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Journal of Chromatography B, 728 (1999) 133–141

JOURNAL OF CHROMATOGRAPHY B

High-performance liquid chromatographic analysis of the anti-tumor agent SCH 66336 in cynomolgus monkey plasma and evaluation of its chiral inversion in animals

Hong Kim[a,*], Paul Likhari[b], Chin-Chung Lin[a], Amin A. Nomeir[a]

[a]*Department of Drug Metabolism and Pharmacokinetics, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA*

[b]*Bioassay Laboratory, Houston, TX 77099, USA*

Received 8 December 1998; received in revised form 10 February 1999; accepted 10 February 1999

Abstract

SCH 66336 is a novel non-cytotoxic anti-tumor agent that is in phase I/II clinical trials for the treatment of solid tumors. This compound is a single enantiomer with one chiral center. Prior to evaluation of this drug candidate in man, it was necessary to evaluate its pharmacokinetics and possible chiral inversion in animals. Thus, high-performance liquid chromatographic (HPLC) methods have been developed for its determination in cynomolgus monkey plasma and for the evaluation of its chiral inversion in rats and cynomolgus monkeys. The achiral HPLC analysis involved extraction with 30% methylene chloride in hexane followed by separation on a CN column and quantitation by UV absorbance at 280 nm. The method was linear over a concentration range of 0.1 to 20 μg/ml in monkey plasma. The chiral HPLC analysis involved the use of a Chiralpak AD column set at 39°C with a mobile phase of hexane–ethanol–diethylamine mixture and a UV detector set at 280 nm. Plasma samples were subjected to solid-phase extraction on a C_2 cartridge prior to HPLC analysis. The method was linear over a concentration range of 0.25 to 10 μg/ml in rat and cynomolgus monkey plasma for both enantiomers. Both methods showed good linearity (r^2>0.99), accuracy (bias<13%) and precision (CV<12%). Chiral HPLC analysis indicated that SCH 66336 was not subjected to chiral inversion in rats and cynomolgus monkeys

Keywords: SCH 66336

1. Introduction

SCH66336, (+) 4-{2-[4-(8-chloro-3, 10-dibromo-6, 11-dihydro-5H-benzo [5,6] cyclohepta [1,2-b] pyridin-11-yl)-1-piperidinyl]-2-oxoethyl}-1-piperidinecarboxamide (Fig. 1), is a potent inhibitor of farnesyl protein transferase (FPT). FPT catalyzes the initial step in the post-translational processing of the *ras* gene, that is, the transfer of the 15-carbon farnesyl group onto a cysteine residue in the carboxy terminal of all Ras proteins [1–6]. The farnesylation step appears to be essential for Ras proteins to become membrane-associated and induces cell transformation [4]. In normal cells, Ras protein in its active GTP-bound state initiates several intracellular signaling pathways that lead to cell proliferation and differentiation. The signal is terminated by the hydrolysis of GTP to the inactive GDP-bound form

*Corresponding author.

0378-4347/99/$ – see front matter
PII: S0378-4347(99)00070-5

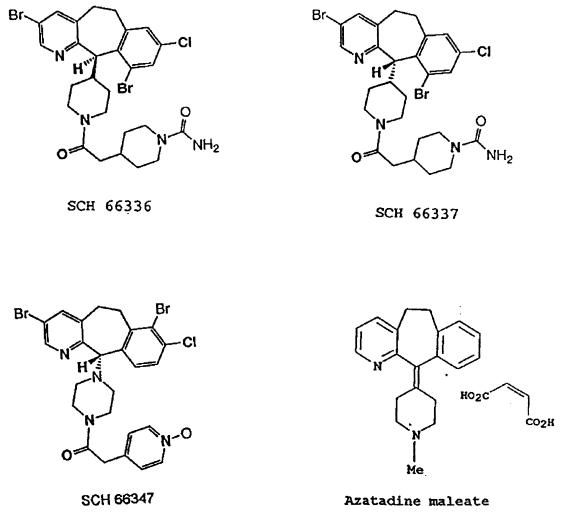

Fig. 1. Chemical structures of SCH 66336, SCH 66337, SCH 66347 and azatadine maleate.

of the Ras protein [7]. The capacity of certain oncogenic forms of Ras to hydrolyze GTP to GDP is greatly reduced, leading to continuous intracellular signaling which results in alteration of cellular proliferation and differentiation [4,8]. Oncogenic forms of Ras proteins have been reported to be associated with nearly one-third of all human cancers, including 50% of colon cancers and 90% of pancreatic cancers [4]. Blocking the farnesylation step by inhibiting FPT is an attractive target for the discovery of compounds with anti-tumor activity [9]. This form of therapy has enormous potential because of the prevalance of mutated *ras* gene in human cancers [4,10].

In vivo studies in nude mice demonstrated that SCH 66336 has a potent oral activity profile in a wide array of human tumor xenograft models including tumors of colon, lung, pancreas, prostate, and urinary bladder origin [11]. When used to treat Ha-Ras transgenic mice with spontaneously occurring tumors prior to tumor onset, SCH 66336 delayed tumor onset, reduced the average number of tumors per mouse and reduced the average tumor weight per animal [11]. In a therapeutic mode, where gavage treatment was initiated after the transgenic mice had developed palpable tumors, significant tumor regression was induced by SCH 66336 in a dose-dependent fashion [11].

The issue of chirality has emerged as a major theme in drug design and discovery [12,13]. Since enantiomers often differ in pharmacological activity, toxicity and pharmacokinetics characteristics, it is important that enantiomeric drug candidates be evaluated in an optically pure form. SCH 66336 is a single enantiomer which contains one chiral center. During the discovery stage of this compound, it was of interest to determine its pharmacokinetics and possible chiral inversion in animals prior to administration in humans. Therefore, these studies were initiated to develop HPLC methods for its determination in cynomolgus monkey plasma and for the evaluation of its chiral inversion in rat and cynomolgus monkey plasma that had been dosed with SCH 66336.

2. Experimental

2.1. Reagents

Hexane, methanol, acetonitrile, methylene chloride, ethanol, diethyl amine and sodium bicarbonate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). SCH 66336, SCH 66337 (enantiomer of SCH 66336) and SCH 66347 (internal standard for the chiral assay) were provided by Schering-Plough Research Institute (Kenilworth, NJ, USA). Azatadine maleate (internal standard for the achiral assay) was obtained from U.S. Pharmacopeial Convention, Inc. (Rockville, MD, USA).

2.2. Calibration standards

For the achiral HPLC analysis, stock solution of SCH 66336 was prepared in methanol at 200 μg/ml; the internal standard was prepared in water at 100 μg/ml. The stock solutions were stored at −20°C. Eight calibration standards (at 0.1, 0.2, 0.5, 1, 2, 5, 10, and 20 μg/ml) were prepared in triplicate on each of three validation days. Three sets of quality control samples (QC samples) at concentration of 0.2 (low), 1.5 (medium) and 15 μg/ml (high) were prepared in bulk from separate weighing, aliquotted and stored at −20°C for use during the entire validation and sample analysis. For the chiral HPLC assay, stock solutions of SCH 66336, SCH 66337

and SCH 66347 were prepared in methanol at 100 μg/ml and stored at −20°C. Three sets of quality control samples at concentration of 0.4 (low), 2.5 (medium) and 8.0 μg/ml (high) were prepared in bulk from separate weighings, aliquotted and stored at −20°C for use during the entire validation and sample analysis.

2.3. Sample preparation

For the achiral assay, a 0.2-ml volume of monkey plasma standard, QC sample or unknown was placed into a 15-ml screw-capped test tube containing 0.2 ml of internal standard solution (2 μg/ml). Saturated sodium bicarbonate solution (0.3 ml) was added followed by 4.0 ml of 30% (v/v) methylene chloride in hexane, mixed on a mechanical vortexer for 10 min and centrifuged. The organic layer was transferred into 15-ml conical test tubes and 20 μl of 0.8 *M* HCl in methanol was added. The samples were evaporated to dryness at 40°C under a gentle stream of nitrogen, the residue was reconstituted in 0.2 ml of mobile phase and 50 μl was injected onto the HPLC column for analysis.

For the chiral analysis, a 0.2-ml volume of rat or monkey plasma standard, QC sample, or unknown was applied onto100 mg of the solid-phase extraction (SPE) cartridge ethyl Bond Elute cartridge which has been pre-washed with 3 ml of methanol and 3 ml of water. The SPE cartridge was washed with 2 ml of water and 2 ml of 10% (v/v) acetonitrile in water. The compounds were eluted with 3 ml of acetonitrile. The eluate was evaporated to dryness under nitrogen and the residue was reconstituted with 0.5 ml of mobile phase; a 0.1 ml aliquot was injected onto the HPLC column for analysis.

2.4. Chromatographic conditions

The HPLC system consisted of a Shimadzu LC-10 AD pump and a Waters 486 absorbance detector set at 280 nm. The achiral separation was accomplished on a Spherisorb CN, 5 μm, 150×4.6 mm column which was preceded by an on-line filter. The Millennium 2020 Chromatography Manager Soft System was used for data handling. The mobile phase consisted of 0.02 *M* potassium phosphate (pH 4)–acetonitrile (65:35, v/v), and was delivered at 0.9 ml/min.

The chiral HPLC method involved the use of a Chiral Pak AD column (Chiral Technologies, Eaton, PA) set at 39°C and a mobile phase of hexane–ethanol–diethylamine (30:70:0.2, v/v/v) at a flow of 0.8 ml/min.

2.5. Administration of SCH 66336

Six male cynomolgus monkeys were dosed orally with a 100-mg experimental tablet of SCH 66336 (approximately 15 mg/kg) for pharmacokinetic evaluation. Also, rats and cynomolgus monkeys were given SCH 66336 at a single oral dose of 300 and 120 mg/kg, respectively, as a suspension in 0.4% methylcellulose for the evaluation of chiral inversion of SCH 66336. Blood samples were collected at intervals after dosing, centrifuged and plasma stored at −20°C pending analysis. Plasma concentrations equal to or above the limit of quantitation (LOQ, 0.1 μg/ml) were used for pharmacokinetic analysis using model-independent methods [14]. The maximum plasma concentration (C_{max}) and time of maximum plasma concentration (T_{max}) were the observed values. The area under the plasma concentration–time curve from time zero to the time of the final measurable sample [AUC(tf)] was calculated using the linear trapezoidal method, and was extrapolated to infinity (I) according to the following equation:

$$\mathrm{AUC}(I) = \mathrm{AUC}(\mathrm{tf}) + C(\mathrm{tf})/k$$

where C(tf) is the estimated concentration at tf using the above regression.

3. Results

3.1. Achiral HPLC

Typical chromatograms of drug-free monkey plasma and plasma spiked with SCH 66336 and the internal standard azatadine maleate are illustrated in Fig. 2. The retention times of SCH 66336 and the internal standard were approximately 7.2 and 10.5 min, respectively. There were no endogenous peaks

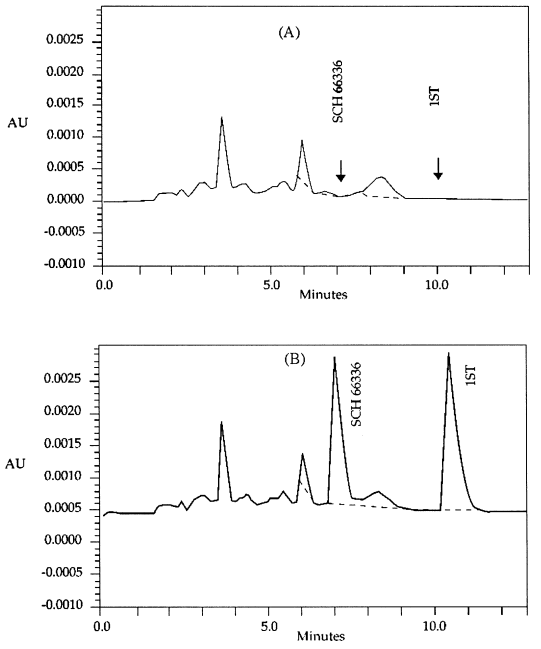

Fig. 2. Typical HPLC chromatograms of blank cynomolgus plasma (A), plasma spiked with SCH 66336 and the internal standard azatadine maleate (B).

in plasma of six undosed monkeys that coeluted with SCH 66336 or the internal standard, indicating that the method was selective. The linearity of the assay was evaluated over a concentration range of 0.1–20 μg/ml. Linear regression parameters of the peak height ratios versus concentrations along with back-calculated concentrations of nine calibration curves are presented in Table 1. The results showed highly reproducible calibration curves with correlation coefficients of >0.99, indicating that the response was linear over the concentration range studied. Intra-day precision and accuracy were evaluated at SCH 66336 concentrations of 0.2, 1.5 and 15 μg/ml. Six samples were analyzed at each concentration on the same day. The results showed satisfactory intra-day precision and accuracy as indicated by a coefficient of variation (CV) of $<2.5\%$ and a bias of $<5.1\%$ (Table 2). Inter-day precision and accuracy were evaluated at the same concentrations as above and the samples were analyzed on three separate days.

Table 2
Intra-day precision and accuracy for the analysis of SCH 66336 in cynomolgus monkey plasma[a]

	Nominal concentration (μg/ml)		
	0.2	1.5	15.0
	Concentration found (μg/ml)[b]		
Mean	0.21	1.56	15.6
Precision (% CV)	2.4	0.8	1.4
Accuracy (% bias)	5.0	4.0	4.0

[a] All samples were analyzed on the same day.
[b] $n=6$.

Table 1
Back-calculated concentrations and calibration curve parameters for the analysis of SCH 66336 in cynomolgus monkey plasma

	Nominal concentration								Slope	Intercept	Correlation coefficient (r)
	0.1	0.2	0.5	1	2	5	10	20			
	Concentration found (μg/ml)										
Day 1	0.11	0.19	0.50	1.00	1.99	5.11	10.00	19.90	0.475181	−0.019654	0.99994
	0.10	0.19	0.49	0.99	1.98	5.11	9.81	20.10	0.480203	−0.025003	0.99989
	0.11	0.20	0.46	1.01	2.02	5.05	9.90	20.10	0.477915	−0.023911	0.99991
Day 2	0.11	0.20	0.49	0.96	1.95	5.07	10.10	19.90	0.464526	−0.018828	0.99993
	0.10	0.21	0.51	0.97	2.00	4.94	10.00	20.00	0.467907	−0.019717	0.99997
	0.10	0.21	0.48	0.98	1.98	4.92	10.00	20.10	0.458017	−0.021231	0.99996
Day 3	0.11	0.19	0.50	0.99	2.00	5.01	9.90	20.10	0.454711	−0.020989	0.99995
	0.10	0.20	0.50	1.01	1.99	4.95	9.89	20.20	0.453478	−0.019740	0.99996
	0.10	0.19	0.51	0.99	2.01	4.95	9.89	20.10	0.448120	−0.023261	0.99995
Mean	0.10	0.20	0.49	0.99	1.99	5.01	9.94	20.10	0.461351	−0.022801	0.99994
Precision (% CV)	5.00	4.00	3.30	1.70	1.00	1.50	0.90	0.50	–[a]	–	–
Accuracy (% bias)	0.00	0.00	−2.00	−1.00	−0.50	0.20	−0.60	0.50	–	–	–

[a] Not appropriate to calculate for these parameters.

Table 3
Inter-day precision and accuracy for the analysis of SCH 66336 in cynomolgus monkey plasma[a]

	Nominal concentration (μg/ml)		
	0.2	1.5	15.0
	Concentration found (μg/ml)[b]		
Mean	0.21	1.58	15.9
Precision (% CV)	3.3	2.9	2.9
Accuracy (% bias)	5.0	5.3	6.0

[a] All samples were analyzed on 3 days.
[b] $n=3$.

The results demonstrated satisfactory inter-day precision and accuracy as shown by CV and bias values of $<3.4\%$ and $<6.1\%$, respectively (Table 3). The LOQ, defined as the lowest concentration in the calibration curve that could be determined with acceptable precision and accuracy, was 0.1 μg/ml. At this concentration, the precision and accuracy from the back-calculated concentrations were satisfactory (CV=5%, bias 0%; Table 1). The recovery was consistent over a concentration range of 0.2–15 μg/ml (>83%). The recovery of the internal standard at the concentration used (2.0 μg/ml) was 88.7%.

The stability of SCH 66336 in plasma was evaluated through three freeze–thaw cycles at concentrations of 0.2, 1.5 and 15 μg/ml. The samples were thawed in a water bath at room temperature and frozen within 5 min of thawing in each cycle. After three cycles, the changes from nominal concentrations were −5.0, +13.3 and +12.7% for the 0.2, 1.5 and 15 μg/ml, respectively, demonstrating that SCH 66336 was stable in plasma through three freeze–thaw cycles. Long-term stability was determined following 316 days of storage at −20°C at the concentrations above. The changes from the nominal concentrations ranged from 0.7–5.0% indicating that SCH 66336 was stable in plasma for at least 316 days. In-process stability was performed for up to 72 h after sample processing. Three sets of processed plasma samples at the above SCH 66336 concentrations were analyzed after storage at room temperature for 72 h. The changes from nominal concentrations were −5.0, 2.0 and 3.3% for the 0.2, 1.5 and 15 μg/ml, respectively, demonstrating that SCH 66336 was stable under the conditions evaluated.

3.2. Chiral HPLC

Typical chromatograms of drug-free rat and cynomolgus monkey plasma and plasma spiked with the internal standard, SCH 66336 and its enantiomer SCH 66337 are illustrated in Figs. 3 and 4, respectively. The retention times of SCH 66337, SCH 66336 and the internal standard were approximately 5.7, 8.3 and 13.4 min, respectively. There were no endogenous peaks in plasma of undosed rats or monkeys that coeluted with SCH 66337, SCH 66336 or the internal standard, indicating that the method was selective. The linearity was evaluated over a concentration range of 0.25–10 μg/ml, the correlation coefficients were >0.99 indicating that the response was linear over the concentration range studied (Tables 4 and 5). Intra-day precision and accuracy were evaluated at plasma SCH 66337 and SCH 66336 concentrations of 0.4, 2.5 and 8.0 μg/ml. Three samples were analyzed at each concentration on the same day; the results are shown in Table 6. Intra-day precision and accuracy were satisfactory as indicated by a CV of <13% and a bias of <12% for both enantiomers. The LOQ was 0.25 μg/ml; at this concentration, the precision and accuracy from the back-calculated concentrations were satisfactory (CV <14%, bias=6%). The recovery values at 10 μg/ml for SCH 66337, SCH 66336 and the internal standard were 83, 80 and 79%, respectively.

4. Discussion

SCH 66336 belongs to the tricyclic class of FPT inhibitors which are structurally distinct from other reported FPT inhibitors, many of which were derived from peptidomimetic approaches [15,16]. These differences, including the absence of a sulfhydryl function, are likely to contribute to the favorable oral pharmacokinetic properties of the tricyclic class. Initial compounds in this series, such as SCH 44342 ($IC_{50}=250$ n*M*), displayed less than optimal pharmacokinetic properties in the mouse, including a very rapid oxidative metabolism [15]. Blocking the susceptible metabolic sites on SCH 44342 greatly improved the pharmacokinetic properties of compounds in this series. SCH 66336 was the lead

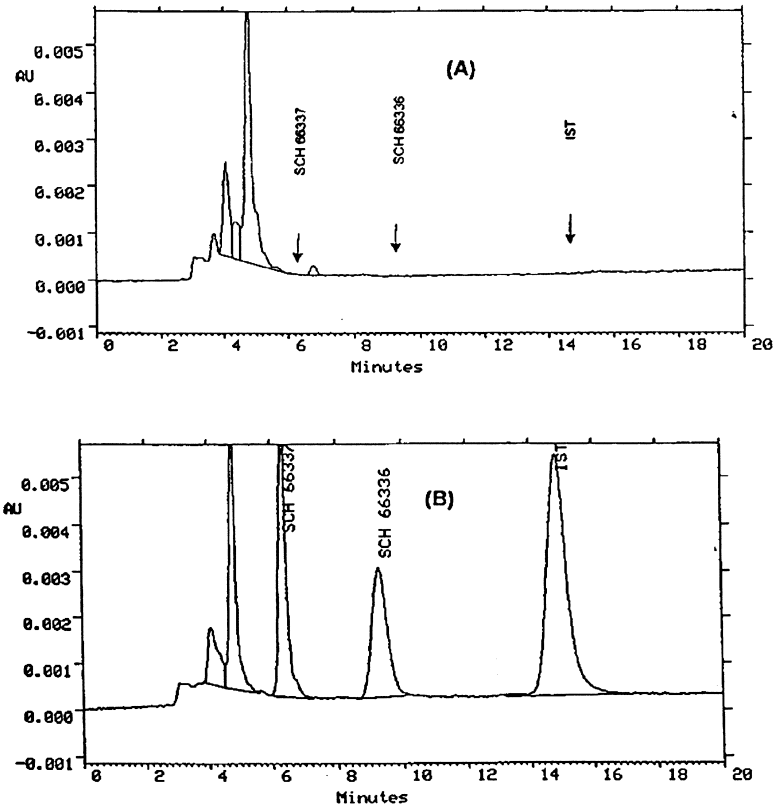

Fig. 3. Typical chiral HPLC chromatograms of blank cynomolgus plasma (A), plasma spiked with SCH 66336, SCH 66337 and the internal standard SCH 66347 (B).

compound that emerged from these efforts. In addition to improved metabolic stability, its intrinsic potency was also improved as indicated by an FPT IC_{50} of 1.9 n*M*. Due to these improvements, SCH 66336 persisted in mouse serum at concentrations greater than its IC_{50} for Ha-Ras processing in Cos cells for over 16 h following a single oral dose of 25 mg/kg [11].

We have attempted to improve the sensitivity of the achiral HPLC method by solid-phase extraction on ethyl column. However, because of the high plasma background, the sensitivity was reduced (LOQ was 0.25 μg/ml). Gas chromatography (GC) with electron capture detection (ECD) appeared to be a suitable mean for developing a sensitive assay, since the compound contains three halogens (Fig. 1). Because SCH 66336 was non-volatile, the GC column temperature needed to be kept above 320°C to observe a signal. However, the compound was thermally unstable at this high temperature.

The achiral analytical method was used to characterize the pharmacokinetic profile of SCH 66336 in the cynomolgus monkey treated orally with a 100-mg experimental capsule of SCH 66336. SCH 66336 was quantifiable in plasma between 2 and 24 h after oral administration (Fig. 5). The mean C_{max} of SCH 66336 was 6.82 μg/ml which was attained at a mean T_{max} of 7.67 h. The area under the plasma concentration–time curve ($AUC_{0-24\ h}$) was 84.5 μg h/ml (Table 7).

Enantiomers of biologically active molecules may differ in potency, pharmacological action, metabolism, toxicity and pharmacokinetics [12]. Therefore, it is highly desirable that only the active enantiomer be the drug candidate. The tools for chiral resolution have been progressively improving by the develop-

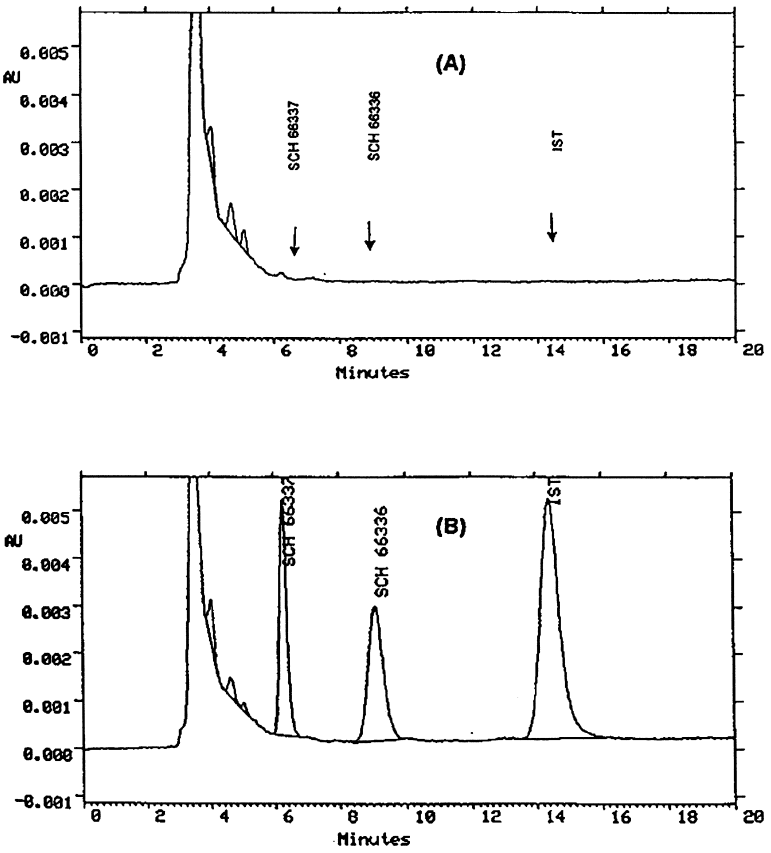

Fig. 4. Typical chiral HPLC chromatograms of blank rat plasma (A), plasma spiked with SCH 66336, SCH 66337 and the internal standard SCH 66347 (B).

ment of a great variety of chiral HPLC-stationary phases. Chiral phases containing cavities such as cyclodextrins or cellulose derivatives have played a significant role in the area of chiral resolution [13]. The chiral recognition of cavity-containing stationary phases is believed to be a result of the insertion of the aromatic portion of the solute into the chiral cavity, in addition to hydrogen bonding interactions. A great number of chiral drugs have been resolved on cyclodextrin (cyclobond), cellulose (chiralcel) or amylose (chiralpak) based columns.

Several chiral HPLC columns were evaluated for the separation of SCH 66336 and SCH 66336 including Cyclobond 1, Chiralcel OD (cellulose carbamate derivative) and Chiralpak AD (amylose carbamate derivative). The Cyclobond 1 and Chiralcel OD columns gave broad peaks with asymmetric peak shape (tailing) without baseline separations. The Chiralpak AD column was selected because of symmetrical peaks and baseline separation resulting in a better sensitivity.

The chiral HPLC method was used to evaluate the possible chiral inversion of SCH 66336 in animals. It should be pointed out that SCH 66336 administered to animals contained approximately 1.5–2.0% SCH 66337 as an impurity. In rat plasma, SCH 66337 concentrations were below the LOQ, while the concentrations of SCH 66336 ranged from 5.5–25.0 μg/ml. In cynomolgus monkey plasma, the concentrations of SCH 66336 were within the range of 0.32– 28.9 μg/ml, while those for SCH 66337 were within the range of below the LOQ to 0.9 μg/ml. The ratios of the concentrations of SCH 66337 to SCH 66337 in the samples that contained quantifiable levels of both analytes were within the range of 2.5–3%, which is similar to the ratio seen as an

Table 4
Back-calculated concentrations and calibration curve parameters for the chiral analysis of SCH 66336 and SCH 66337 in rats

Compound	Nominal concentration						Slope	Intercept	Correlation coefficient (r)
	0.25	0.5	1	2	5	10			
	Concentration found (μg/ml)								
SCH 66336	0.25	0.50	0.94	1.90	5.03	10.10	0.012123	−0.000401	0.999636
	0.28	0.43	0.91	1.99	5.00	10.11	0.116821	−0.008833	0.999639
	0.26	0.49	0.98	1.96	5.02	9.99	0.103509	−0.006071	0.999958
Mean	0.26	0.47	0.94	1.95	5.02	10.07	0.077485	−0.005102	0.999744
Precision (% CV)	5.80	8.00	3.70	2.40	0.30	0.70	–[a]	–	–
Accuracy (% bias)	5.10	−5.60	−6.00	−2.60	0.30	0.70	–	–	–
SCH 66337	0.29	0.45	1.13	2.03	5.23	9.75	0.107376	0.019340	0.998325
	0.24	0.50	1.00	2.03	5.12	9.85	0.099922	0.007260	0.999447
	0.23	0.49	1.11	1.98	5.15	9.79	0.103509	0.003007	0.998828
Mean	0.25	0.48	1.08	2.01	5.17	9.80	0.103602	0.009869	0.998867
Precision (% CV)	12.70	5.51	6.48	1.43	1.10	0.51	–[a]	–	–
Accuracy (% bias)	1.30	−4.20	7.40	0.70	3.20	−2.10	–	–	–

[a] Not appropriate to calculate for these parameters.

impurity in SCH 66336. Therefore, it is concluded that SCH 66336 is not subjected to chiral inversion in rats and cynomolgus monkeys.

In conclusion, the HPLC assay for the determination of SCH 66336 in cynomolgus monkey plasma was shown to be accurate and reliable over a concentration range of 0.1 to 20 μg/ml with an average CV of 6% and an average bias of 7%. The assay was used successfully to monitor plasma concentrations of SCH 66336 in cynomolgus monkey. A reliable chiral HPLC method was also developed to separate SCH 66336 from its enantiomer SCH 66337 in monkey and rat plasma. Chiral HPLC analysis indicated that SCH 66336 was not subjected to chiral inversion in rats and cynomolgus monkeys.

Table 5
Back-calculated concentrations and calibration curve parameters for the chiral analysis of SCH 66336 and SCH 66337 in c-monkeys.

Compound	Nominal concentration						Slope	Intercept	Correlation coefficient (r)
	0.25	0.5	1	2	5	10			
	Concentration found (μg/ml)								
SCH 66336	0.27	0.54	0.96	1.94	4.69	10.38	0.085505	−0.004286	0.996714
	0.24	0.49	0.98	1.89	5.04	10.14	0.084063	−0.003829	0.999461
	0.27	0.46	0.99	2.02	4.91	10.10	0.086208	−0.006383	0.999731
Mean	0.26	0.50	0.98	1.95	4.88	10.21	0.085258	−0.004833	0.998635
Precision (% CV)	6.70	8.14	1.56	3.36	3.60	1.48	–[a]	–	–
Accuracy (% bias)	3.80	−0.70	−2.40	−2.60	−2.50	2.00	–	–	–
SCH 66337	0.26	0.48	0.99	2.06	5.01	9.96	0.085788	−0.005390	0.999920
	0.24	0.50	1.08	1.90	5.01	10.20	0.086408	−0.008890	0.999073
	0.27	0.44	1.03	2.07	5.04	9.91	0.084192	−0.007384	0.999712
Mean	0.26	0.47	1.03	2.01	5.02	10.02	0.085463	−0.007221	0.999568
Precision (% CV)	6.00	6.50	4.40	4.70	0.30	1.50	–	–	–
Accuracy (% bias)	2.60	−5.60	3.20	0.50	0.40	0.20	–	–	–

[a] Not appropriate to calculate for these parameters.

Table 6
Intra-day precision and accuracy for the analysis of SCH 66336 and SCH 66337[a]

Species	Compound	Nominal Concentration (μg/ml)	Concentration found (μg/ml)[b]	Precision (% CV)	Accuracy (% bias)
Rat	SCH 66336	0.4	0.36	10.0	−11.1
		2.5	2.48	3.2	−0.8
		8.0	8.07	3.0	0.8
	SCH 66337	0.4	0.36	5.8	−12.1
		2.5	2.43	2.9	−2.7
		8.0	8.02	1.9	0.3
C-Monkey	SCH 66336	0.4	0.43	5.3	7.7
		2.5	2.39	1.5	−4.7
		8.0	8.22	2.8	2.6
	SCH 66337	0.4	0.42	11.6	5.5
		2.5	2.44	9.5	−2.6
		8.0	8.15	1.9	1.8

[a] All samples were analyzed on the same day.
[b] $n=3$.

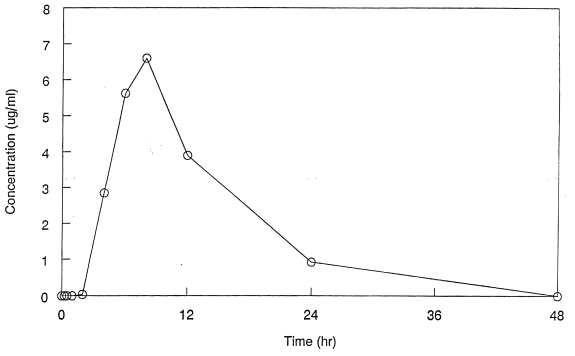

Fig. 5. Mean plasma concentration–time profile of SCH 66336 in cynomolgus monkeys after oral administration at a 100-mg tablet.

Table 7
Mean pharmacokinetic parameters of SCH 66336 in fed cynomolgus monkeys following oral administration of a 100-mg tablet

Parameter (unit)	Mean ($n=6$)	% CV
C_{max} (μg/ml)	6.82	39.93
T_{max} (h)	7.67	10.65
AUC $_{0-48\ h}$ (μg h/ml)	84.46	37.29

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