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DETERMINATION OF PIRLINDOLE IN PLASMA AND URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A high-performance liquid chromatographic method is described for the analysis of pirlindole [2,3,3a,4,5,6-hexahydro-8-methyl-1H-pyrazino(3,2,1-*jk*)carbazole hydrochloride], a new antidepressive drug. The drug was extracted from plasma into dichloromethane, and the analysis was carried out on a reversed-phase column, the effluent being monitored by fluorescence detection. The method is selective and sensitive (limit of detection 1—2 ng/ml plasma). Urine analysis was done by direct injection of the diluted sample. The method was applied to the analysis of plasma and urine samples of eight healthy male volunteers who received a 75-mg oral dose of a tablet formulation of pirlindole. The method was also applied to a study in three beagle dogs which received pirlindole (1 mg/kg) by infusion (0.1 mg/kg/min) and orally (10 mg/kg) to estimate the absolute bioavailability of the drug.

INTRODUCTION

Pirlindole* [2,3,3a,4,5,6-hexahydro-8-methyl-1H-pyrazino(3,2,1-*jk*)carbazole hydrochloride] is a new antidepressive drug. Its clinical efficacy is comparable to amitriptyline. Imipramine, desipramine and maprotiline, but the mechanism of action is different. Pirlindole has in vitro and in vivo monoamine oxidase A inhibitory activity which is reversible and short-lasting. The drug also inhibits

*Euthym®, manufactured by Cassella Riedel Pharma GmbH, Frankfurt/Main, F.R.G.;

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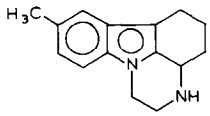

Pirlindole

the neuronal re-uptake of norepinephrine and serotinin into presynaptic vesicles. It does not possess anticholinergic activity [1, 2].

Up to now no selective analytical method has been available to measure the pharmacokinetics of the unchanged drug in body fluids. This paper describes a selective and sensitive high-performance liquid chromatographic (HPLC) method for the determination of pirlindole in plasma and urine and its application to man and dog.

EXPERIMENTAL

Apparatus and materials

The chromatograph consisted of a pump (M6000, Waters, Eschborn, F.R.G.), an automatic injector (WISP 710, Waters), and a fluorescence detector (650-10LC variable-wavelength monitor, Perkin Elmer, Offenbach, F.R.G.) operated at excitation 295 nm and emission 340 nm. The separation column was a 300 × 3.9 mm I.D. stainless-steel tube packed with μBondapak C_{18} reversed phase (particle size 10 μm, Waters).

The mobile phase consisted of the components acetonitrile (HPLC grade S, Rathburn Chemicals, Walkerburn, U.K.), methanol (Riedel, Seelze, F.R.G., No. 32 213), and 0.05 *M* phosphoric acid (Riedel, No. 30 417), buffered at pH 3.5 with aqueous tetraethylammonium hydroxide (Riedel, No. 16 262). All chemicals and solvents were of analytical reagent grade at least and used without further pretreatment. Stock solutions of pirlindole were freshly prepared in distilled water every day.

Analysis of plasma and urine samples

To 1.0 ml of plasma were added 0.1 ml of an aqueous solution of 0.1 *M* Na_2—EDTA (Riedel, No. 34 549, Idranal III) and 5 ml of dichloromethane (Riedel, No. 32 222) saturated with 0.1 *M* aqueous Na_2-EDTA. The mixture was agitated in stoppered 10-ml glass tubes for 20 sec. After centrifuging (6000 *g*, 5 min, 5° C) to separate the layers, 4 ml of the organic phase were transferred to a tapered tube and mixed with 0.1 ml of a freshly prepared methanolic solution of 0.01 *M* Na_2—EDTA. This solution was evaporated to dryness in a stream of nitrogen at 55° C in about 25 min. The dry residue was taken up in 0.2 ml of the mobile phase, acetonitrile—methanol—phosphoric acid (40:1:60, v/v), agitated using a Vortex mixer for 10 sec and centrifuged for 1 min before injection into the chromatograph. The above procedure was duplicated for each plasma sample.

Pirlindole peak height was measured and plasma concentration calculated by reference to a calibration curve. Urine samples (50 μl diluted in 500 μl of mobile phase, acetonitrile—methanol—phosphoric acid—PIC B-7, 35:1:70:1.3, v/v) were analysed by direct injection into the chromatograph. Plasma and urine samples are stable in the frozen state and dark at −20° C for at least one year.

The HPLC method was applied to the analysis of pirlindole in plasma and urine samples of eight healthy male volunteers (27—57 years, body weight 63—88 kg), who had given informed consent. All subjects received the drug as a 75-mg tablet. The method was also applied to a study in three fasted beagle dogs, two male and one female, body weight 14—18 kg, which received 1.0—1.1 mg/kg pirlindole by infusion (0.1 mg/kg/min) and 10 mg/kg pirlindole per os (aqueous solution).

Calculation of pharmacokinetic parameters

Calculation of the area under the plasma concentration—time curve, AUC_∞, the terminal half-life of elimination, $t_{½}$, total plasma clearance, Cl_{tot}, and the absolute bioavailability, f, were based on the RIP-fitting procedure [3] using a desk computer (WANG 2200). When analysing human data, areas were calculated by means of the trapezoidal rule. Mean transit times of pirlindole after intravenous (T_{vss}) and oral (T_{sys}) administration were estimated to obtain information about the steady-state volume of distribution V_{ss} ($V_{ss} = Cl_{tot} \times T_{vss}$) and the rate constant k_a of the drug's entrance ("invasion") into the central compartment from the gastrointestinal tract. k_a does not relate to absorptive processes alone, but rather reflects the joint effects of several subprocesses including most often distribution into readily accessible side compartments [4]. The concept of moment analysis from plasma data is valid if the drug was cleared exclusively from the central compartment, and if invasion and elimination were strictly first-order processes.

Statistics

Mean values are given as arithmetical means together with their standard deviations (S.D.). Statistical analysis of calibration curves was done by linear-regression routine on a desk computer (WANG 2200). A new non-parametric regression procedure [5] was used for comparison of two analytical methods for determination of pirlindole in plasma.

RESULTS AND DISCUSSION

Chromatography and recovery from plasma

Pirlindole was added to human plasma and extracted at physiological pH. Good separation of the drug from the endogenous matrix was obtained on an octadecylsilyl reversed-phase column, eluting with an acetonitrile—methanol—phosphoric acid mobile phase as described above (Fig. 1).

This chromatographic procedure is also suitable for dog and rat plasma. The recovery of pirlindole from plasma was determined by referring the peak height to a calibration curve obtained by analysing aqueous solutions of the drug, and amounted to 78 ± 3%.

Calibration

Pirlindole was added to plasma to give six replicate samples at five concentrations between 5 and 100 ng/ml (C_{added}). This is the concentration range typically found in the plasma of human subjects following an oral dose of 75 mg. The samples were processed as described and the peak height of the

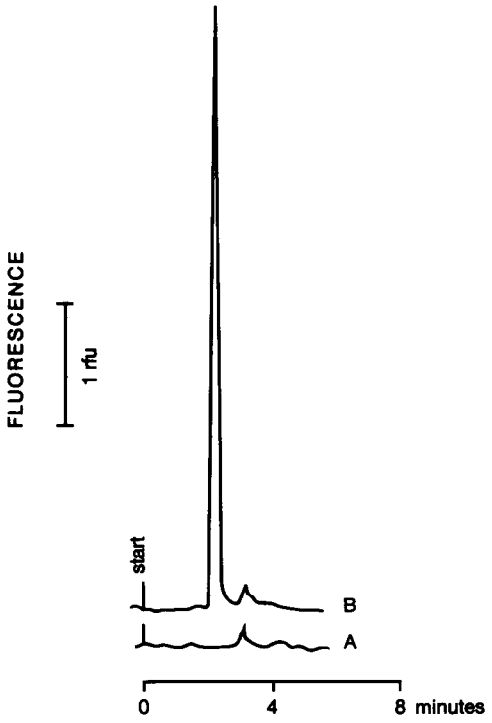

Fig. 1. (A) Chromatogram of an extract of human plasma (blank). (B) Chromatogram of an extract of human plasma to which had been added pirlindole to give a plasma concentration of 50 ng/ml (rfu = relative fluorescence units).

fluorescence signal (h) depends linearly on the concentration of pirlindole. The linearity of the calibration curve holds up to 500 ng/ml as the analysis of supplementary samples has shown.

Precision

The precision of the method [8] was expressed as standard deviation (S.D.) or relative standard deviation (C.V.) of the measured concentration values C_{found}. The within-run precision changed with C_{found} in an approximately

TABLE I

PRECISION (C.V.) OF THE HPLC METHOD FOR DETERMINATION OF THE CONCENTRATION OF PIRLINDOLE IN PLASMA (C_{found})

$C_{found} \approx (0.99 \pm 0.02) \times C_{added}$.

C_{added} (ng/ml)	C_{found} ± S.D. (ng/ml)	Coefficient of variation (C.V., %)
5	5.1 ± 1.1	21.6
10	9.0 ± 0.9	10.0
20	19.4 ± 1.3	6.7
50	45.2 ± 2.3	5.1
100	99.7 ± 5.1	5.1

linear relationship S.D. $\approx 0.4 + 0.047 \times C_{found}$ in the concentration range of therapeutic relevance (Table I). The between-run precision was 6.9% for a spiked plasma sample.

Accuracy

Accuracy [6] — referred to as bias of ($C_{added} - C_{found}$) of each of the concentrations — was tested from the same samples that were used for testing precision (Table I). Regression of C_{added} versus C_{found} shows that C_{found} is very similar to C_{added} [$C_{found} \approx (0.99 \pm 0.02) \times C_{added}$].

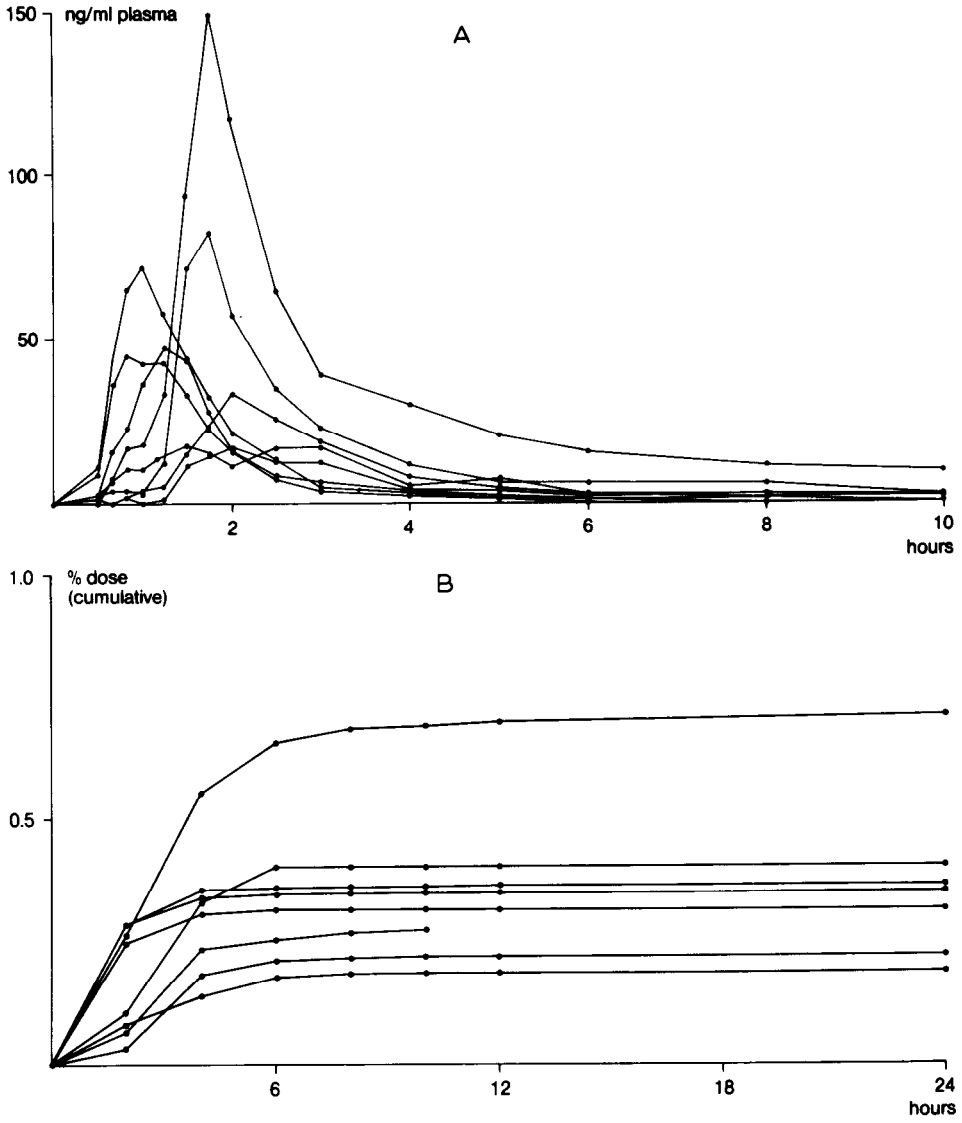

Fig. 2. Plasma levels (A) and urine excretion (B) of pirlindole in eight fasted healthy male volunteers after a single oral dose of 75 mg of pirlindole (tablet).

Sensitivity

As the standard deviations of six blank samples and of six replicates in the low concentration range are nearly equal (S.D. ≈ 0.4 ng/ml), the limit of detection (L.D.) was taken as 3.0 × S.D. This means the L.D. is in the range of 1–2 ng/ml pirlindole when extracting 1 ml plasma for analysis.

Selectivity

The selectivity of the method was imparted by the chromatographic system and the fluorescence detection wavelength. Additional evidence for the selectivity is that the fluorescence of the main metabolites in plasma of man, dog and rat do not interfere in the chromatographic analysis of pirlindole. The HPLC procedure has been validated by reference to a second analytical technique measuring fluorescence [7]. Human plasma samples were prepared by admixing known amounts of pirlindole. Equivalent results were obtained from both analytical methods measuring the plasma concentration of pirlindole (slope B = 0.987), as could be shown by non-parametric linear-regression analysis [5].

Application of the HPLC method

In routine practice, plasma and urine samples are analysed as independent duplicates. Calibration of the instrument is adjusted daily. By processing five freshly prepared external plasma or urine standards containing a known amount of pirlindole (C_{stand}) in the upper expected concentration range, the calibration line $h_{stand} = f \times C_{stand}$ is constructed by plotting the peak heights of the blank-corrected fluorescence signals of the five samples against C_{stand} and joining the mean peak height at C_{stand} with the zero coordinates. For purposes of quality control, another series of deep-frozen plasma or urine samples with a lower but known content of pirlindole ($C_{control}$) between zero

TABLE II

PHARMACOKINETIC PARAMETERS OF PIRLINDOLE CALCULATED FROM CONCENTRATIONS IN PLASMA AND URINE OF FASTED HEALTHY MALE VOLUNTEERS AFTER A SINGLE DOSE OF A 75-mg TABLET

Volunteer No.	C_{pmax} (ng ml^{-1})	t_{max} (h)	$t_{½,\,terminal}$ (h)	AUC_∞ (ng ml^{-1} h)	AUC_{ex} (%)	Cl_{tot}/f (l h^{-1})	Cl_r (l h^{-1})
1	17.1	2.00	0.98	37.2	4.3	2016.1	4.5
2	47.4	1.25	1.07	69.1	2.9	1085.4	3.4
3	82.2	1.75	3.10	148.8	5.7	503.9	1.4
4	72.3	1.00	1.90	98.2	5.9	763.1	2.7
5	42.8	1.25	2.00	67.1	0	1117.7	4.1
6	148.8	1.75	6.51	383.4	4.9	195.5	1.4
7	33.7	2.00	5.19	87.5	14.5	856.6	3.4
8	17.5	1.50	2.06	67.2	5.3	1115.9	2.1
Median	45.1		2.03	78.3		971.0	3.1
Minimum	17.1	1.00	0.98	37.2		195.5	1.4
Maximum	148.8	2.00	6.51	383.4		2016.1	4.5
$\bar{X}$	57.7		2.85	119.8		956.8	2.9
S.D.	43.6		1.99	111.3		537.5	1.2

and C_{stand} are measured. Whenever there is a tendency of exceeding 2 S.D. of $C_{control}$, routine analysis is stopped in favour of a trouble-shooting programme.

The HPLC method has been applied to the analysis of pirlindole in plasma and urine following a 75-mg oral dose as a tablet to eight healthy male volunteers. The plasma concentration—time course (Fig. 2) could be measured in all cases, and pharmacokinetic parameters were calculated (Table II). Only 0.4 ± 0.2% of the administered drug was excreted unchanged in the urine (Fig. 2 and Table II). The mean value of renal clearance was low ($Cl_r = 2.9 \pm 1.21$ l/h). The drug was also administered intravenously (1 mg/kg) and orally (10 mg/kg)

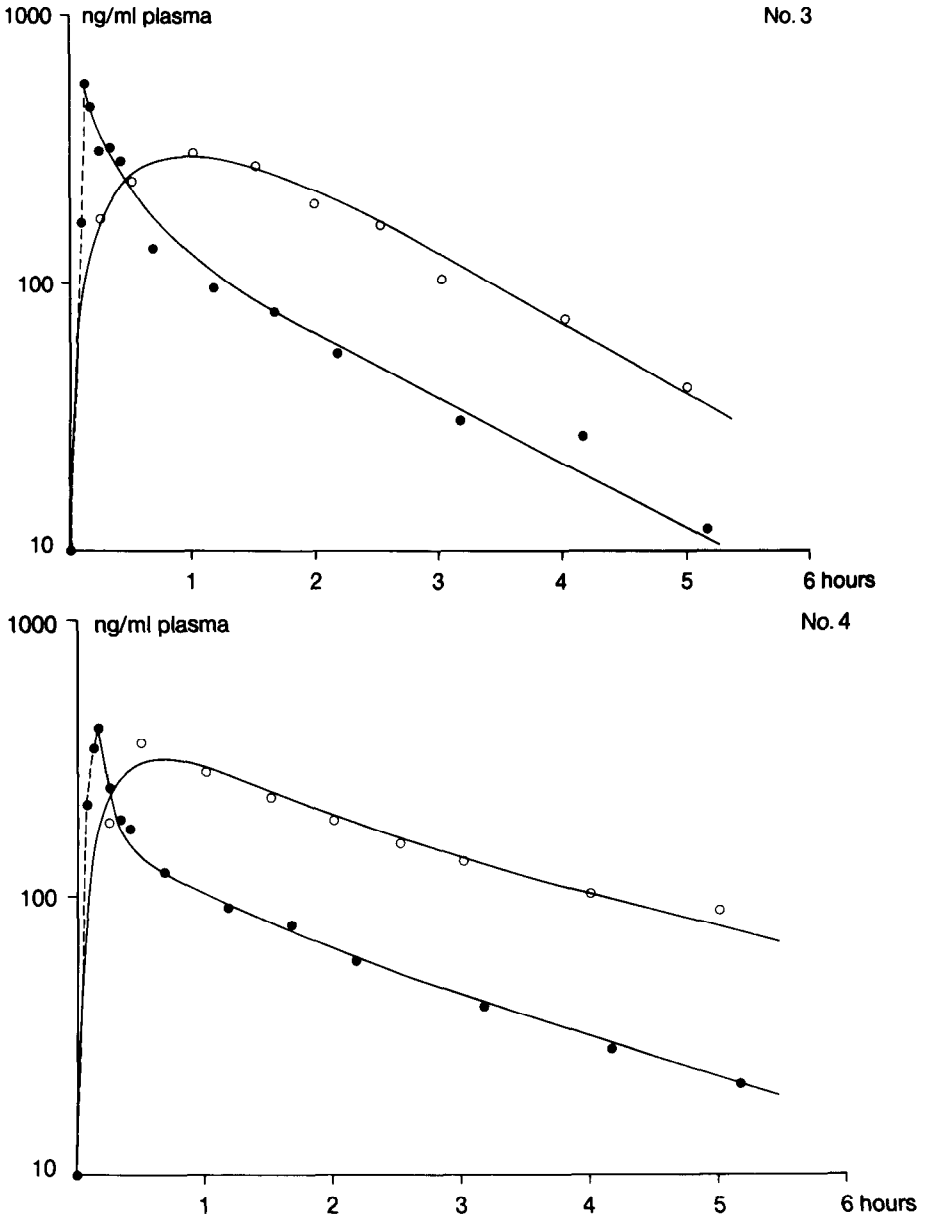

Fig. 3.

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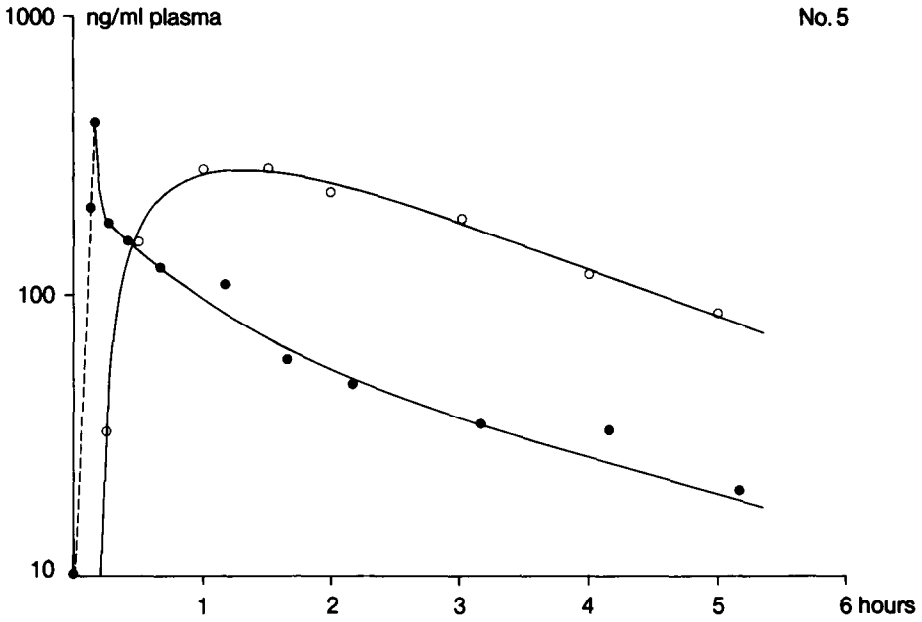

Fig. 3. Pirlindole plasma levels in three beagle dogs which received a single dose of 1 mg/kg by infusion (0.1 mg/kg/min) (•) and 10 mg/kg (aqueous solution) orally (○). (—), RIP-fitting [3].

TABLE III

PHARMACOKINETIC PARAMETERS OF PIRLINDOLE CALCULATED FROM PLASMA CONCENTRATIONS IN FASTED BEAGLE DOGS WHICH RECEIVED THE DRUG (1 mg/kg) BY INFUSION (0.1 mg/kg/min) AND ORALLY (10 mg/kg AS AQUEOUS SOLUTION)

T_0 = lag-time, $T_{abs} = T_{sys} - T_{vss}$.

Dog No.	Dose (mg/kg)	C_{pmax} (ng/ml) (infusion end)	AUC_∞ (ng/ml h)	AUC_{ex} (%)	Cl_{tot} (l/h)	T_{vss} (h)	V_{ss} (l)	$t_{½}$ terminal (h)
Infusion								
3 M	1.14	400.2	434.2	15.0	36.82	2.71	99.7	2.12
4 M	1.11	446.6	433.4	4.9	46.08	1.61	74.2	1.26
5 F	1.03	420.0	409.2	15.0	39.06	2.76	107.8	2.27
$\bar{X}$		422.3	425.6		40.63	2.36	93.9	1.88
S.D.		19.0	14.2		4.83	0.65	17.5	0.55

	C_{pmax} (ng/ml)	t_{max} (h)	AUC_∞ (ng/ml h)	AUC_{ex} (%)	T_{sys} (h)	T_{abs} (h)	k_a (l/h)	T_0 (h)	$t_{½}$ terminal (h)	f (%)
Oral administration										
3 M	355.7	0.5	1154.3	25.7	3.74	1.03	0.9709	0.01	2.69	30.3
4 M	308.2	1.0	876.0	7.2	2.22	0.61	1.6393	0.00	1.12	22.4
5 F	285.1	1.0	1100.6	19.2	3.19	0.53	1.8868	0.21	1.75	27.7
$\bar{X}$	316.3		1043.6		3.05	0.72	1.4990	0.07	1.85	26.8
S.D.	36.0		147.6		0.77	0.26	0.4737	0.12	0.79	4.0
Range		0.5—1.0								

to three beagle dogs to estimate the absolute bioavailability of pirlindole (26.8 ± 4.0%). The plasma levels after single oral dosing were characterized by the absorption constant k_a = 1.5 ± 0.5 h^{-1}, C_{pmax} = 316.3 ± 36.0 ng/ml, t_{max} = 0.5—1.0 h, AUC_∞ = 1043 ± 148 ng/ml h^{-1}, and the terminal half-life of elimination, $t_{½}$ = 1.85 ± 0.79 h. Total plasma clearance (Cl_{tot} = 40.7 ± 4.8 l/h) and steady-state volume of distribution (V_{ss} = 93.9 ± 17.5 l) were estimated from the corresponding intravenous data (Fig. 3 and Table III).

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