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High-performance liquid chromatographic analysis of the D_4 receptor antagonist SCH 66712 in rat plasma

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Abstract

SCH 66712 is a potent and selective dopamine D_4 receptor antagonist. An HPLC method was developed for the analysis of SCH 66712 in the plasma of rats, a species used for safety evaluation of this compound. The method involved solid-phase extraction on an ethyl cartridge and HPLC separation on a reversed-phase C_8 column with quantitation using a fluorescence detector. The calibration curve was linear over a concentration range of 5–100 ng/ml. The limit of quantitation was 5 ng/ml, where the coefficient of variation (C.V.) was 2.9% and the bias was 6%. The precision of the method was satisfactory as indicated by an intra-day C.V. of ≤4% and an inter-day C.V. of ≤6%. The accuracy was also satisfactory as shown by an intra-day bias of ≤8% and an inter-day bias of ≤9%. The assay was shown to be sensitive, specific, accurate, precise, and reliable for use in pharmacokinetic or toxicokinetic studies.

Keywords: SCH 66712; Dopamine D_4 receptor antagonist

1. Introduction

Excessive dopaminergic activity in the brain has been proposed as a factor in disease states such as schizophrenia [1]. Classical neuroleptics, which are presumed to act by antagonism of dopamine D_2 receptors, are useful for the treatment of the positive symptoms of schizophrenia, but cause major movement disorders which are thought to be due to blockade of D_2 receptors in the striatum [1,2]. The recent discovery of additional dopamine receptor subtypes (D_1–D_5) has renewed interest into this area to develop antipsychotic agents with enhanced efficacy and/or an improved safety profile [1]. Several investigators have examined the role of D_4 receptors in schizophrenia and their interaction with antipsychotic drugs. Patients with schizophrenia have been shown to have an elevated density of D_4 receptors in postmortem brain tissue [1–3]. Most antipsychotic drugs block D_4 receptors, although for all but one compound, the affinity for D_4 receptors is less than that for D_2 receptors [2]. The exception, clozapine, is a potent D_4 antagonist (K_i=9 n*M*) and has 5- to 20-fold higher affinity for D_4 than that for D_2 receptors [2]. The observation that the atypical antipsychotic clozapine has high affinity for D_4 receptors touched off a new phase of antipsychotic research [2].

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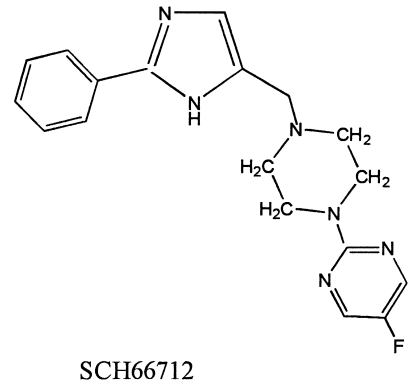
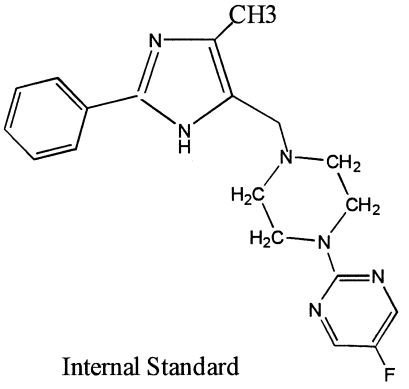
SCH 66712, 5-fluoro-2-[4-[(2-phenyl-1H-imidazol-5-yl)methyl]-1-piperazinyl]pyrimidine (Fig. 1), is a potent inhibitor of the dopamine D_4 receptor. The affinity of SCH 66712 for the D_4 receptor is 6.6 n*M*, and it is at least 400-fold selective for D_4 over other dopamine receptor subtypes [4]. In vivo, SCH 66712 potently antagonizes apomorphine-induced blockade of pre-pulse inhibition of a startle response in rats, with a minimal effective dose of 0.03–0.125 mg/kg [4]. This test, while not proven to be predictive of efficacy in schizophrenia, is thought to reflect activity against some of the symptoms observed in patients.

A sensitive, precise and accurate analytical method for the analysis of SCH 66712 in various biological matrices was needed in order to evaluate the pharmacokinetics and toxicokinetics of this drug candidate in various species. Since the rat is one of the toxicology species, a method was developed in rat plasma as the first biological matrix. This report describes an HPLC method developed for the analysis of SCH 66712 in rat plasma.



SCH66712



Internal Standard

Fig. 1. Chemical structures of SCH 66712 and internal standard.

2. Experimental

2.1. Reagents

SCH 66712 and NGB-1595 (internal standard) were provided by Neurogen Corporation (Branford, CT, USA). The chemical structures of these compounds are shown in Fig. 1. All other chemicals were obtained from Fisher Scientific (Springfield, NJ, USA) and were HPLC grade. Ethyl Bond Elute columns were purchased from Jones Chromatography (Lakewood, CO, USA).

2.2. Calibration standards

Stock solutions of SCH 66712 and the internal standard were prepared in methanol at 1000 ng/ml. The stock solutions were stored at −20°C. Five calibration curve standards (at 5, 10, 20, 50 and 100 ng/ml) were prepared on each of five validation days. Three sets of quality control samples (QC samples) at concentrations of 8 (low), 25 (medium) and 80 ng/ml (high) were prepared in bulk from a separate weighing, aliquotted and stored at −20°C for use during the entire validation.

2.3. Sample preparation

A 0.2-ml volume of plasma containing SCH 66712 at various concentrations was diluted with 1.5 ml of 0.25 *M* potassium phosphate buffer, pH 8.0, followed by the addition of 20 μl of the internal standard. The diluted plasma sample was slowly applied on a 1 ml disposable ethyl cartridge (Jones Chromatography) which had been successively prewashed with 2 ml of methanol, 1 ml of acetonitrile, 1 ml of water and 1 ml of 0.25 *M* phosphate buffer, pH 8.0, using a vacuum manifold (3–5 mmHg, Spe-ed Mate™-30, Applied Separations, Allentown, PA, USA). The loaded cartridge was washed with 1 ml phosphate buffer followed by 2 ml of water, then dried by airflow through under vacuum for 5 min. SCH 66712 was eluted from the cartridge with 2×

0.6 ml of methyl-*t*-butyl ether (saturated with water) containing 8% of triethylamine, and the eluate was evaporated to dryness under nitrogen. The residue was dissolved in 0.4 ml of mobile phase and a 90-μl aliquot was injected onto the HPLC column.

2.4. Chromatographic conditions

The HPLC system consisted of a Hitachi Model L-7100 solvent delivery system, a YMC basic column (150×4.6 mm, 5 μm), a Hitachi Model L-7480 fluorescence detector and a Waters LIMS Monitor (VT340). The detector was set at an excitation wavelength of 260 nm and an emission wavelength of 400 nm. The mobile phase consisted of 35% acetonitrile and 65% 0.05 *M* ammonium acetate and delivered at 1.0 ml/min. All analyses were carried out at ambient temperature. Peak integrations were performed using Waters Expert Ease version 2.3. The slopes, intercepts and correlations (r^2) were determined using the least-squares linear regression analysis with a weighting factor of $1/y^2$.

2.5. Administration of SCH 66712 and sample collection

The toxicokinetics of SCH 66712 were evaluated in the rat. The compound was administered orally to male and female rats (two male and two female rats/time-point) as a solution in sterile water (for irrigation, USP) at daily doses of 5 and 30 mg/kg. Blood samples were obtained at 0.25, 0.5, 1, 2, 4, 6, 8, 10 and 24 h after dosing on day 1 and at 0.5, 1, 6, 8, 24 h post dose on day 57 (week 9). The five sampling times selected on day 57 were based on a sparse sampling assessment from the day 1 data [5].

3. Results and discussion

HPLC methods with fluorescence detection have been widely used to determine the plasma and serum concentrations of drugs [6,7]. Preliminary studies using conventional HPLC analysis by UV absorbance at 260 nm (λ_{max}) showed that the limit of quantitation (LOQ) was 50 ng/ml of plasma which could not provide the adequate sensitivity to conduct pharmacokinetic studies in animals and man. GC analysis using nitrogen–phosphorus detection appeared to be the most suitable means of developing a very sensitive assay since the compound contains six nitrogen atoms (Fig. 1). The GC assay for SCH 66712 involved extraction with organic solvent followed by separation on a capillary column (Carbowax amine). The method was more sensitive with a limit of quantitation of 10 ng/ml, but the column gave asymmetric peak shapes (tailing). HPLC assay with fluorescence detection (excitation wavelength of 260 nm and emission wavelength of 400 nm) after a single extraction with organic solvent had a limit of quantitation of 20 ng/ml due to plasma background. Reproducible calibration curves with correlation coefficients of >0.99 indicated that the response was linear over a concentration range of 20–400 ng/ml. This method was relatively simple and accurate for measuring high plasma concentrations. However, it was necessary to develop a more sensitive assay for the determination of the compound in plasma at low doses. The best result was obtained by solid-phase extraction using an ethyl column.

Typical chromatograms of extracted control plasma and control plasma containing SCH 66712 and the internal standard are shown in Fig. 2. The retention times for SCH 66712 and the internal standard were 5.7 and 6.7 min, respectively. The method was specific as demonstrated by the absence of interference peaks in blank plasma of six different rats. The linearity was determined by the analysis of plasma samples containing 5–100 ng/ml of SCH 66712. Linear regression analysis of the peak height ratio (y) versus nominal concentration (x) gave the following equation: $y=0.01541x-0.0131$. The correlation coefficient (0.9995) demonstrated excellent linearity over the range studied. The LOQ, defined as the lowest concentration in the calibration curve that could be determined with acceptable precision and accuracy, was 5 ng/ml. At this concentration, the precision and accuracy from the back-calculated concentrations were satisfactory (C.V.=2.9%, bias=6.0%, Table 1). The intra-day precision (expressed as coefficient of variation,%C.V.) and accuracy (expressed as % bias) were determined by assaying three concentrations (10, 25 and 50 ng/ml) with five replicates each in rat plasma on the same day. The intra-day precision was demonstrated by a low coefficient of variation range (0.1–3.9%). The bias

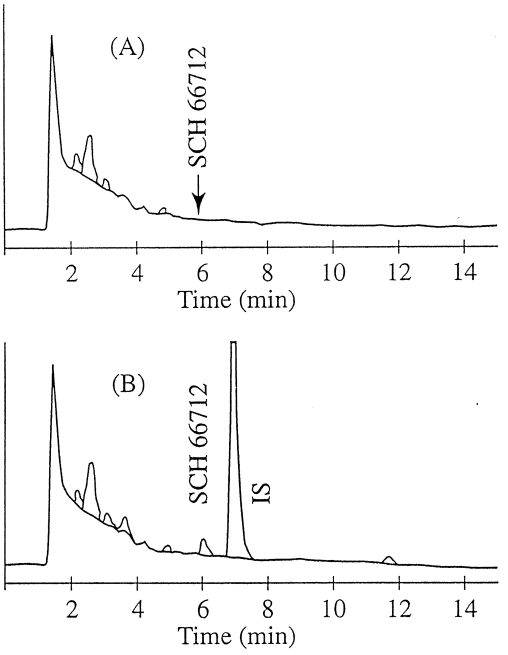


Fig. 2. Typical chromatogram of blank rat plasma (A) and blank rat plasma spiked with both SCH 66712 at 5 ng/ml (LOQ) and the internal standard at 50 ng/ml (B).

range was −7.6 to 2.4%, indicating satisfactory accuracy (Table 2).

The inter-day precision and accuracy were evaluated at concentrations of 8, 25 and 80 ng/ml. The samples were analyzed on five consecutive days. The inter-day precision and accuracy were satisfactory as indicated by a C.V. of ≤7% and a bias of ≤9%. The absolute recovery was determined by comparing the peak height of extracted SCH 66712 to the peak height of non-extracted standards at 10, 25 and 50 ng/ml. The average recovery of SCH 66712 was 83% (Table 3). The average recovery of the internal standard was 85%. The stability of SCH 66712 in plasma was evaluated through three freeze/thaw cycles at 60 ng/ml. After three cycles, the changes from nominal concentration were less than 5%, demonstrating that SCH 66712 was stable in plasma through three freeze/thaw cycles.

Table 2
Intra-day precision and accuracy for the analysis of SCH 66712 in rat plasma[a]

Parameter	Nominal concentration (ng/ml)		
	10	25	50
	Concentration found (ng/ml)[b]		
Mean	9.8	23.1	51.2
Precision (%C.V.)	0.1	3.0	3.9
Accuracy (% bias)	−2.0	−7.6	2.4

[a] All samples were analyzed on the same day.
[b] $n=5$.

Table 3
Recovery of SCH 66712 and internal standard from rat plasma

Concentration (ng/ml)	Mean % recovery[a]	%C.V.
10	81.9	4.6
25	81.0	3.8
50	84.9	3.5
I.S., 50.0	85.0	5.7

[a] $n=5$.

Table 1
Back-calculated concentrations and calibration curve parameters for the analysis of SCH 66712 in rat plasma

Standard curve used	Nominal concentration					Slope	Intercept	Correlation coefficient (r)
	5	10	20	50	100			
	Concentration found (ng/ml)							
1	5.4	9.8	18.4	50.8	99.9	0.015252	−0.0122	0.99984
2	5.5	10.0	17.4	51.7	99.6	0.015061	−0.0185	0.99959
3	5.4	9.8	17.7	50.3	99.5	0.015486	−0.0165	0.99974
4	5.3	10.3	17.77	49.8	100.5	0.015653	−0.0175	0.99958
5	5.1	9.6	17.9	54.3	106.3	0.015582	−0.0009	0.99851
Mean	5.3	9.9	17.9	51.4	101.2	0.015407	−0.0131	0.99945
Precision (%C.V.)	2.9	2.7	2.0	3.5	2.9	1.6	54.8	0.1
Accuracy (% bias)	6.0	−1.0	−10.5	2.8	1.2	–[a]	–	–

[a] Not appropriate to calculate for these parameters.

Table 4
Inter-day precision and accuracy of SCH 66712 calibration curve standards during sample analysis[a]

Parameter	Nominal concentration (ng/ml)					
	30	50	100	300	500	800
	Concentration found (ng/ml)[b]					
Mean	27.2	50.5	112.4	285.8	495.8	808.1
Precision (%C.V.)	8.8	9.6	5.1	3.2	4.3	4.9
Accuracy (% bias)	−9.4	1.0	12.4	−4.7	−0.8	1.0

[a] Samples were analyzed on 6 days.
[b] $n=18$.

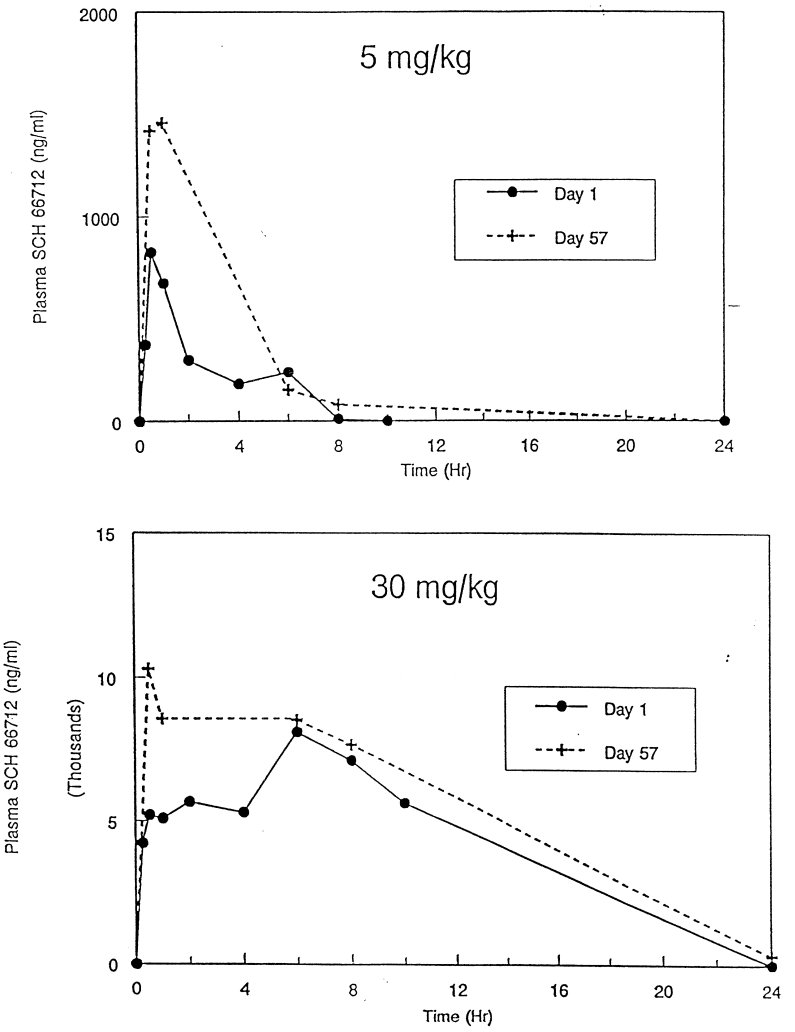


Fig. 3. Mean ($n=4$) plasma concentration–time profiles of SCH 66712 in rats after single and multiple oral doses.

The analytical method was used to evaluate the toxicokinetics of SCH 66712 in rats following oral administration in drinking water at daily doses of 5 and 30 mg/kg for 3 months. The linear range of the calibration curve for the toxicokinetic study was extended by the analysis of plasma samples containing 30–800 ng SCH 66712/ml. The relationship between detector response and the concentration of SCH 66712 was linear and reproducible from 30 to 800 ng SCH 66712/ml of rat plasma (Table 4). Plasma samples with concentrations of SCH 66712 greater than 800 ng/ml were diluted with control rat plasma and re-analyzed. Inter-day precision and accuracy of quality control samples were evaluated at plasma SCH 66712 concentrations of 60, 320 and 640 ng/ml. Inter-day precision and accuracy were satisfactory as indicated by a C.V. of $<10\%$ and a bias of $<7\%$.

Mean ($n=4$) plasma concentration–time profiles of SCH 66712 after single and multiple doses are illustrated in Fig. 3. Dose-related increases in plasma concentrations of SCH 66712 were noted between 5 and 30 mg/kg on both sampling days. Female rats generally had somewhat higher plasma concentrations than the males at both sampling intervals. Although SCH 66712 concentrations were somewhat higher on day 57 than on day 1 at some time-points, there was considerable overlap indicating little or no accumulation of SCH 66712 occurred in the plasma of rats with multiple dosing.

In conclusion, an HPLC assay for the determination of SCH 66712 in rat plasma was developed and was shown to be linear over the ranges studied with satisfactory precision and accuracy. The method was used for monitoring plasma SCH 66712 levels in rats following single or multiple oral doses.

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