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Short communication

A chiral liquid chromatographic method for the determination of the enantiomers of the racemic triazole antifungal drug (SCH 39304) in human plasma

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1. Introduction

The compound, SCH 39304, (I)-(2*R**,3*R**)-2-(2,4-difluorophenyl)-3-methylsulfonyl-1-(1,2,4-triazol-1-yl)butan-2-ol, is a novel, potent triazole antifungal agent which has shown activity both orally and topically. It is active in vitro and in vivo against a broad range of fungal pathogens, including Aspergillus, Candida, Cryptococcus, Histoplasma and Trichophyton. SCH 39304 is a racemate which contains 50% of an active RR isomer (SCH 42427) and 50% of an inactive SS isomer (SCH 42426) (Fig. 1).

Although microbiological assays have been used to determine the in vitro activity of several imidazole drugs, the assays are variable, and are strongly influenced by the composition of various media. The assays also lack specificity in the presence of stereoisomers, active metabolites or co-administered antifungal agents.

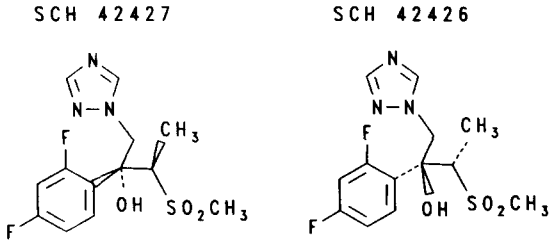

Fig. 1. Chemical structures of SCH 42427 and SCH 42426.

Recently, conventional LC and GLC methods have been developed to determine concentrations of SCH 39304 in human plasma. However, both methods determine the concentrations of the RR isomer (SCH 42427) and SS isomer (SCH 42426) collectively, and cannot quantitate the two stereoisomers selectively.

The separation of enantiomers of racemic drugs by LC has recently received considerable attention. A number of approaches including the use of chiral mobile phase addition [1], ligand exchange [2,3], charge transfer complex formation [4], chiral stationary phases [5] and derivatization of the enantiomer with a chiral reagent [6] have been reported. However, the use of a stable cyclodextrin bonded phase appears to be a more practical and widely applicable approach. Cyclodextrin bonded phase LC columns have been shown to be useful in separating enantiomers [7,8], diastereomers [8,9], and structural isomers [9,10]. The selectivity of cyclodextrin columns is often different from that of conventional reverse-phase columns, because the separation mechanism is based on an inclusion complex formation. A chiral liquid chromatography (LC) method was developed to evaluate the pharmacokinetic profile of the active RR isomer and the inactive SS isomer of SCH 39304 in man.

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2. Experimental

2.1. Reagents

SCH 39304, SCH 42426 and SCH 42427 were obtained from Sumitomo Corp., Japan. Anhydrous diethyl ether, acetonitrile and methanol were obtained from Mallincknodt Inc. (Paris, KY).

2.2. Sample preparation

A 0.5 ml aliquot of human plasma was diluted with 1 ml of water and extracted with 6 ml of anhydrous diethyl ether by mixing for 1 min on a Vortex mixer. After centrifugation, 5.5 ml of the organic layer was transferred into a clean centrifuge tube and evaporated to dryness under nitrogen at room temperature. The residue was then dissolved in 0.6 ml of water and 30 μl of this solution was injected into the LC system.

2.3. Chromatographic conditions

The chromatographic system consisted of a Waters model 6000A pump and a Model 480 absorbance detector set at a wavelength of 205 nm (Waters Association, Milford, MA). Separation was accomplished on a Cyclobond I (β-cyclodextrin) column (4.6 mm × 25 cm). The absorbance detector output was monitored with a 10 mV recorder and detector sensitivity was set at 0.01 absorbance units (full scale). The mobile phase was acetonitrile–methanol–water (7:15:80, v/v/v) at a flow-rate of 0.7 ml min^{-1}. All separations were carried out at ambient temperature.

2.4. Calculations

Peak height, retention time and concentration were calculated using an HP-3357 Laboratory automation system (Hewlett Packard Co., Palo Alto, CA). The slopes, intercepts and correlation coefficients were determined by least-squares linear regression analysis using a weighing factor of $1/y$.

3. Results and discussion

Typical chromatograms of extracted control plasma and control plasma spiked with SCH 42427 and SCH 42426 are shown in Fig. 2. The retention times for SCH 42427 and SCH 42426 were 9.8 min and 10.7 min, respectively. The retention times of endogenous substances in the plasma were compared with those of SCH 42427 and SCH 42426. Possible interference was determined by injecting solutions of the following drugs: fluconazole, miconazole, econazole, ketoconazole, sulconazole, terconazole, griseofulvin, aspirin, pseudoephedrine, chlorpheniramine, and acetaminophen. The method was specific, as demonstrated by the absence of interference from endogenous substances in the plasma and from many analogs and some drugs likely to be used concomitantly. The linearity of the assay was determined by the analysis of plasma samples containing 0.2–40 μg ml^{-1} of SCH 42427 and SCH 42426. Linear regression analysis of the peak height (y) versus the theoretical concentration (x) gave the following equations: $y = 1201x - 17.8$, $r^2 = 0.9999$ for SCH 42427; $y = 1073x + 9.6$, $r^2 = 0.9997$ for SCH 42426. The correlation coefficient (0.9999 for SCH 42427, and 0.9997 for SCH 42426) demonstrated the excellent linearity of the method over the concentration range analyzed.

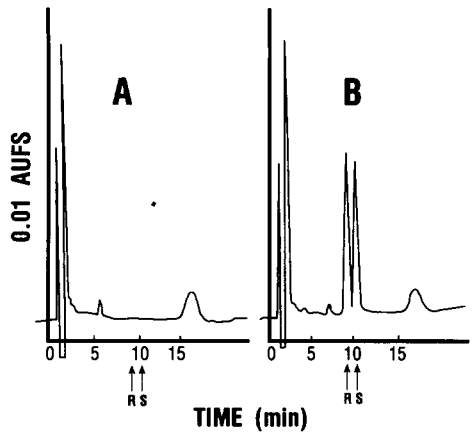

Fig. 2. Typical chromatogram of SCH 42427 (R) and SCH 42426 (S) from (A) control human plasma and (B) control human plasma spiked with 2.0 μg of SCH 42427 and SCH 42426 per ml. The y axis represents the detector response and the x axis represents the retention time.

The intra-day precision (expressed as RSD) and accuracy (expressed as % bias) were determined by assaying three concentrations (0.5, 2.0 and 5.0 μg ml^{-1}) of SCH 42427 and SCH 42426 in plasma with five replicates at each concentration on the same day.

Table 1
Intra-day precision and accuracy of the LC assay for SCH 42427 and SCH 42426 in human plasma

Compound	Theoretical concentration ($\mu g\ ml^{-1}$)	Observed concentration ($\mu g\ ml^{-1}$) [a]	RSD (%)	Bias (%)
SCH 42427	0.5	0.48	1.69	−3.34
	2.0	2.03	1.51	1.42
	5.0	5.09	1.58	1.77
SCH 42426	0.5	0.49	2.78	−1.00
	2.0	2.04	1.27	1.84
	5.0	5.00	1.87	−0.10

[a] Mean of five determinations.

Table 2
Inter-day precision of the LC assay for SCH 42427 and SCH 42426 in human plasma

Compound	Theoretical concentration ($\mu g\ ml^{-1}$)	Observed concentration ($\mu g\ ml^{-1}$)					RSD (%)
		Day 1	Day 2	Day 3	Day 4	Day 5	
SCH 42427	0.5	0.49	0.49	0.47	0.49	0.53	4.4
	2.0	2.03	2.02	2.01	1.98	2.05	1.3
	5.0	5.09	5.15	4.86	4.89	5.34	3.9
SCH 42426	0.5	0.50	0.48	0.50	0.53	0.54	4.8
	2.0	2.04	2.07	2.01	1.98	2.03	1.7
	5.0	5.00	5.27	4.89	4.95	5.25	3.5

The intra-day precision of the method was demonstrated by a low RSD (1.5–1.7% for SCH 42427 and 1.3–2.8% for SCH 42426) (Table 1). The bias was 1.4–3.3% for SCH 42427 and 0.1–1.8% for SCH 42426, indicating excellent accuracy of the methods (Table 1).

The inter-day precision was evaluated by assaying three concentrations (0.5, 2.0 and 5.0 $\mu g\ ml^{-1}$) of SCH 42427 and SCH 42426 in plasma with five replicate samples at each concentration over a period of five days. The inter-day precision of the method was also demonstrated by the low RSD (1.3–4.4% for

Table 3
Limit of quantitation [a] of the LC method for SCH 42427 and SCH 42426 in human plasma

Compound	Sample No.	Concentration added ($\mu g\ ml^{-1}$)	Concentration determined ($\mu g\ ml^{-1}$)	RSD (%)
SCH 42427	1	0.2	0.20	
	2	0.2	0.21	
	3	0.2	0.23	
	4	0.2	0.19	
	5	0.2	0.21	
	6	0.2	0.20	
	Mean		0.21	6.6
SCH 42426	1	0.2	0.21	
	2	0.2	0.21	
	3	0.2	0.22	
	4	0.2	0.20	
	5	0.2	0.20	
	6	0.2	0.20	
	Mean		0.21	4.0

[a] Signal-to-noise > 10.

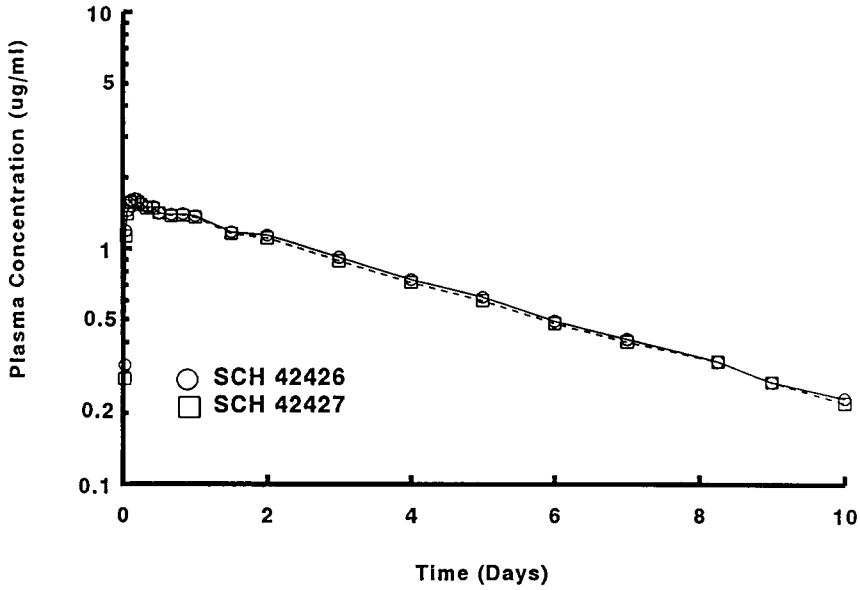

Fig. 3. Plasma concentrations of SCH 42426 and SCH 42427 in man following single administration of 200 mg SCH 39304.

Table 4
Pharmacokinetics of SCH 42426 and SCH 42427 in man following a single oral dose of 200 mg SCH 39304

Parameter	Mean (RSD (%))		
	SCH 42426 (I)	SCH 42427 (II)	Ratio I/II
C_{max} ($\mu g\ ml^{-1}$) [a]	1.7 (11)	1.7 (11)	1.00
T_{max} (h) [b]	4.0 (59)	4.2 (53)	
AUC (I) ($\mu g\ h\ ml^{-1}$) [c]	199.4 (16)	195.0 (16)	0.98
$t_{1/2}$ (h) [d]	83.3 (23)	82.9 (18)	

[a] Maximum plasma concentration.
[b] Time of maximum plasma concentration.
[c] Area under plasma concentration–time curve from time zero to infinity.
[d] Terminal phase half-life.

SCH 42427 and 1.7–4.8% for SCH 42426) (Table 2).

The limit of quantitation (LOQ) of the LC method was 0.2 $\mu g\ ml^{-1}$, which was the lowest concentration tested with a RSD of only 4–7% (Table 3). The recovery of SCH 42427 and SCH 42426 from plasma was determined by comparing the peak heights of SCH 42427 and SCH 42426 from spiked plasma with those from standard solutions of SCH 42427 and SCH 42426. The recovery of the drug at 1 $\mu g\ ml^{-1}$ was determined to be 87.5% for SCH 42427 and 88.3% for SCH 42426.

The method was used to analyze plasma samples from healthy volunteers following oral administration of a single dose of 200 mg SCH 39304. Mean plasma concentration–time curves for SCH 42427 and SCH 42426 were superimposable (Fig. 3), indicating similar pharmacokinetics for the two isomers. Further, the C_{max}, T_{max}, $t_{1/2}$, and AUC(I) values of SCH 42427 (active RR isomer) following oral administration of SCH 39304 (racemic mixture) were similar to the respective values for SCH 42426 (inactive SS isomer) (Table 4), indicating that the bioavailability and pharmacokinetics of the two isomers after oral administration of the racemate are similar.

In conclusion, a chiral LC assay for SCH 42427 and SCH 42426, the enantiomers of the racemic triazole antifungal agent SCH 39304, has been developed and validated in human plasma. The validated LC method has been utilized to evaluate the pharmacokinetics of the enantiomers following oral administration of a

single 200 mg dose of SCH 39304 to healthy human subjects.

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