CHAPTER 4

RESULTS

4.1 Validation Study

Risperidone and internal standard were clearly separated at the retention time of 4.6 and 6.4 minutes, respectively. The chromatogram of blank plasma and the chromatograms of risperidone 20 ng/ml and the internal standard are shown in Figure 4.

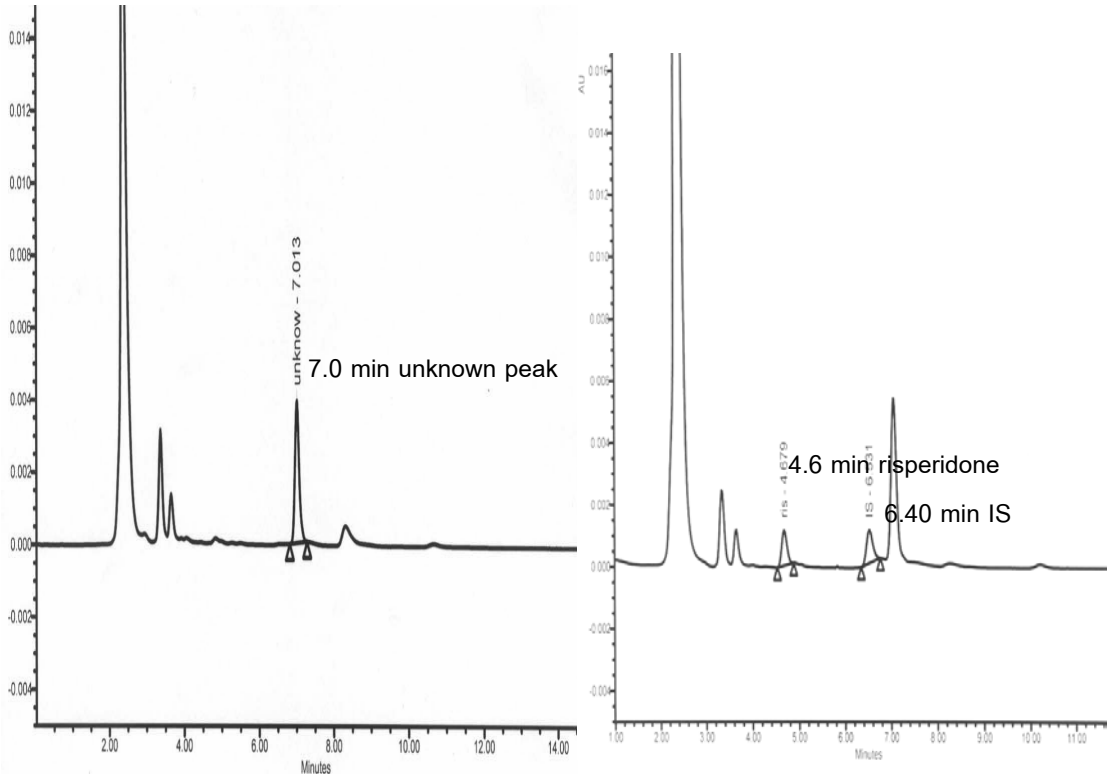

Figure 4. Representative chromatograms of human plasma samples

Key : (A) blank human plasma ; (B) spiked with standard risperidone 20 ng/ml and internal standard (clozapine 20 ng/ml)

4.1.1 Linearity of the standard curve

The calibration curve of standard risperidone was linear over the range of 2-100 ng/ml (Figure 5).

4.1.2 Precision and accuracy

The lower limit of quantitative (LOQ) was 2 ng/ml (Table 1) and the average intra-day and inter-day assay coefficient of variations were 7.69% and 8.83% respectively (Table 2, 3). The mean recoveries were 109.52%, 106.10%, and 98.72% at the drug concentrations of 5, 20 and 100 ng/ml respectively. The overall mean recovery was 104.78% (Table 4)

Table 1. The lower limit of quantitative (LOQ) of risperidone in plasma

Conc. (ng/ml)	**N**	Minimum peak area	Maximum peak area	Mean peak area	S.D.
2 ng/ml	5	0.05	0.07	0.062	0.0084

%CV = SD/Mean x 100

= (0.008/0.062) x 100

= 13.39%

Table 2. The intra-day variance of three different risperidone concentrations in plasma

Concentration[a] (ng/ml)	Mean peak area (n=5)	S.D.	CV (%)[b]
5	0.20	0.02	8.19
20	0.84	0.09	10.54
100	4.23	0.19	4.36
Mean			7.69

[a]Various concentrations of standard risperidone were added to drug-free human plasma samples prior to precipitation as described in the text.

[b]Standard deviation divided by mean, expressed in percent.

Table 3. The inter-day variance of three different risperidone concentrations in plasma

Concentration[a] (ng/ml)	Mean peak area (n=5)	S.D.	CV (%)[b]
5	0.21	0.03	13.94
20	0.84	0.07	8.43
100	4.41	0.18	4.12
Mean			8.83

[a]Various concentrations of standard risperidone were added to drug-free human plasma samples prior to precipitation as described in the text.

[b]Standard deviation divided by mean, expressed in percent.

Table 4. Relative percent recovery of standard risperidone in human plasma

Concentration (ng/ml)	Mean peak area in mobile phase (n=5)	Mean peak area in plasma (n=5)	%Recovery[a]
5	0.21	0.23	109.52
20	0.82	0.87	106.10
100	4.70	4.64	98.72

[a]Mean peak area in plasma divided by mean peak area in mobile phase, expressed in percent.

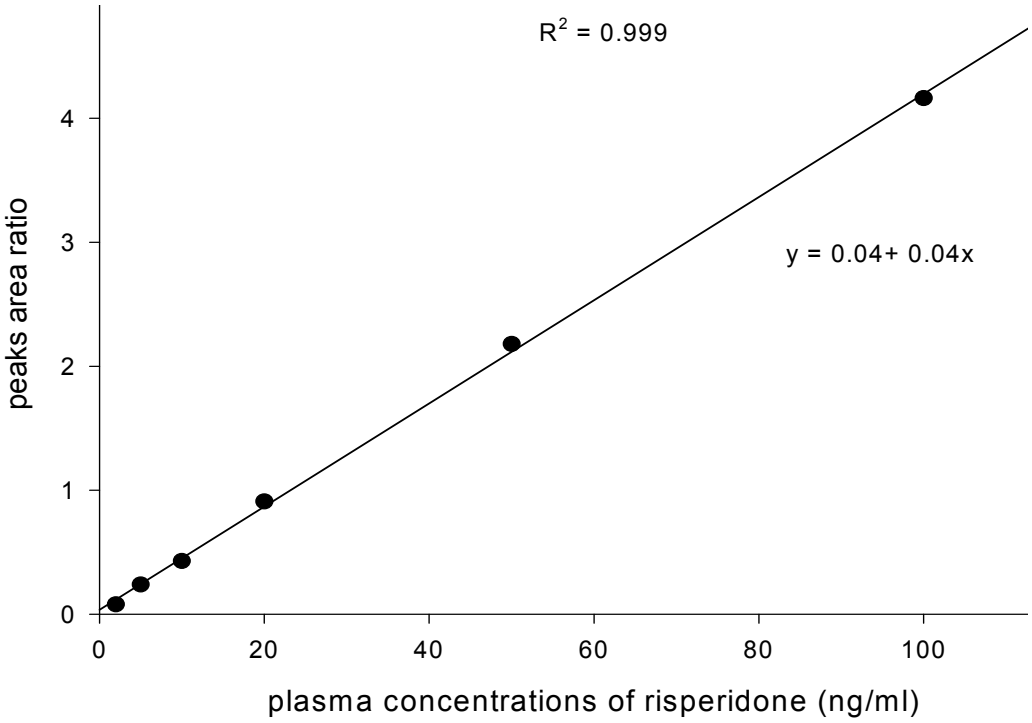

Figure 5. The calibration curve of standard risperidone

4.2 Pharmacokinetic Study

4.2.1 The chromatograms of human plasma samples.

The chromatograms showed that the peak of risperidone was well separated from the other peaks in plasma (Figures 4). There was no interference from the peak of rifampin in this analytical method. The retention time for risperidone and clozapine (internal standard) were approximately 4.6 and 6.4 min, respectively.

4.2.2 Pharmacokinetics of risperidone alone in the single dose.

After a single oral dose of 4 mg of risperidone alone in ten subjects, the mean C_{max} value of 32.44 $\pm$ 6.05 ng/ml was reached at 0.95 $\pm$ 0.13 hours. The mean values for AUC_{0-48}, $AUC_{0-\infty}$, $t_{1/2}$, Cl/f, Vd/f, and $MRT_{0-\infty}$ were 157.49 $\pm$ 48.80 ng/l.hr, 179.27 $\pm$ 53.76 ng/l.hr, 4.02 $\pm$ 1.36 hr, 0.05 $\pm$ 0.01 l/hr/kg, 0.26 $\pm$ 0.08 l/kg and 7.84 $\pm$ 2.19 hours, respectively (Table 7)

4.2.3 Pharmacokinetics of risperidone in subjects after pretreatment with rifampin compared with risperidone alone.

The mean plasma concentration-time profile of risperidone was shown in Figure 6. The mean values of C_{max} , AUC $_{0\text{-}48}$, $AUC_{0\text{-}\infty,}$ t_{max}, $t_{1/2}$, Cl/f, Vd/f and $MRT_{0\text{-}\infty}$ of risperidone in the ten subjects after rifampin pretreatment were 16.16 $\pm$ 2.73 ng/ml, 42.66 $\pm$ 7.81 ng/l.hr, 51.41 $\pm$ 8.54 ng/l.hr, 0.88 $\pm$ 0.07 hr, 2.39 $\pm$ 0.30 hr, 0.27 $\pm$ 0.18 l/hr/kg, 0.41 $\pm$ 0.11 l/kg and 3.75 $\pm$ 0.45 hr, respectively. The results indicated that C_{max} , AUC $_{0\text{-}48}$, $AUC_{0\text{-}\infty,}$ Cl/f, Vd/f and $MRT_{0\text{-}\infty}$ in the ten subjects after pretreatment with rifampin were significantly decreased compared with those values when risperidone was administered alone. However, there were no significant difference in K_e, t_{max} and $t_{1/2}$ (Table 7). For individual plasma concentration-time profile of risperidone was shown in Figure 7.

4.2.4 The period and sequence of study

No significance of period and sequence effect were observed in this study. The interaction between period and sequence were evaluated by using two-way ANOVA analysis.

4.2.5 Demographic data

All subjects were considered to be physically healthy based on essential laboratory tests (blood chemistry and hematology). Demographic characteristics of subjects were presented in Table 5.

4.2.6 Adverse effects.

Ten adult healthy Thai male volunteers were enrolled and completed in this study. No serious side effects were observed after taking 600 mg of rifampin. In the single oral dose of risperidone, all subjects were somnolent on the average of 1 hour after taking 4 mg of risperidone and two subjects reported dizziness, nasal congestion, mild headache and three subjects reported orthostatic hypotention (Table 6). The symptoms were subsided within one day and did not required any specific treatment. Moreover, all subjects were well tolerated to all drugs throughout the study. No marked laboratory abnormality occurred in all subjects, and physical examinations revealed no abnormal finding at the end of the study.

Table 5. Dermographic characteristics of 10 male subjects in this study

Characteristics	Subject number										**mean±SD.**
	01	02	03	04	05	06	07	08	09	10	
Age (yr)	25	26	40	37	33	26	22	33	38	25	**30.5±6.45**
Height (cm)	178	170	166	165	165	175	168	166	167	173	**169.3±4.57**
Weight (kg)	76	69	65	58	63	56	66	66	55	59	**63.3±6.5**
BMI (18-24 kg/m^2)	23.9	23.8	23.6	21.3	23.2	18.3	23.4	24.0	19.7	19.7	**22.09±2.15**
Hct (40-50 %)	44	44	43	44	42	45	42	45	42	45	**43.6±1.26**
Hb (13.3-16 g%)	14.5	14.5	14.3	14.5	14	15	14	15	14	15	**14.48±0.41**
WBC (5000-10,000 $/mm^3$)	6150	6150	5100	4200	4150	6550	5550	4900	4050	5300	**5210±899.63**
ALP (39-117 u/l)	79	67	86	65	74	69	75	92	63	60	**73±10.31**
SGOT (3-37 u/l)	17	17	26	27	24	25	26	18	19	28	**22.7±4.42**
SGPT (7-45 U/L)	17	17	31	43	15	24	22	23	18	27	**23.7±8.42**
Direct Bilirublin (0-0.3 mg/dl)	0.1	0.1	0.1	0.3	0.1	0.1	0.1	0.1	0.1	0.3	**0.15±0.11**
Total Bilirublin (0.2-1.1 mg/dl)	0.15	0.18	0.15	0.94	0.25	0.27	0.47	0.75	0.3	1	**0.45±0.33**
BUN (7-24 mg/dl)	15.4	14.9	14.2	11.6	17.3	13	12.1	10.6	13	7.4	**12.95±2.77**
Creatinine (0.8-1.4 mg/dl)	1.2	0.9	1	1	1.2	0.9	0.9	1	1	0.7	**0.98±0.15**
FBS (70-110 mg/dl)	76	68	76	74	78	84	81	78	79	75	**76.9±4.31**

Table 6. Adverse effects were observed in ten subjects after receiving a single oral dose of 4 mg risperidone alone, and after pretreatment with 600 mg rifampin orally for 5 days.

Adverse Effects	risperidone alone (%)	risperidone after rifampin (%)
somnolence	100	90
dizziness	20	20
mild headache	20	20
syncope	30	0

Table 7. Pharmacokinetic parameters (mean ± S.E.) of risperidone in ten subjects after receiving a single oral dose of 4 mg risperidone alone, and after pretreatment with 600 mg rifampin orally for 5 days.

Parameter	risperidone alone	risperidone after rifampin	P-value
AUC_{0-48} (ng/l/hr)	157.49 ± 48.80	42.66 ± 7.81	P< 0.01
$AUC_{0-\infty}$ (ng/l/hr)	179.27 ± 53.76	51.41 ± 8.54	P< 0.01
Ke (hr^{-1})	0.22 ± 0.04	0.40 ± 0.12	NS
$t_{1/2}$ (hr)	4.02 ± 1.36	2.39±0.30	NS
t_{max} (hr)	0.95 ± 0.13	0.88 ± 0.07	NS
C_{max} (ng/ml)	32.44 ± 6.05	16.16 ± 2.73	P< 0.01
Vd/f (l/kg)	0.26 ± 0.08	0.41 ± 0.11	P< 0.01
Cl/f (l/hr/kg)	0.05 ± 0.01	0.27 ± 0.18	P< 0.05
$MRT_{0-\infty}$ (hr)	7.84 ± 2.19	3.75 ± 0.45	P< 0.05

NS; no significant differences from control when compared to risperidone alone (Wilcoxon signed rank test)

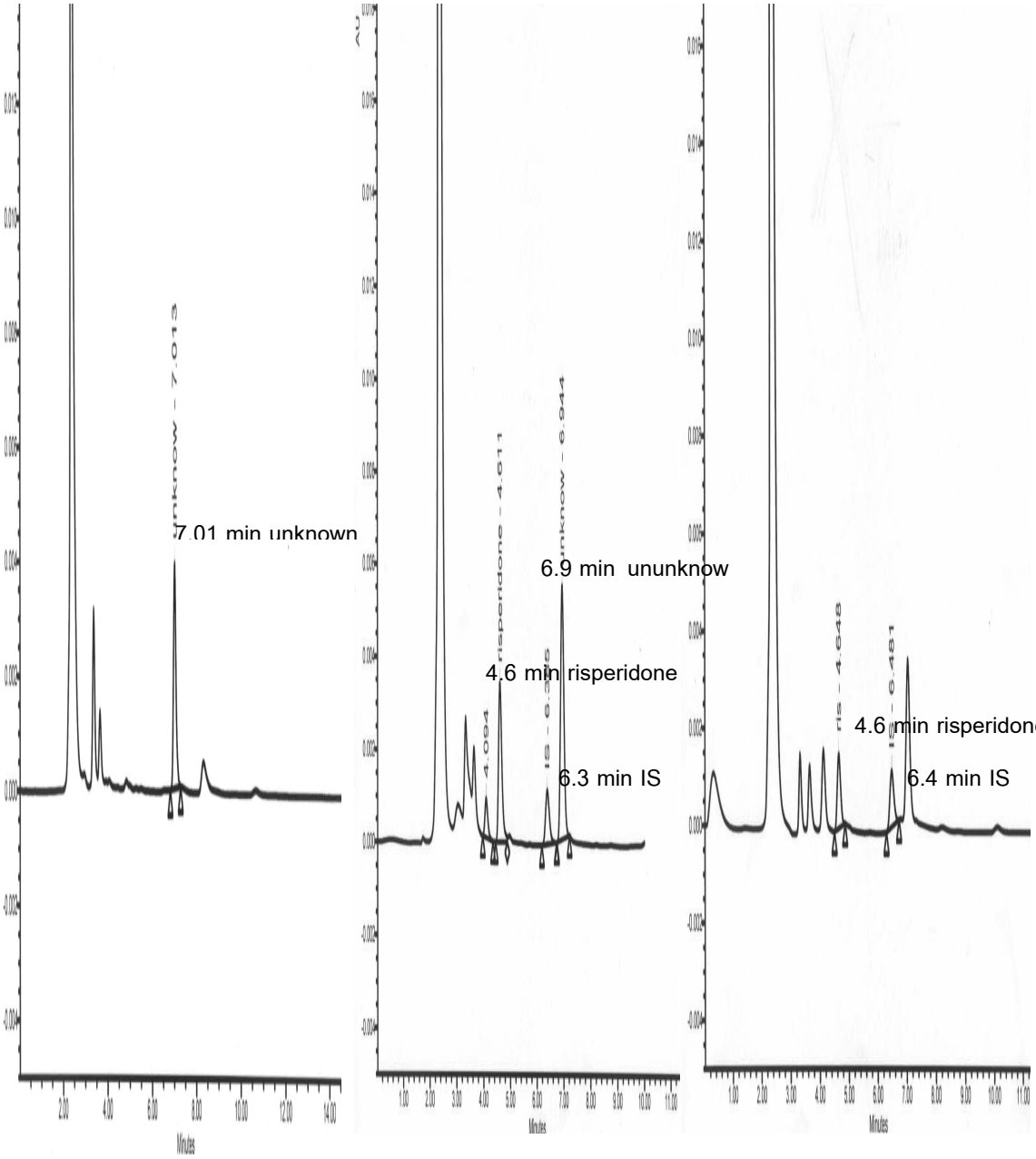

Figure 6. Representative chromatograms of human plasma samples

Key : (A) blank human plasma ; (B) plasma obtained from a subject who received 4 mg of risperidone alone at 2 hours ; (C) plasma obtained from a subject who received 4 mg of risperidone at 2 hours after pretreatment with rifampin

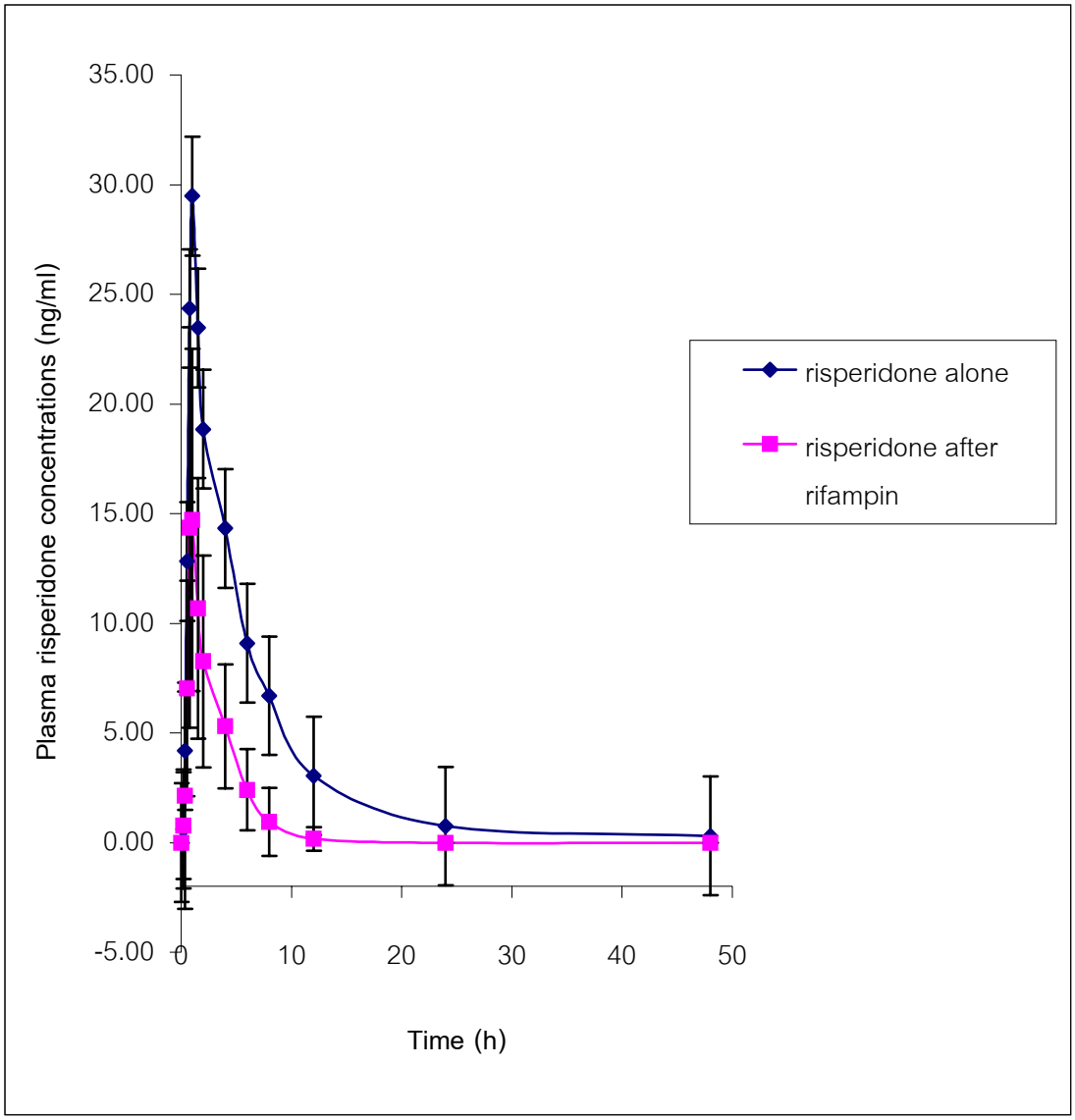

Figure 7. Mean plasma risperidone concentrations after a single oral dose of 4 mg risperidone administration alone and after pretreatment with 600 mg rifampin once daily for 5 days.

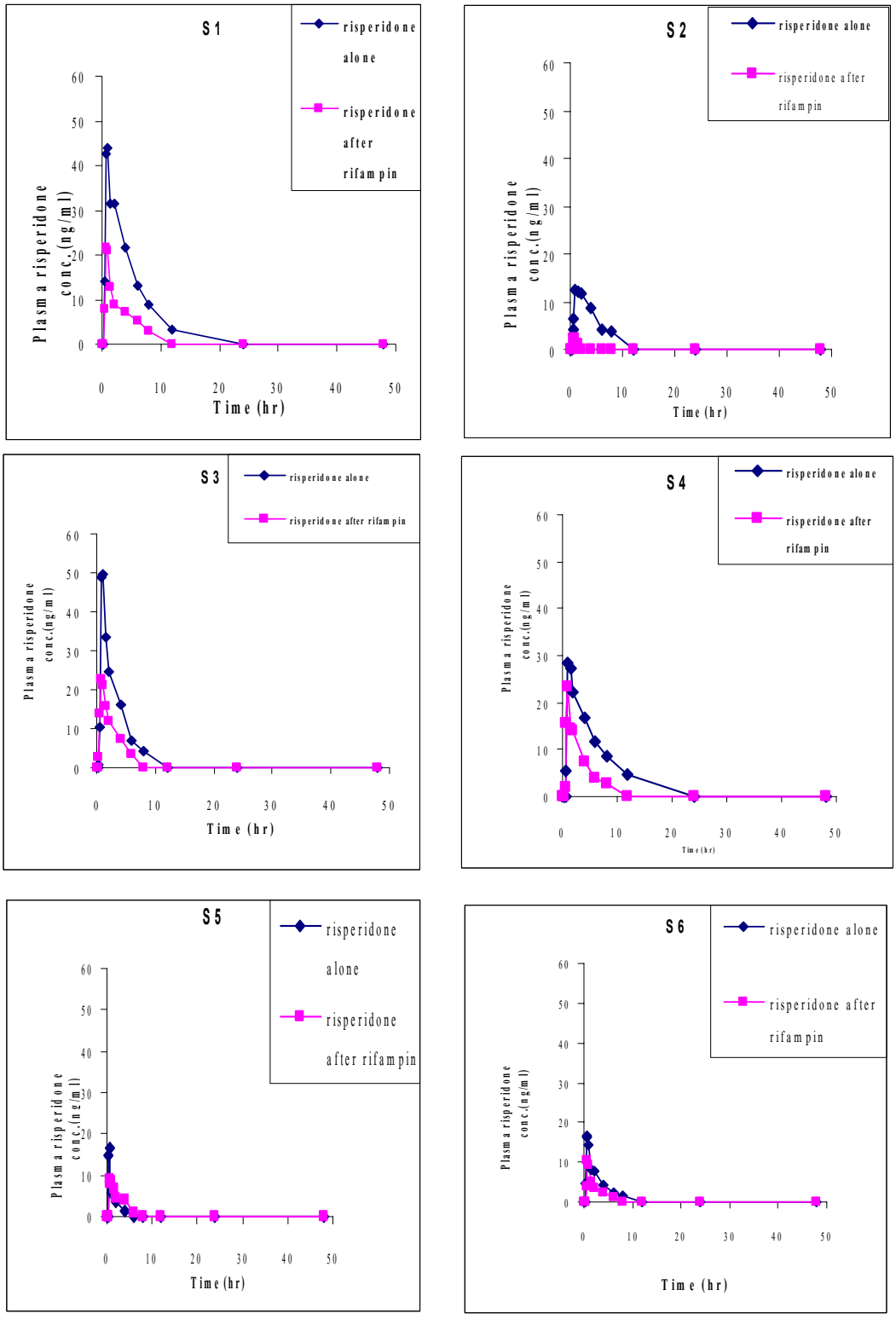

Figure 8. Plasma concentration – time profiles of risperidone after a single oral dose of 4 mg risperidone alone and after pretreatment with rifampin in each subject

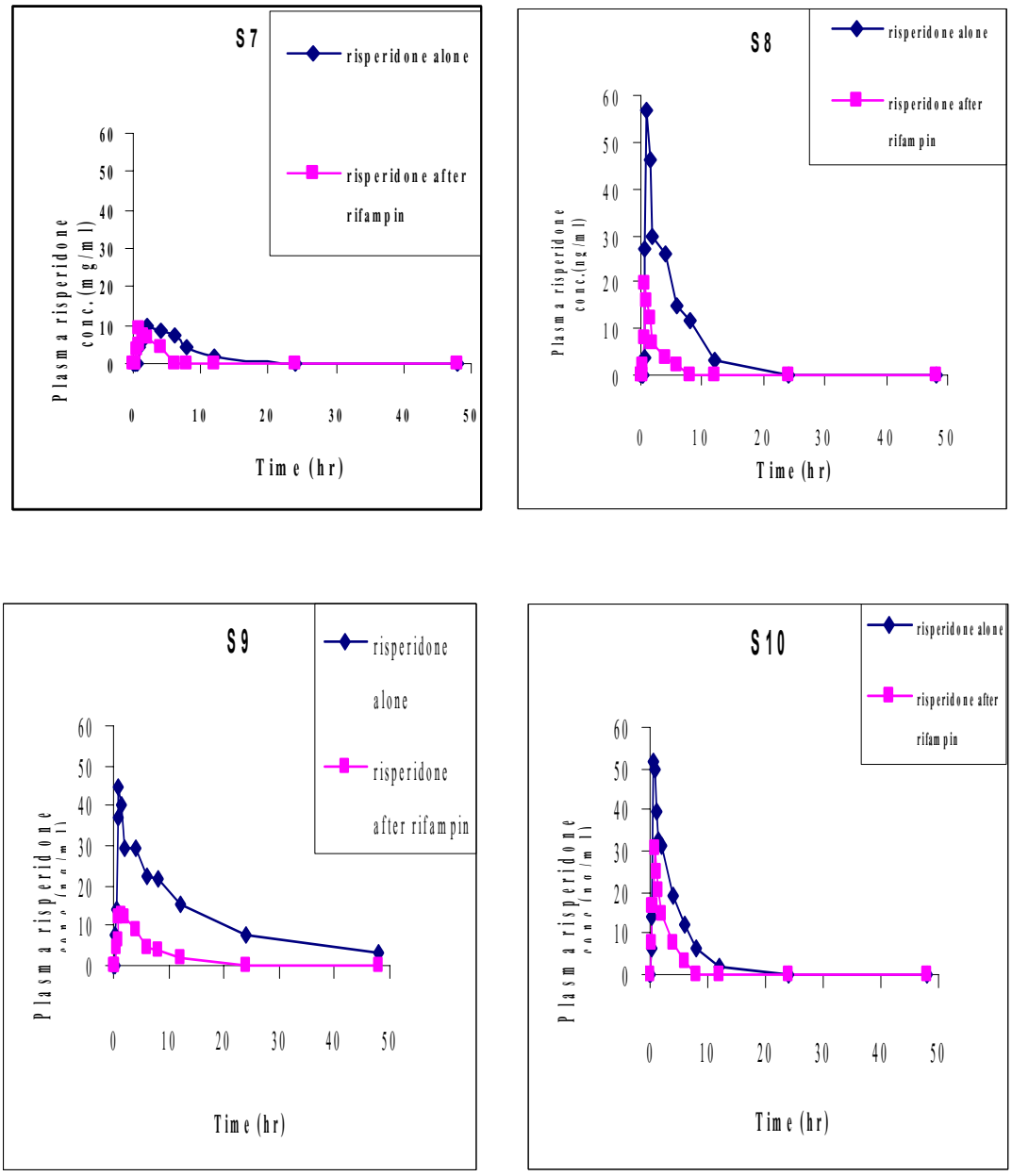

Figure 8. (continued.)

Table 8. Pharmacokinetic parameters derived from non-compartment model analysis of risperidone in ten subjects receiving a single oral dose of 4 mg risperidone alone.

Subject No.	AUC_{048} (ng/l.hr)	$AUC_{0-\infty}$ (ng/l.hr)	K_e (hr^{-1})	$t_{1/2}$ (hr)	C_{max} (ng/ml)	t_{max} (hr)	Cl/f (l/hr/kg)	Vd/f (l/kg)	$MRT_{0-\infty}$ (h)
1	187.80	201.62	0.24	2.95	43.96	1	0.02	0.08	4.83
2	40.09	86.30	0.05	1.41	5.64	1	0.04	0.94	2.08
3	131.00	144.98	0.31	2.22	49.62	1	0.03	0.09	3.58
4	144.66	178.57	0.14	4.84	28.51	1	0.02	0.16	7.40
5	18.57	22.02	0.45	1.55	16.78	0.5	0.18	0.41	2.10
6	41.64	47.44	0.26	2.63	16.83	0.5	0.08	0.32	3.73
7	67.22	78.29	0.18	3.67	9.5	2	0.05	0.27	6.81
8	212.94	226.05	0.25	2.74	56.78	1	0.02	0.07	4.84
9	550.32	621.20	0.04	16.00	44.77	1	0.01	0.15	20.43
10	180.64	186.27	0.32	2.19	51.99	0.5	0.02	0.07	3.78
Mean	**157.49**	**179.27**	**0.22**	**4.02**	**32.44**	**0.95**	**0.05**	**0.26**	**7.84**
S.D.	**154.33**	**170.02**	**0.13**	**4.33**	**19.16**	**0.44**	**0.05**	**0.27**	**6.93**
S.E.	**48.80**	**53.76**	**0.04**	**1.36**	**6.05**	**0.13**	**0.01**	**0.08**	**2.19**

Table 9 Pharmacokinetic parameters derived from non-compartment model analysis of risperidone in ten subjects receiving a single oral dose of 4 mg risperidone after pretreatment with 600 mg rifampin orally for 5 days.

Subject No.	AUC_{0-48} (ng/l.hr)	$AUC_{0-\infty}$ (ng/l.hr)	K_e (hr^{-1})	$t_{1/2}$ (hr)	C_{max} (ng/ml)	t_{max} (hr)	Cl/f (l/hr/kg)	Vd/f (l/kg)	$MRT_{0-\infty}$ (h)
1	60.55	74.19	0.22	3.15	21.78	0.75	0.05	0.24	4.87
2	1.19	1.89	1.49	0.46	2.35	0.75	1.99	1.33	1.05
3	57.56	68.38	0.32	2.17	22.58	0.75	0.06	0.18	3.41
4	63.85	73.72	0.27	2.53	23.22	1	0.05	0.20	4.12
5	25.18	27.97	0.36	1.91	9.04	0.75	0.14	0.38	3.04
6	19.36	24.55	0.24	2.85	10.49	0.75	0.16	0.67	3.92
7	22.15	44.78	0.20	3.46	9.46	1	0.09	0.45	5.56
8	36.95	42.60	0.33	2.09	19.55	0.75	0.09	0.28	3.03
9	68.76	77.82	0.19	3.67	12.61	1.5	0.05	0.27	5.83
10	71.06	78.18	0.41	1.67	30.53	0.75	0.05	0.12	2.67
Mean	**42.66**	**51.41**	**0.40**	**2.39**	**16.16**	**0.88**	**0.27**	**0.41**	**3.75**
S.D.	**24.72**	**27.01**	**0.39**	**0.95**	**8.64**	**0.24**	**0.60**	**0.36**	**1.44**
S.E.	**7.81**	**8.54**	**0.12**	**0.30**	**2.73**	**0.07**	**0.18**	**0.11**	**0.45**