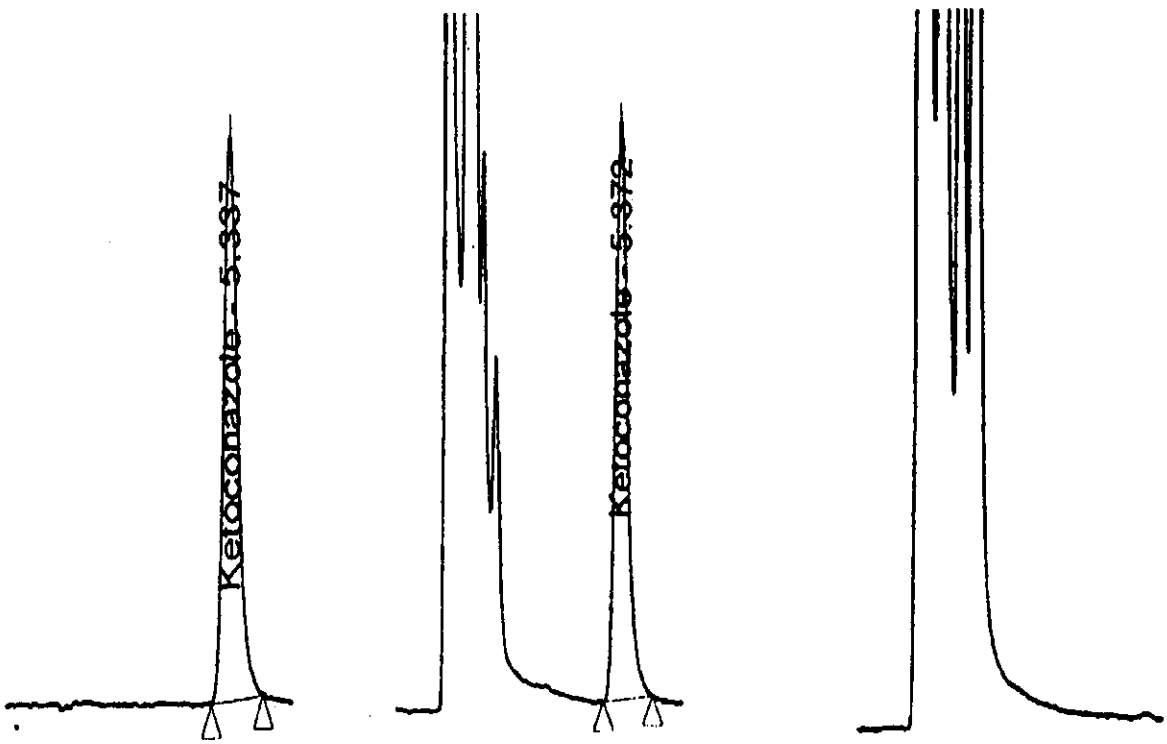
CHAPTER 4

RESULTS

Analysis of Ketoconazole in Plasma

Chromatograms representative of the assay are presented in **Figure 4** and shown results for ketoconazole 8 μg/ml in methanol (a); ketoconazole 8 μg/ml in plasma (b); and drug free plasma (c). Ketoconazole eluted after 5 minutes as sharp and symmetrical peak.



(a) (b) (c)

Figure 4 HPLC chromatograms of ketoconazole 8 μg/ml in methanol (a); ketoconazole 8 μg/ml in plasma (b); and drug-free plasma (c).

Linearity

Calibration curves for plasma analysis were constructed for ketoconazole in drug-free plasma to achieve the final concentrations of 0.1, 0.5, 2 and 8 μg/ml. Then, calibration curves were plotted between the peak area of ketoconazole versus plasma ketoconazole concentration (μg/ml), as shown in **Figure 5**. Using the least-square linear regression analysis, the correlation coefficient (R-square) was 0.9999 and the linear regression equation was:

$$Y = 7.9111 \times 10^{-5} X - 0.0196$$

where :

X = the peak area of ketoconazole

Y = plasma ketoconazole concentration (μg/ml)

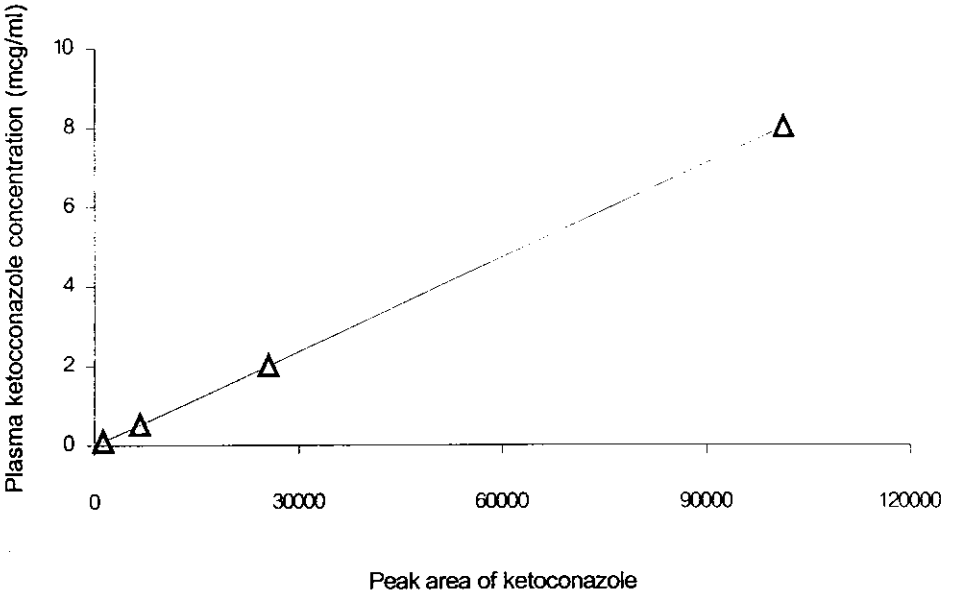


Figure 5 Calibration curve of ketoconazole in plasma

($Y = 7.9111 \times 10^{-5} X - 0.0196$, R-square = 0.9999)

Recovery

Efficacy of deproteinization procedure was assessed from the percentage recovery, as shown in **Table 2**. The mean percentage recovery of ketoconazole at the concentration of 1, 2, and 8 μg/ml was 105.60, 109.84, and 102.67, respectively. These results were showed a good efficiency of deproteinization procedure owing to the high percentages recovery with the low %CV.

Table 2 Recovery of ketoconazole

Plasma concentration (μg/ml)	Recovery (%) (n = 6)	
	Mean	% CV
1	105.60	4.34
2	109.84	4.14
8	102.67	0.69

Limit of Quantitation

The limit of quantitation was obtained in this chromatographic condition was found to be 0.1 μg/ml.

Precision

The precision of the assay procedure was assessed from %CV of area under the peak ketoconazole and retention times from intraday and interday results, as shown in **Table 3**. %CV of intraday precision for area under the peak of ketoconazole was found in the range of 0.77 to 3.17% and retention time was found in the range of 0.09 to 0.19. %CV of interday precision for area under the peak of ketoconazole was found in the range of 5.34 to 6.54% and retention time was found in the range of 1.98 to 2.07%. Although the study did not use internal standard, the results demonstrated a good precision of the assay method.

Table 3 Precision of the analysis method, intraday and interday precision

Concentrations (μg/ml)	Mean ± SD of area under ketoconazole peak	%CV	Mean ± SD of Retention time (min)	%CV
Intraday (n = 6)				
1	13158.0 ± 416.9	3.17	5.22 ± 0.005	0.09
2	26894.6 ± 253.3	0.94	5.26 ± 0.010	0.19
8	108966.7 ± 839	0.77	5.22 ± 0.006	0.11
Interday (n = 6)				
1	12742.2 ± 699.3	5.49	5.11 ± 0.101	1.98
2	25555.6 ± 1670.3	6.54	5.12 ± 0.106	2.07
8	111114.4 ± 5938	5.34	5.10 ± 0.103	2.01

Patients

Twelve HIV infected patients who had CD4 T-lymphocyte absolute cell count less than 200 cell/mm^3 and met the inclusion criteria were enrolled into the study. One patient dropped out from the study due to denied NG tube insertion at the second visit. Eight patients were male and three patients were female; the mean age was 33.7 years (ranging from 27 to 53 years) and the mean body weight was 54.5 kg (ranging from 42.9 to 80.2 kg). According to patients' CD4 T-lymphocyte absolute cell count less than 200 cell/mm^3 (ranging from 6 to 113 cell/mm^3), seven patients received cotrimoxazole for *Pneumocystis carinii* Pneumonia (PCP) prophylaxis prior the study. The others had active opportunistic infection and received different medication as described in **Table 4**.

Table 4 Demographic data of the patients in the study

Patient No.	Sex	Age (years)	Wt (kg)	CD4 (cell/mm^3)	observed gastric pH		Mean gastric pH of each patient
					Treatment		
					A	B	
1	F	30	42.9	6	6.8	1.7	4.25
2	M	30	58.2	9	6.4	7.3	6.85
3	M	29	67.2	10	1.7	3.0	2.35
4[a]	M	31	61.0	18	6.4	6.7	6.55
5	M	36	56.2	19	3.5	7.6	5.55
6[b]	M	53	57.6	21	6.6	4.8	5.7
7	M	37	45.9	16	7.6	7.8	7.7
8	M	28	80.2	113	7.4	7.4	7.4
9[c]	M	34	48.1	86	7.4	2.8	5.1
10[d]	F	27	47.2	8	4.2	2.0	3.1
11	F	36	46.1	63	1.7	1.6	1.65
Mean		33.7	55.5	33.5	5.43	4.79	5.11
SD		7.3	11.2	36.6	2.25	2.62	2.05

a : Patient was treated for PCP infection with cotrimoxazole (400/80) 4 tablet orally three time daily, and prednisolone 40 mg once daily prior treatment A, the dose of prednisolone was taper to 10 mg prior treatment B.

b : Patient was on oral ciprofloxacin 750 mg twice daily. and dapsone 100 mg once daily. Patient has history of Stevens-Johnson syndrome after erythromycin, rifampicin, and cotrimoxazole taken.

c : no medication prior study

d : Besides cotrimoxazole, patient was treated for vaginitis with ceftriaxone 250 mg IM single dose, acyclovir 200 mg five times daily, norfloxacin 200 mg twice daily, metronidazole 400 mg four times daily and clotrimazole vaginal tablet 100 mg at bedtime. prior treatment A. Before treatment B, she received only cotrimoxazole.

Intragastric acidity

Thirty minutes prior to ketoconazole administration, NG tube was inserted into patient's stomach and gastric content was aspirated for pH study. Nine patients from treatment A and eight from treatment B showed gastric hypoacidity (gastric pH > 3). The mean ± SD patients' gastric pH of treatment A and B were 5.45 ± 2.27 and 4.79 ± 2.59, respectively. No statistically significant differences were found between the two treatments ($p = 0.575$). The mean ± SD of all patients' gastric pH was 5.11 ± 2.05.

Plasma ketoconazole concentrations

Eleven patients completed the study without adverse effects. Plasma ketoconazole concentrations were measured; all plasma drug concentrations from treatment A are presented in **Table 5** and those of treatment B are presented in **Table 6**. The mean ± SD of C_{max}, , T_{max}, $t_{1/2}$ and $AUC_{0-\alpha}$ of treatment A were 2.69 ± 1.95 μg/ml, 1.30 ± 0.42 h, 1.62 ± 0.38 h, and 10.22 ± 7.35 μg.h/ml, respectively. For treatment B, the mean ± SD of C_{max} , T_{max} , $t_{1/2}$ and $AUC_{0-\alpha}$ were 3.91 ± 1.54 μg/ml, 1.36 ± 0.60 h, 3.91 ± 1.54 h, 1.61 ± 0.58 h, and 13.26 ± 6.58 μg.h/ml, respectively. Comparison between treatment, C_{max} showed statistically significant difference ($p = 0.016$), while the others were not as shown in **Table 7**. The mean plasma ketoconazole concentration-time data of the two treatments are depicted in **Figure 6**, and individual patient profiles are shown in **Appendix D**.

According to patients' gastric pH, patients were partitioned into the group of gastric pH greater than 3.1 (mean ± SD was 2.37 ± 0.73, ranging from 1.65 to 3.10), and the group of gastric pH less or equal to 3.1 (mean ± SD was 6.14 ± 1.19, ranging from 4.25 to 7.70). The mean ± SD of $AUC_{0-\alpha}$, C_{max}, T_{max}, and $t_{1/2}$ of treatment A and B are shown in **Table 8**. In the group of gastric pH greater than 3.1, C_{max} showed statistically significant difference between the two treatment group ($p = 0.007$). But the other parameters were not shown the difference, as same as the group of gastric pH less or equal to 3.1.

Furthermore, patients were partitioned into two groups according to disease state. First, the group of patient with stable illness (n = 8), these patients had no active opportunistic infection. Seven of them were on cotrimoxazole for PCP prophylaxis and the other was not on any medication. Second, the group of patients had progressive illness (n = 3), they were diagnosed and treated for opportunistic infection. Patient No. 4, 6, and 10 was diagnosed PCP infection, rhodococcosis, and multi-organism vaginal infection, respectively. Their medications are presented in **Table 4.** The mean $\pm$ SD of $AUC_{0-\alpha}$, C_{max}, T_{max}, and $t_{1/2}$ of treatment A and B from each group are shown in **Table 9.** Compared the two treatment, the stable illness group was shown statistically significant difference of $AUC_{0-\alpha}$ (p = 0.029) and C_{max} (p = 0.007), power of test with alpha 0.005 were 0.614 and 0.896, respectively. The progressive illness group was not shown the difference.

Table 5 Plasma ketoconazole concentration for each patient during the treatment A

Patient No.	Plasma ketoconazole concentration (μg/ml) at each time of blood drawn (h)											
	0.5	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**6**	**8**	**12**	**24**
1	3.74	4.99	5.26	4.79	4.13	3.70	3.12	2.90	1.06	0.45	ND	ND
2	0.54	1.71	3.50	3.98	3.33	3.15	2.78	2.45	0.94	0.36	0.20	ND
3	2.65	2.19	2.06	1.89	1.78	1.76	1.30	1.28	0.65	0.41	0.11	ND
4	0.95	1.36	2.22	1.98	1.82	1.69	1.31	1.33	0.51	0.25	ND	ND
5	0.10	0.34	0.31	0.33	0.28	0.26	0.20	0.16	ND	ND	ND	ND
6	1.97	2.65	4.00	3.53	3.06	2.80	2.30	2.28	1.09	0.54	ND	ND
7	1.09	1.10	1.02	0.89	0.65	0.53	0.37	0.38	ND	ND	ND	ND
8	ND	0.81	0.81	0.69	0.59	0.51	0.44	0.34	0.15	ND	ND	ND
9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10	2.66	2.81	3.66	3.13	2.63	2.13	1.75	1.46	0.39	0.15	ND	ND
11	*	1.84	5.57	5.15	3.59	3.18	2.41	2.12	0.26	0.22	ND	ND
Mean	1.25	1.80	2.58	2.39	1.99	1.79	1.45	1.34	0.54	0.23	0.03	0.00
SD	1.31	138	1.95	1.82	1.46	1.32	1.10	0.01	0.41	0.19	0.07	0.01

ND = Not detectable, the limit of quantitation was 0.10 μg/ml.

Table 6 Plasma ketoconazole concentration for each patient during the treatment B

Patient No.	Plasma ketoconazole concentration (μg/ml) at each time of blood drawn (h)											
	0.5	1	1.5	2	2.5	3	3.5	4	6	8	12	24
1	2.14	5.78	5.31	5.17	4.22	3.60	3.12	2.66	1.05	0.49	ND	ND
2	3.39	3.62	5.78	3.53	2.95	2.25	2.10	2.31	0.88	0.33	ND	ND
3	3.37	3.71	4.00	2.80	3.22	3.00	2.77	2.63	1.40	1.12	0.50	ND
4	0.68	3.10	2.12	1.91	1.50	1.26	0.86	0.73	0.24	ND	ND	ND
5	0.68	1.92	1.48	1.30	1.09	1.04	0.90	0.80	0.33	0.17	ND	ND
6	0.66	3.13	3.98	2.62	2.11	1.87	1.68	1.33	0.52	0.26	0.08	ND
7	2.82	4.87	4.22	3.93	3.43	2.93	2.51	2.00	0.58	0.28	ND	ND
8	1.91	2.58	2.65	1.90	1.64	1.29	1.24	1.07	0.26	0.14	ND	ND
9	0.72	2.29	1.78	1.38	1.15	0.84	0.67	0.54	ND	ND	ND	ND
10	0.38	0.35	0.61	0.68	0.58	2.50	2.40	2.01	0.53	0.17	ND	ND
11	0.62	6.12	4.95	4.52	3.68	3.31	2.52	2.25	0.67	0.25	ND	ND
Mean	1.58	3.41	3.35	2.70	2.33	2.17	1.89	1.67	0.59	0.30	0.06	0.00
SD	1.18	1.70	1.71	1.44	1.22	0.97	0.86	0.79	0.39	0.31	0.15	0.00

ND = not detectable, the limit of quantitation was 0.10 μg/ml.

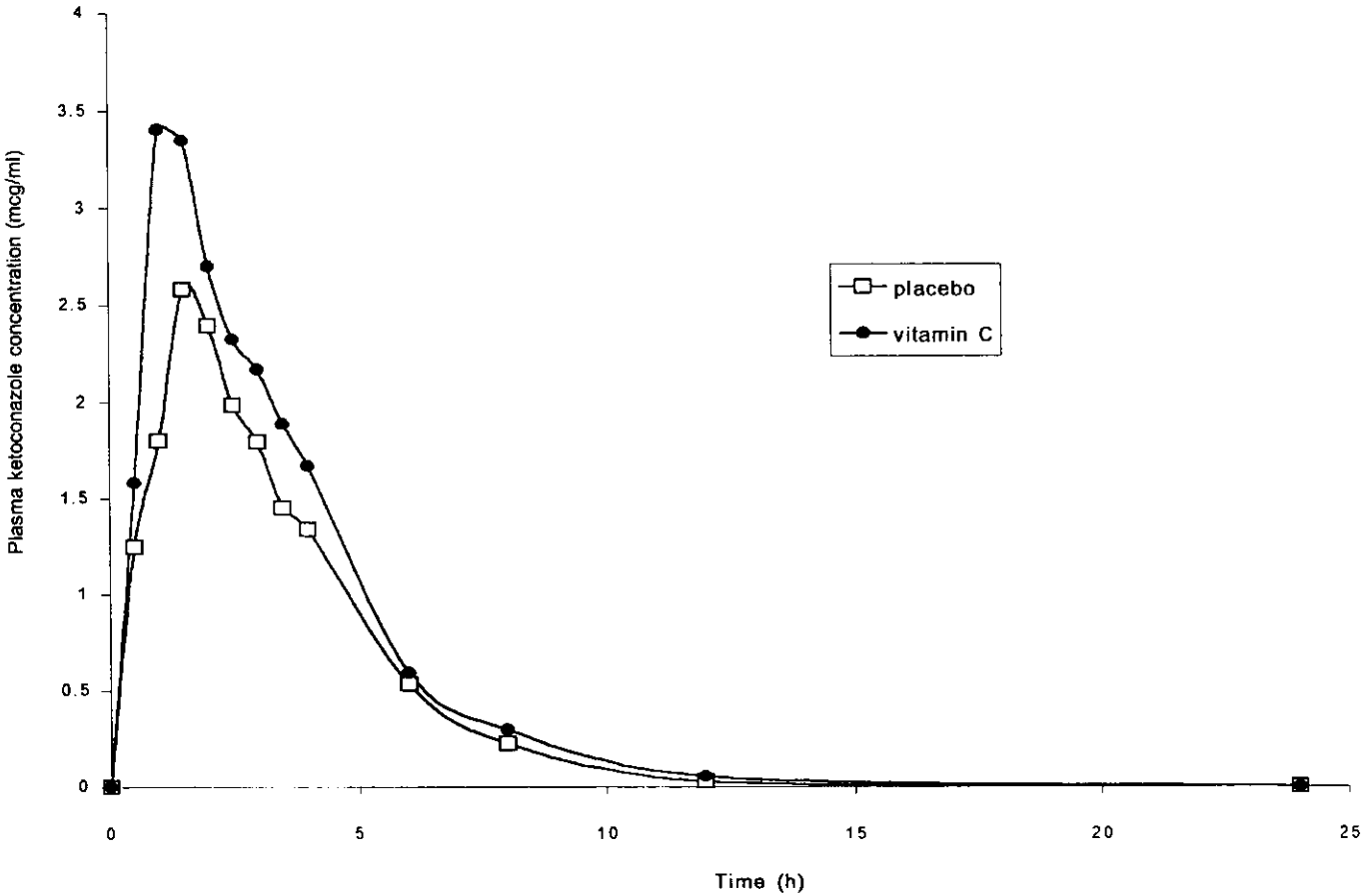


Figure 6 The mean of plasma ketoconazole concentration profiles at the time of blood drawn

Table 7 Effects of vitamin C on ketoconazole absorption in AIDS patients

Patient No.	$AUC_{0-\alpha}$ (μg.h/ml)		C_{max} (μg/ml)		T_{max} (h)		$t_{1/2}$ (h)		AUC for treatment B / AUC for treatment A
	Treatment		Treatment		Treatment		Treatment		
	A	B	A	B	A	B	A	B	
1	22.05	21.75	5.26	5.78	1.5	1.0	1.49	1.64	0.98
2	15.58	17.50	3.98	5.78	2.0	1.5	1.49	1.43	1.12
3	11.50	24.22	2.65	4.00	0.5	1.5	2.27	3.26	2.11
4	9.19	7.35	2.22	3.10	1.5	1.0	1.65	1.34	0.80
5	1.64	6.50	0.34	1.92	1.0	1.0	1.96	1.83	3.96
6	17.83	11.63	4.00	3.98	1.5	1.5	2.09	1.67	0.65
7	3.59	16.84	1.10	4.87	1.0	1.0	1.21	1.37	4.69
8	2.91	8.88	0.81	2.65	1.0	1.5	1.69	1.35	3.01
9	x	5.65	ND	2.29	x	1.0	x	1.43	
10	12.39	7.77	3.66	2.50	1.5	3.0	1.20	1.14	0.63
11	15.71	17.73	5.57	6.12	1.5	1.0	1.19	1.30	1.13
Mean	10.22	13.26	2.69	3.91	1.30	1.36	1.62	1.61	1.91
SD	7.35	6.58	1.95	1.54	0.42	0.60	0.38	0.58	1.48
***p*-value**	0.155		0.016		0.726		0.721		

ND = not detectable, the limit of quantitation was 0.10 μg/ml. x = could not perform

Table 8 Effects of vitamin C on ketoconazole absorption in AIDS patients, partitioned into two groups according to gastric pH

Patient No.	The mean gastric pH	$AUC_{0-\alpha}$ (μg.h/ml)		C_{max} (μg/ml)		T_{max} (h)		$t_{1/2}$ (h)	
		Treatment		Treatment		Treatment		Treatment	
		A	B	A	B	A	B	A	B
The mean gastric pH > 3.1									
1	4.25	22.05	21.75	5.26	5.78	1.5	1.0	1.49	1.64
2	6.85	15.58	17.50	3.98	5.78	2.0	1.5	1.49	1.43
4	6.55	9.19	7.35	2.22	3.10	1.5	1.0	1.65	1.34
5	5.55	1.64	6.50	0.34	1.92	1.0	1.0	1.96	1.83
6	5.7	17.83	11.63	4.00	3.98	1.5	1.5	2.09	1.67
7	7.7	3.59	16.84	1.10	4.87	1.0	1.0	1.21	1.37
8	7.4	2.91	8.88	0.81	2.65	1.0	1.5	1.69	1.35
9	5.1	x	5.65	ND	2.29	x	1.0	x	1.43
Mean	6.14	9.10	12.01	2.21	3.80	1.36	1.19	1.65	1.51
SD	1.19	8.39	5.99	1.97	1.54	0.38	0.26	0.30	0.18
***p*-value**		0.206		0.007		0.175		0.356	

ND = not detectable, the limit of quantitation was 0.10 μg/ml. x = could not performed

Table 8 (continued)

Patient No.	The mean gastric pH	$AUC_{0-\alpha}$ (μg.h/ml)		C_{max} (μg/ml)		T_{max} (h)		$t_{1/2}$ (h)	
		Treatment		Treatment		Treatment		Treatment	
		A	B	A	B	A	B	A	B
The mean gastric pH ≤ 3.1									
3	2.35	11.50	24.22	2.65	4.00	0.5	1.5	2.27	3.26
10	3.1	12.39	7.77	3.66	2.50	1.5	3.0	1.20	1.14
11	1.65	15.71	17.73	5.57	6.12	1.5	1.0	1.19	1.30
Mean	2.37	13.2	16.57	3.96	4.21	1.17	1.83	1.55	1.90
SD	0.73	2.22	8.29	1.48	1.82	0.58	1.04	0.62	1.18
***p*-value**		0.573		0.771		0.398		0.383	

Table 9 Effects of vitamin C on ketoconazole absorption in AIDS patients, partitioned into two groups according to disease state

Patient No.	$AUC_{0-\alpha}$ (μg.h/ml)		C_{max} (μg/ml)		T_{max} (h)		$t_{1/2}$ (h)	
	Treatment		Treatment		Treatment		Treatment	
	A	B	A	B	A	B	A	B
Stable illness								
1	22.05	21.75	5.26	5.78	1.5	1.0	1.49	1.64
2	15.58	17.50	3.98	5.78	2.0	1.5	1.49	1.43
3	11.50	24.22	2.65	4.00	0.5	1.5	2.27	3.26
5	1.64	6.50	0.34	1.92	1.0	1.0	1.96	1.83
7	3.59	16.84	1.10	4.87	1.0	1.0	1.21	1.37
8	2.91	8.88	0.81	2.65	1.0	1.5	1.69	1.35
9[a]	x	5.65	ND	2.29	x	1.0	x	1.43
11	15.71	17.73	5.57	6.12	1.5	1.0	1.19	1.30
Mean	9.12	14.88	2.46	4.18	1.21	1.21	1.61	1.74
SD	8.16	7.02	2.24	2.46	0.49	0.27	0.39	0.70
***p*-value**	0.029		0.007		1.000		0.460	

ND = not detectable, the limit of quantitation was 0.10 μg/ml. x = not performed

Table 9 (continued)

Patient No.	$AUC_{0-\alpha}$ (µg.h/ml)		C_{max} (µg/ml)		T_{max} (h)		$t_{1/2}$ (h)	
	Treatment		Treatment		Treatment		Treatment	
	A	B	A	B	A	B	A	B
Progressive illness								
4 [b]	9.19	7.35	2.22	3.10	1.5	1.0	1.65	1.34
6 [c]	17.83	11.63	4.00	3.98	1.5	1.5	2.09	1.67
10 [d]	12.39	7.77	3.66	2.50	1.5	3.0	1.20	1.14
Mean	13.14	8.92	3.29	3.19	1.50	1.83	1.65	1.38
SD	4.37	2.36	0.94	0.74	0.00	1.04	0.44	0.27
***p*-value**	0.08		0.881		0.635		0.635	

a : no medication

b : Patient was treated for PCP infection with cotrimoxazole (400/80) 4 tablet orally three time daily. and prednisolone 40 mg once daily prior treatment A, the dose of prednisolone was taper to 10 mg prior treatment B.

c : Patient took oral ciprofloxacin 750 mg twice daily. and dapsone 100 mg once daily. Patient has history of cotrimoxazole hypersensitivity, Stevens-Johnson syndrome.

d : Besides cotrimoxazole, patient was received ceftriaxone 250 mg IM single dose, acyclovir 200 mg five times daily, norfloxacin 200 mg twice daily, metronidazole 400 mg four times daily and clotrimazole vaginal tablet 100 mg at bedtime during treatment A. Before treatment B, she left only cotrimoxazole.