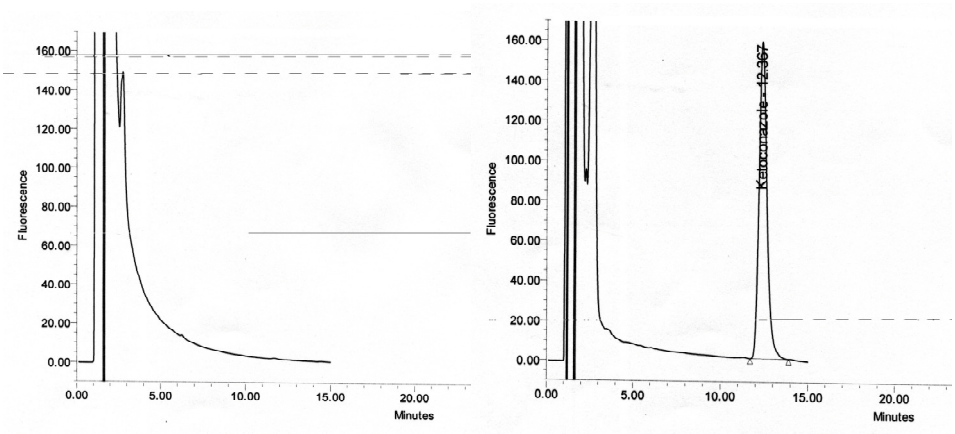
CHAPTER 4

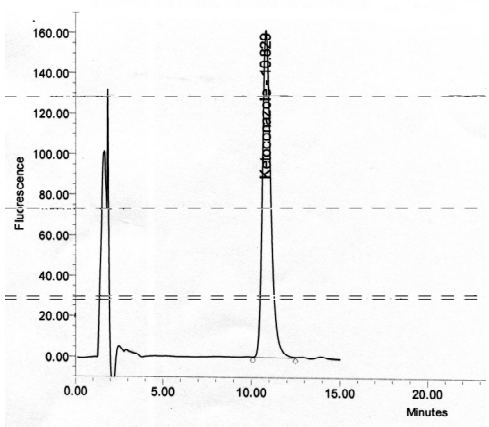
RESULTS

4.1. Analysis of Ketoconazole in Plasma

Chromatograms of drug free plasma (a); ketoconazole 8 μg/ml in plasma (b); and ketoconazole 8 μg/ml in methanol (c) are shown in Figure 13. Ketoconazole was eluted at 11 minutes as sharp and symmetrical peak.



(a) (b)



(c)

Figure 13 HPLC chromatograms of drug free plasma (a); ketoconazole 8 μg/ml in plasma (b); and ketoconazole 8 μg/ml in methanol (c).

4.1.1. Linearity

Calibration curves for plasma analysis were constructed for ketoconazole in drug-free plasma to achieve the final concentrations of 0.01, 0.1, 0.4, 2, 8 and 16 μg/ml. Then, calibration curves were plotted between the peak area of ketoconazole versus plasma ketoconazole concentration (μg/ml), as shown in Figure 14. Using the least-square linear regression analysis, the correlation coefficient (R-square) was 0.9999 and the linear regression equation was :

$$Y = 534041\ X + 12823$$

Where :

X = plasma ketoconazole concentration (μg/ml)

Y = the peak area of ketoconazole

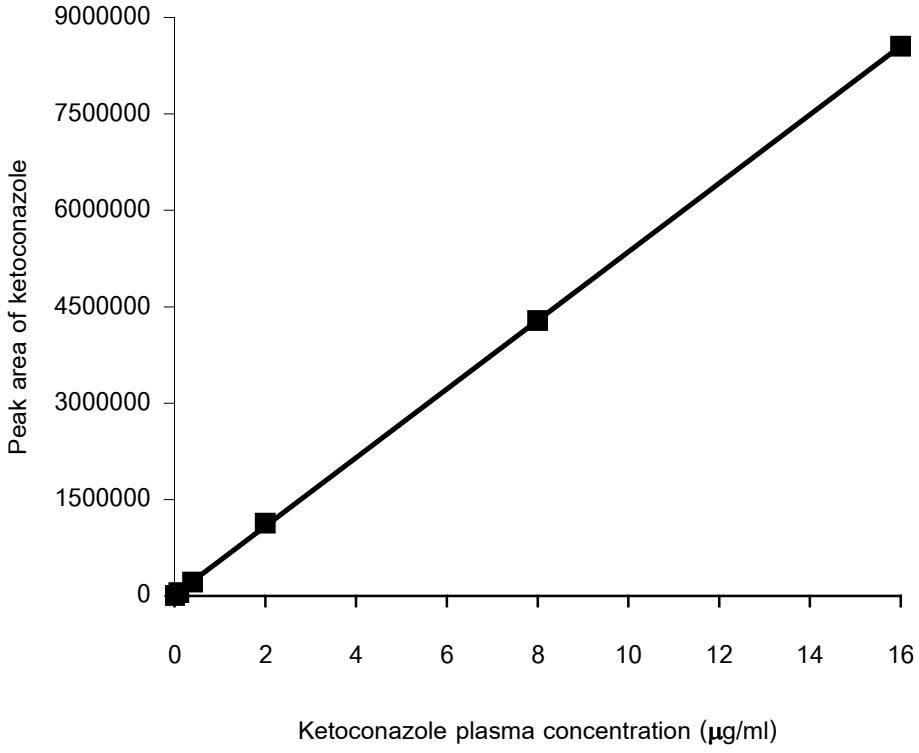


Figure 14 Calibration curve of ketoconazole in plasma

(Y = 534041 X + 12823, r^2 = 0.9999)

4.1.2. Recovery

Efficacy of deproteinization procedure was assessed from the percentage recovery, as shown in Table 1. The mean percentage recovery of ketoconazole at the concentration of 0.4, 2 and 8 μg/ml were 101.43, 102.59 and 100.11%, respectively. These results had shown a good efficiency of deproteinization procedure owing to the high percentages of recovery with %CV less than 5%.

Table 1 Recovery of ketoconazole

Plasma concentration (μg/ml)	Recovery (%) (n=5)	
	Mean	%CV
0.4	101.43	3.16
2	102.59	3.57
8	100.11	1.98

4.1.3. Lower limit of quantitation

The lower limit of quantitation (LLOQ) was obtained in this chromatographic condition and was found to be 0.01 μg/ml.

4.1.4. Precision

The precision of the assay procedure was assessed from %CV of area under the curve ketoconazole and retention times from intraday and interday results, as shown in table 4-2. %CV of intraday precision for area under the peak of ketoconazole was found in the range of 1.00 to 1.67 % and retention time was found in the range of 0.02 to 0.09 %. %CV of interday precision for area under the peak of ketoconazole was found in the range of 3.58 to 4.17 % and retention time was found in the range of 1.00 to 1.87 %. Although internal standard was not used in this study, the results demonstrated a good precision of the assay method.

Table 2 Precision of the analytical method, intraday and interday precision

Concentration (μg/ml)	Mean ± SD of area under ketoconazole peak	%CV	Mean ± SD of Retention time	%CV
Intraday (n=5)				
0.4	221736 ±5911	2.67	11.25 ± 0.007	0.06
2	1144402 ±11408	1.00	11.23 ± 0.002	0.02
8	4476478±76330	1.71	11.20 ± 0.010	0.09
Interday (n=10)				
0.4	217243 ± 8853	4.08	11.21 ± 0.112	1.00
2	1098212 ± 45863	4.17	11.22 ± 0.210	1.87
8	4312581 ± 154645	3.58	11.21 ± 0.189	1.69

4.2. Patients

Fourteen HIV infected patients who had CD_4 T-lymphocyte absolute cell count less than 350 cell/mm^3 and met the inclusion criteria were enrolled into the study. Two patients dropped out from the study due to adverse effects, both patients reported rash and headache. Three patients were male and nine patients were female. The mean age was 33.7±8.9 years (ranging from 22 to 55 years) and the mean body mass index was 22.56±3.45 kg/m^2 (ranging from 20.34 to 28.04 kg/m^2). CD_4 T-lymphocyte counts were in the range from 46 to 345 cell/mm^3. Demographic data of the patients in the study are summarized in Table 3.

Table 3 Demographic data of the patients in the study

Patient No.	Sex	Age (years)	BMI (kg/m^2)	CD_4 ($cell/mm^3$)	WBC	Hb	Hct	Lymph	Mono	BUN mg%	Cr mg%	SGOT U/L	SGPT U/L	Alb g%	Preg. test
1	F	41	20.75	336	6,300	11.9	36	31	5	14.0	0.95	20	25	4.0	Neg
2	F	22	20.99	345	7,100	15.4	43	18	7	10.2	1.12	30	34	4.1	Neg
3	F	55	28.04	296	6,500	11.5	33	41	7	10.8	1.07	33	31	4.2	Neg
4	F	26	20.81	252	7,100	11.9	38	35	5	13.7	1.04	34	35	4.3	Neg
5	F	28	21.02	57	4,700	11.1	34	28	10	12.8	1.15	24	14	4.0	Neg
6	M	34	22.19	272	3,600	11.9	32	5	5	9.2	1.20	33	37	4.1	NA
7	F	26	26.56	298	2,900	12.3	37	45	6	14.1	0.70	18	8	4.0	Neg
8	M	34	16.07	46	4,400	10.1	32	12	6	16.7	1.06	35	29	3.6	NA
9	F	27	26.71	250	5,600	13.6	42	29	10	9.7	0.65	21	5	4.6	Neg
10	F	36	25.51	343	4,400	12.9	38	39	9	13.1	0.66	27	11	4.4	Neg
11	M	37	20.34	239	5,400	15.6	45	27	11	15.6	1.04	35	34	4.4	NA
12	F	39	21.72	90	5,240	12.3	37	52	10	9.2	0.66	34	32	3.8	Neg
Mean	-	33.75	22.56	235.33	5,270.0	12.5	37.3	30.17	7.58	12.43	0.94	28.67	24.58	4.13	-
SD	-	8.97	3.45	109.54	1,336.3	1.6	4.3	13.63	2.27	2.55	0.21	6.39	11.72	0.28	-

F = Female , M = Male , Neg = Negative , ND = Not applicable

4.2. Plasma ketoconazole concentrations

Twelve patients completed the study without serious adverse effects. Plasma ketoconazole concentration were measured; all plasma drug concentration from Phase 1 are presented in Table 4 and those of Phase 2 are presented in Table 5. The pharmacokinetic parameters of ketoconazole in Phase 1 and 2 were showed in Table 6 and Table 7, respectively. The mean ± SD of C_{max}, AUC_{0-24}, $AUC_{0-\infty}$, Cl/f and $t_{½}$ of Phase 1 were 10.62±4.23 μg/ml, 68.53±52.89 μg/ml.h, 70.95±57.18 μg/ml.h, 10.76± 11.17 L/h and 5.00±3.99 h, respectively. For Phase 2, the mean ± SD of C_{max}, AUC_{0-24}, $AUC_{0-\infty}$, Cl/f and $t_{½}$ were 5.95±3.20 μg/ml, 19.18±9.76 μg/ml.h, 19.28±9.74 μg/ml.h, 32.46±36.73 L/h and 2.08±1.27 h, respectively. All parameters showed statistically significant difference ($p<0.05$) as shown in Table 8. The mean plasma ketoconazole concentration-time data of two phases are depicted in figure 4-1, and individual patient profile are shown in appendix E. Coadministration efavirenz resulted in a decrease in ketoconazole C_{max}, AUC_{0-24} and $t_{½}$ in all patients as shown in appendix F.

Table 4 Plasma ketoconazole concentration for each patient during phase 1

Patient	Plasma ketoconazole concentration (μg/ml) at each time of blood drawn (h)											
No.	0.5	1	1.5	2	2.5	3	3.5	4	6	8	12	24
1	6.25	6.33	5.60	4.69	4.44	3.78	3.37	2.94	1.11	0.51	0.12	0.05
2	0.97	8.60	11.20	12.20	12.71	10.01	10.50	7.53	4.90	2.94	0.57	0.08
3	4.78	8.79	8.74	8.30	7.93	7.43	7.17	6.43	3.08	2.51	1.41	0.33
4	0.10	2.03	4.18	5.25	3.11	0.90	0.34	0.19	0.11	0.04	0.03	0.02
5	4.11	9.19	16.45	16.02	14.98	15.25	14.65	14.06	6.89	6.71	3.66	0.54
6	3.78	6.41	7.45	7.36	6.24	5.89	5.55	4.64	1.65	0.77	0.13	0.06
7	0.61	7.58	9.97	13.34	12.26	11.14	11.00	10.21	5.84	3.80	1.38	0.03
8	4.99	8.34	9.62	12.46	16.47	16.08	14.79	15.88	12.89	11.14	8.49	1.90
9	1.94	6.33	10.72	10.33	8.82	7.73	7.48	6.98	3.01	1.77	0.54	0.02
10	0.32	1.62	3.27	6.99	10.11	15.87	13.96	13.33	6.99	4.83	2.17	0.12
11	8.29	8.15	7.69	6.88	6.06	5.76	5.54	4.87	3.02	2.21	1.17	0.35
12	2.57	5.79	5.13	4.64	3.63	3.27	2.93	2.63	0.89	0.42	0.16	0.03
Mean	3.23	6.60	8.34	9.04	8.90	8.59	8.11	7.47	4.20	3.14	1.65	0.29
SD	2.59	2.49	3.64	3.76	4.46	5.13	4.86	4.95	3.59	3.20	2.40	0.53

Table 5 Plasma ketoconazole concentration for each patient during phase 2

Patient	Plasma ketoconazole concentration (μg/ml) at each time of blood drawn (h)											
No.	0.5	1	1.5	2	2.5	3	3.5	4	6	8	12	24
1	0.24	2.50	4.60	6.23	7.12	5.66	3.87	2.82	0.34	0.07	0.02	0.01
2	1.66	6.99	10.02	11.26	6.78	4.83	3.64	2.36	0.23	0.10	0.04	ND
3	1.57	1.87	5.66	4.72	3.76	3.00	2.43	1.92	0.49	0.16	0.13	ND
4	0.42	0.93	0.64	0.49	0.58	0.56	0.61	0.40	0.06	ND	ND	ND
5	2.38	5.11	10.21	10.68	11.78	9.53	7.76	6.42	1.79	0.45	0.06	0.02
6	1.06	2.95	2.98	3.02	3.26	3.32	2.54	2.44	1.27	0.82	0.35	0.02
7	2.17	2.68	2.51	4.64	6.40	6.33	5.01	3.75	0.71	0.16	0.01	ND
8	0.02	0.13	2.46	2.12	1.72	1.45	1.49	2.03	0.98	0.36	0.10	ND
9	0.25	0.76	4.21	3.54	4.12	4.85	4.23	5.06	0.91	0.25	0.02	ND
10	0.12	1.22	2.93	2.18	3.08	4.66	6.47	6.18	1.57	0.36	0.06	0.02
11	4.43	6.66	6.70	5.43	3.95	2.67	2.03	1.34	0.10	0.03	0.01	ND
12	0.47	0.89	2.04	4.27	2.13	1.87	1.81	1.68	0.44	0.09	0.03	0.01
Mean	1.23	2.72	4.58	4.88	4.56	4.06	3.49	3.03	0.74	0.26	0.08	0.02
SD	1.30	2.33	3.06	3.25	3.04	2.46	2.12	1.92	0.57	0.23	0.10	0.01

ND = Not detectable, the limit of quantitation was 0.01 μg/ml.

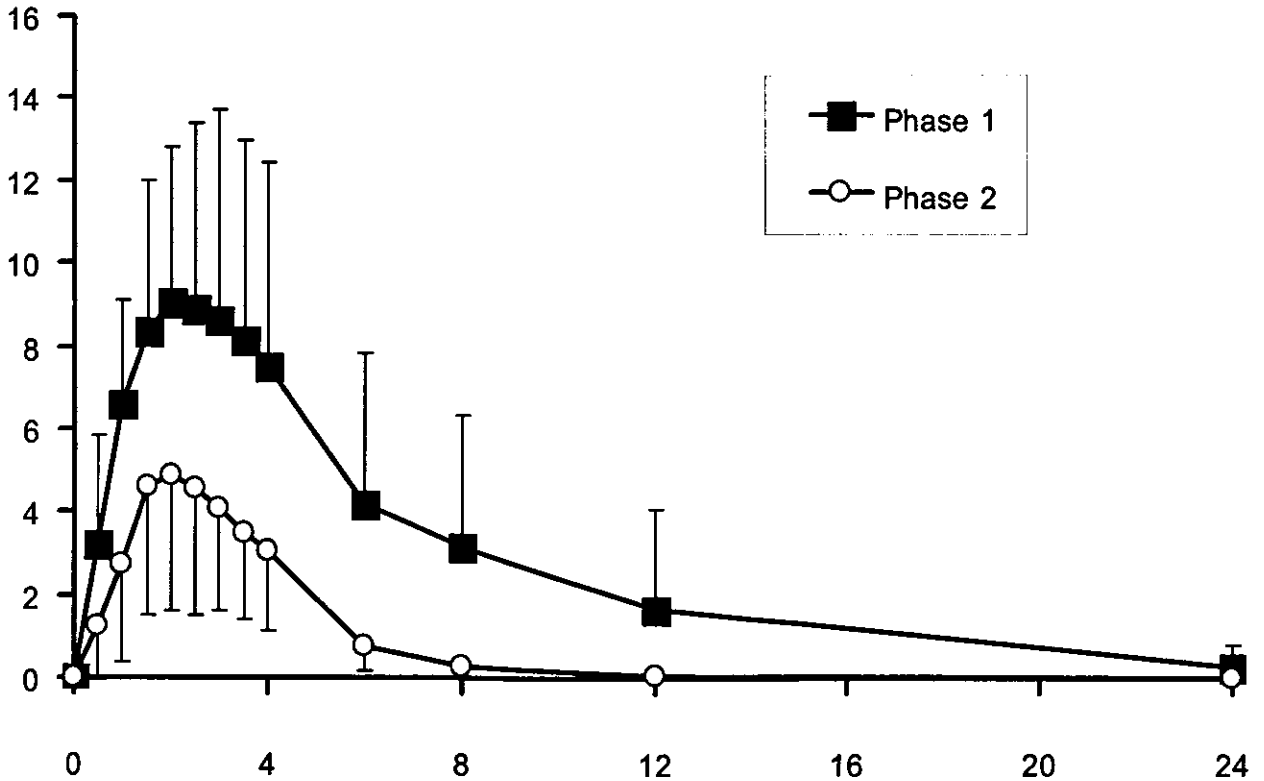


Figure 15 The mean plasma concentration-time profiles of ketoconazole in 12 HIV-infected patients after a singal dose of 400 mg ketoconazole alone, Phase 1 (■) and combination of 150 mg lamivudine and 30 or 40 mg stavudine twice daily, plus 600 mg efavirenz once daily, Phase 2 (O).

Table 6 Pharmacokinetic parameters of ketoconazole in each of the twelve patients during phase 1

Parameters	Patient number (S1-S12)													
	1	2	3	4	5	6	7	8	9	10	11	12	Mean	SD
C_{max} (μg/ml)	6.33	12.71	8.79	5.25	16.45	7.45	13.34	16.47	10.72	15.87	8.29	5.79	10.62	4.23
AUC_{0-24} (μg/ml.h)	25.92	66.08	61.56	8.89	129.33	34.15	80.01	199.75	51.17	89.28	54.40	21.77	68.53	52.89
$AUC_{0-\infty}$ (μg/ml.h)	26.13	66.41	64.19	9.34	132.75	34.40	80.11	217.68	51.24	89.80	57.38	21.95	70.95	57.18
λ_z (h^{-1})	0.23	0.24	0.13	0.04	0.16	0.24	0.31	0.11	0.28	0.23	0.12	0.16	0.19	0.08
$t_{½,Z}$ (h)	2.97	2.87	5.54	16.85	4.38	2.88	2.26	6.54	2.48	2.99	5.90	4.35	5.00	3.99
T_{max} (h)	1.0	2.5	1.0	2.0	1.5	1.5	2.0	2.5	1.5	3.0	0.5	1.0	1.67	0.75
Cl/f (L/h)	15.31	6.02	6.23	42.65	3.01	11.63	4.99	1.84	7.81	4.45	6.97	18.22	10.76	11.17
V_z /f (L/kg)	65.50	24.92	49.85	1037.14	19.08	48.30	16.30	17.34	27.98	19.27	59.35	114.53	124.96	288.68

Table 7 Pharmacokinetic parameters of ketoconazole in each of the twelve patients during phase 2

Parameters	Patient number (S1-S12)													
	1	2	3	4	5	6	7	8	9	10	11	12	Mean	SD
C_{max} (μg/ml)	7.12	11.26	5.66	0.93	11.78	3.32	6.40	2.46	5.06	6.47	6.70	4.27	5.95	3.20
AUC_{0-24} (μg/ml.h)	19.75	26.38	15.62	2.68	42.28	20.54	21.48	10.47	19.92	22.88	17.92	10.29	19.18	9.76
$AUC_{0-\infty}$ (μg/ml.h)	19.78	26.52	15.94	2.74	42.34	20.62	21.49	10.74	19.95	22.95	17.93	10.37	19.28	9.74
λ_z (h^{-1})	0.32	0.28	0.41	0.93	0.32	0.23	0.71	0.38	0.64	0.28	0.68	0.13	0.44	0.24
$t_{½,Z}$ (h)	2.14	2.45	1.69	0.74	2.16	2.99	0.98	1.82	1.09	2.44	1.01	5.44	2.08	1.27
T_{max} (h)	2.5	2.0	1.5	1.0	2.5	3.0	2.5	1.5	4.0	3.5	1.5	2	2.29	0.89
Cl/f (L/h)	20.23	15.08	25.09	146.02	9.46	19.39	18.61	37.32	20.05	17.43	22.30	38.58	32.46	36.73
V_z /f (L/kg)	62.51	53.37	61.49	156.41	29.46	83.82	26.29	98.06	31.55	61.32	32.62	304.32	83.44	78.68

Table 8 Effect of efavirenz on ketoconazole pharmacokinetics in each of twelve HIV-infected patients

Patient No.	C_{max} (μg/ml)		AUC_{0-24} (μg/ml.h)		$AUC_{0-\infty}$ (μg/ml.h)		T_{max} (h)		$t_{½,Z}$ (h)		Cl/f (L/h)	
	Phase		Phase		Phase		Phase		Phase		Phase	
	1	2	1	2	1	2	1	2	1	2	1	2
1	6.33	7.12	25.92	19.75	26.13	19.78	1.0	2.5	2.97	2.14	15.31	20.23
2	12.71	11.26	66.08	26.38	66.41	26.52	2.5	2.0	2.87	2.45	6.02	15.08
3	8.79	5.66	61.56	15.62	64.19	15.94	1.0	1.5	5.54	1.69	6.23	25.09
4	5.25	0.93	8.89	2.68	9.34	2.74	2.0	1.0	16.85	0.74	42.65	146.02
5	16.45	11.78	129.33	42.28	132.75	42.34	1.5	2.5	4.38	2.16	3.01	9.46
6	7.45	3.32	34.15	20.54	34.40	20.62	1.5	3.0	2.88	2.99	11.63	19.39
7	13.34	6.40	80.01	21.48	80.11	21.49	2.0	2.5	2.26	0.98	4.99	18.61
8	16.47	2.46	199.75	10.47	217.68	10.74	2.5	1.5	6.54	1.82	1.84	37.32
9	10.72	5.06	51.17	19.92	51.24	19.95	1.5	4.0	2.48	1.09	7.81	20.05
10	15.87	6.47	89.28	22.88	89.80	22.95	3.0	3.5	2.99	2.44	4.45	17.43
11	8.29	6.70	54.40	17.92	57.38	17.93	0.5	1.5	5.90	1.01	6.97	22.30
12	5.79	4.27	21.77	10.29	21.95	10.37	1.0	2.0	4.35	5.44	18.22	38.58
Mean	10.62	5.95	68.53	19.18	70.95	19.28	1.67	2.29	5.00	2.08	10.76	32.46
SD	4.23	3.20	52.89	9.76	57.18	9.74	0.75	0.89	3.99	1.27	11.17	36.73
p-value	0.002		0.006		0.008		0.063		0.049		0.018	