capsules) of tritiated sulconazole nitrate after an overnight fast. A 20-ml water wash immediately followed the dose, and feeding was resumed 4 hr after dosing. Blood samples were drawn at 0.5, 1, 2, 4, 6, 8, 24, 48, 72, 96, and 168 hr following drug administration. Plasma levels of sulconazole nitrate radioequivalents and unchanged sulconazole were determined in these samples.

Total Sulconazole Radioequivalent Determination—Total sulconazole radioequivalents in dog plasma were determined by liquid scintillation spectrometry. Plasma samples of 0.1–0.2 ml were mixed with 10 ml of scintillation fluid[9]. Corrections were made for quenching and machine[10] efficiency by the automatic external standard method.

RESULTS AND DISCUSSION

A sensitive and specific HPLC method was developed that utilized the natural 229-nm UV-absorbing property of sulconazole, measuring levels of ≥0.5 μg/ml.

A structurally similar compound miconazole nitrate 1-[2-(2,4-dichlorophenyl)-2-(2,4-dichlorobenzyloxy)ethyl]imidazole mononitrate (II), was used as the chromatographic internal standard. This compound possesses UV, extraction, and chemical properties similar to sulconazole and was chromatographically resolved under the described conditions.

Good chromatography was achieved by employing a relatively nonpolar extracting solvent containing no oxidants and a solvent system at a slightly basic pH. Reproducibility of this method was shown by construction of calibration curves over the concentration range of 0.5–5.0 μg/ml. The coefficients of variation obtained for the calibration curve points were 3.92, 2.05, 1.20, 2.5, and 1.09%, respectively (n = 5). A chromatogram of an unfortified plasma sample is shown in Fig. 1. Chromatograms of plasma samples fortified with 0.5 and 5 μg/ml of sulconazole are shown in Figs. 2 and 3, respectively. A chromatogram of a plasma sample taken from a dog 6 hr after oral dosing with a 1000-mg capsule of sulconazole nitrate is shown in Fig. 4.

A plasma sample from a dog dosed with sulconazole was extracted with methanol and subjected to TLC. An authentic sulconazole standard was run alongside on the same plate. The plate was developed with an ammonium hydroxide–chloroform–methanol system (1:99:1). After drying, 1-cm sections of the plate were scraped into liquid scintillation vials and counted for radioactivity. Based on R_f values, it was determined that the percentage of radioactivity at the sulconazole R_f value represented the same fraction of the total radioactivity as the level of sulconazole obtained by HPLC. These TLC data support the identity of the HPLC peak at the retention time of sulconazole.

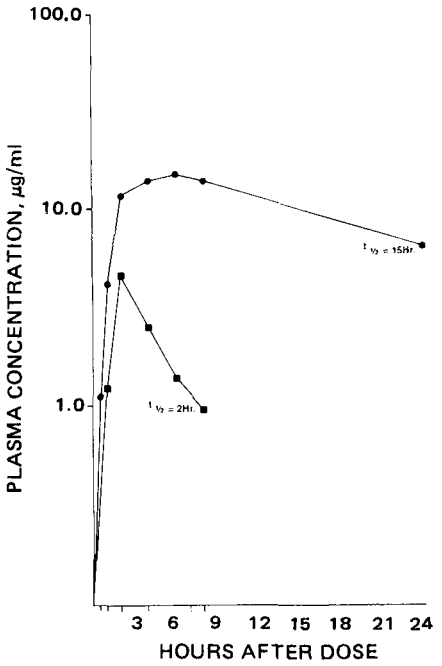


Figure 5—*Plasma levels of total sulconazole nitrate radioequivalents (●) and concentration of unchanged drug (■) in a dog given 1000 mg of tritium-labeled sulconazole nitrate.*

The overall recovery of sulconazole through the method was established by spiking plasma with tritiated sulconazole and processing the samples through the procedure. Radioactivity in the sample prior to injection onto the chromatograph was determined, and the recovery of sulconazole was 85%.

The developed method was applied to the analysis of plasma samples from a dog dosed with 1000 mg of tritiated sulconazole nitrate. Concentrations of total sulconazole radioequivalents and unchanged sulconazole in plasma are shown in Table 1 and Fig. 5. The peak unchanged sulconazole plasma level occurred at 2 hr following dose administration, and the peak total sulconazole radioequivalent level occurred at 6 hr after dosing. The half-life of unchanged sulconazole in this dog was 2 hr. The half-life observed for the total sulconazole radioequivalents was 15 hr. The data demonstrate that in the one animal studied, orally administered sulconazole is rapidly and extensively metabolized.

REFERENCES

(1) R. Brodie, L. Chasseaud, and L. Walmsley, *J. Chromatogr.*, **155**, 209 (1978).

(2) "Basic Gas Chromatography," 5th ed., H. M. McNair and E. J. Bonelli, Eds., Consolidated Printers, Berkeley, Calif., 1967, p. 33.

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[9] Oxifluor-H_2O, New England Nuclear, Boston, Mass.
[10] Scintillation counter, Packard Instrument Co., Chicago, Ill.