CHAPTER IV

RESULTS AND DISCUSSION

In Vitro Studies

Based on the described chromatographic conditions for tablet assay, the retention times of ketoconazole and of diazepam (internal standard) were 6.56 ± 0.05 and 4.69 ±0.03 min, respectively (Figure 2). The resolution between two major peaks was more than 2 and the coefficient of variation of peak height ratios of five replicate standard solution chromatograms was 1.24% (Figure 2), so the quantitative HPLC method of assaying ketoconazole tablets employed in the present study met the system suitability as specified by the the United State Pharmacopoeia XXII.

Table 1 shows the results of weight variation and test stipulated by the United State Pharmacopoeia XXII for ketoconazole tablets. Ketoconazole content in tablets of the nine brands, expressed in terms of the percent of labeled amount actually found by analysis, were within United State Pharmacopoeia XXII limits of 90.0-110.0 % of the labeled content. For content uniformity analysis, the United State Pharmacopoeia requires that the assay of ten individual tablets of each lot should indicate that not less than nine of the ten tablets are within 85-115% of
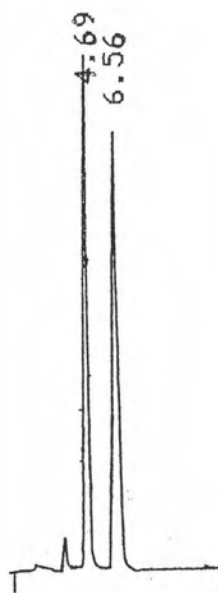
Figure 2. Chromatogram of standard solution containing ketoconazole (retention time 6.56 min) and diazepam (retention time 4.69 min).

Using chart speed of 0.50 cm/min revealed that the resolution between two compounds was 2.137 ,The peak height ratios of five replicate injection were 0.8282 , 0.8037 , 0.8090 , 0.8076 and 0.8045 , or 0.8106±0.0100 (mean±S.D.) and %C.V. was 1.24.

Table 1.   Weight variation and tests required by the United State
Pharmacopoeia XXII for ketoconazole tablets.

PRODUCT	WEIGHT[a] VARIATION	ASSAY OF %[b] CONTENT	CONTENT RANGE	UNIFORMITY[c] MEAN+C.V.	DISINTE[d] GRATION
A	311.66±2.99	97.36±2.36	94.97-103.68	99.10±3.46	1.92±0.14
B	359.59±3.70	99.82±0.52	100.62-104.92	102.33±1.52	0.39±0.03[e]
C	355.78±7.74	103.15±3.15	99.00-110.81	103.90±4.24	1.22±0.23
D	399.26±8.28	105.18±3.97	96.64-111.07	103.91±3.96	6.08±0.31[e]
E	299.11±6.30	100.83±3.10	94.25-112.04	103.07±5.57	2.32±0.31
F	321.33±8.20	101.28±0.79	102.01-111.91	106.32±3.45	2.36±0.27
G	315.18±3.30	98.40±3.71	97.11-105.37	101.16±3.01	3.68±0.57
H	325.05±4.82	100.82±3.76	96.94-104.59	101.37±2.43	3.33±0.40[e]
I	378.39±6.46	97.81±2.64	91.51-100.53	96.10±3.13	1.21±0.11

a   Means(±S.D.) are based on 20 determinations. (mg)

b   Means(±S.D.) are based on 3 determinations. (%)

c   Means(±C.V.) are based on 10 determinations. (%)

d   Means(±S.D.) are based on 6 determinations. (min)

e   undergo disintegration test for coated-tablets , The others
were tested using procedure for plain tablets.

the average of the tolerances specified in the potency definition in the monograph and that the content of none of the tablets falls outside the limits of 75-125% of that average and the percent C.V. of those content must be less than or equal to 6.0%. In no instance did any of the product fail to meet these United State Pharmacopoeia specifications. The last testing required by the United State Pharmacopoeia is the disintegration testing having limits of 10.0 min. All products tested disintegrated well within the ten min time of limit. Results in table 1 show that all nine products met the specifications for assay, content uniformity and disintegration of the United State Pharmacopoeia XXII. This supported the assumption that all products were pharmaceutical equivalent.

Not required by the United State Pharmacopoeia but being a crucial factor of systemic drug availability, the dissolution testing was performed in two media representing biologic gastric and intestinal fluids. Major differences were observed for the rate of dissolution in the two media and the disintegration time in carbon dioxide-free water. Tables 2 and 3 Figures 3 and 4 show mean percent dissolved of six ketoconazole tablets in media used at each sampling time. Table 4 summarizes the dissolution rates in the two media and the disintegration times of nine products whose statistical differences were shown in table 5, 6, 7 and 8. Multiple t-test results in table 8 indicates that the dissolution in acidic medium (pH 1.2) may be approximately

Table 2. Mean percent dissolved of ketoconazole in simulated gastric fluid without pepsin (pH 1.2) at each sampling time.

SAMPLING TIME (min)	MEAN PERCENT DISSOLVED[a]								
	A	B	C	D	E	F	G	H	I
2.5	6.20 (0.88)	1.35 (1.11)	4.89 (2.76)	1.75 (0.22)	2.19 (0.32)	17.06 (7.03)	2.34 (0.46)	2.56 (0.75)	10.91 (3.52)
5.0	26.46 (2.51)	25.42 (10.37)	50.50 (13.72)	2.15 (0.19)	11.16 (2.20)	81.44 (7.93)	47.74 (2.63)	35.24 (7.30)	61.33 (9.46)
7.5	48.27 (4.22)	60.06 (8.56)	74.46 (11.84)	10.67 (4.17)	20.56 (2.29)	99.52 (5.60)	39.26 (8.23)	63.26 (11.31)	81.17 (8.02)
10	65.79 (1.25)	79.38 (5.65)	85.71 (6.83)	34.75 (9.43)	28.58 (3.52)	104.27 (4.21)	60.67 (8.88)	87.21 (10.80)	93.93 (6.92)
12.5	76.92 (2.79)	89.39 (4.42)	91.24 (3.36)	66.00 (13.31)	39.99 (4.95)	106.18 (2.22)	76.90 (13.38)	98.39 (6.34)	99.47 (3.28)
15	90.37 (4.78)	90.87 (2.69)	92.80 (2.94)	85.53 (10.19)	50.50 (8.61)	106.83 (2.56)	85.41 (13.65)	104.46 (3.67)	103.0 (1.19)
20	97.63 (1.92)	92.08 (1.97)	93.86 (1.04)	100.55 (2.65)	58.50 (7.87)	108.00 (2.20)	92.95 (13.21)	104.60 (3.58)	104.9 (1.24)
25	99.98 (3.47)	93.10 (1.10)	93.79 (0.94)	105.91 (1.22)	74.00 (3.85)	107.52 (2.22)	95.07 (11.44)	105.2 (2.68)	100.85 (1.66)
30	100.66 (0.24)	91.76 (1.35)	92.26 (1.66)	106.85 (1.52)	86.51 (0.53)	108.27 (2.38)	97.46 (7.80)	106.37 (1.48)	102.21 (2.24)
35	100.87 (1.47)	92.64 (1.35)	92.80 (1.36)	105.63 (1.50)	95.80 (0.64)	107.26 (3.20)	98.98 (8.21)	104.27 (2.00)	102.09 (0.83)
40	100.11 (1.14)	94.68 (1.73)	93.56 (1.65)	107.06 (1.50)	96.58 (9.23)	108.21 (1.47)	101.04 (4.84)	107.06 (0.70)	102.50 (1.68)
45	100.96 (3.51)	93.04 (1.66)	92.66 (1.21)	104.76 (1.98)	105.18 (5.07)	104.90 (2.83)	101.00 (4.78)	107.08 (0.70)	99.40 (1.68)
50	100.57 (2.87)	92.18 (2.44)	92.69 (1.81)	106.23 (3.41)	106.56 (4.28)	104.78 (2.58)	99.99 (4.59)	105.45 (1.06)	99.08 (1.10)
60	97.12 (1.44)	93.27 (1.77)	92.46 (1.80)	105.40 (1.61)	102.52 (3.77)	104.57 (2.23)	99.71 (2.49)	105.42 (2.21)	97.95 (1.27)

a   Means (S.D.) are based on 6 determinations.
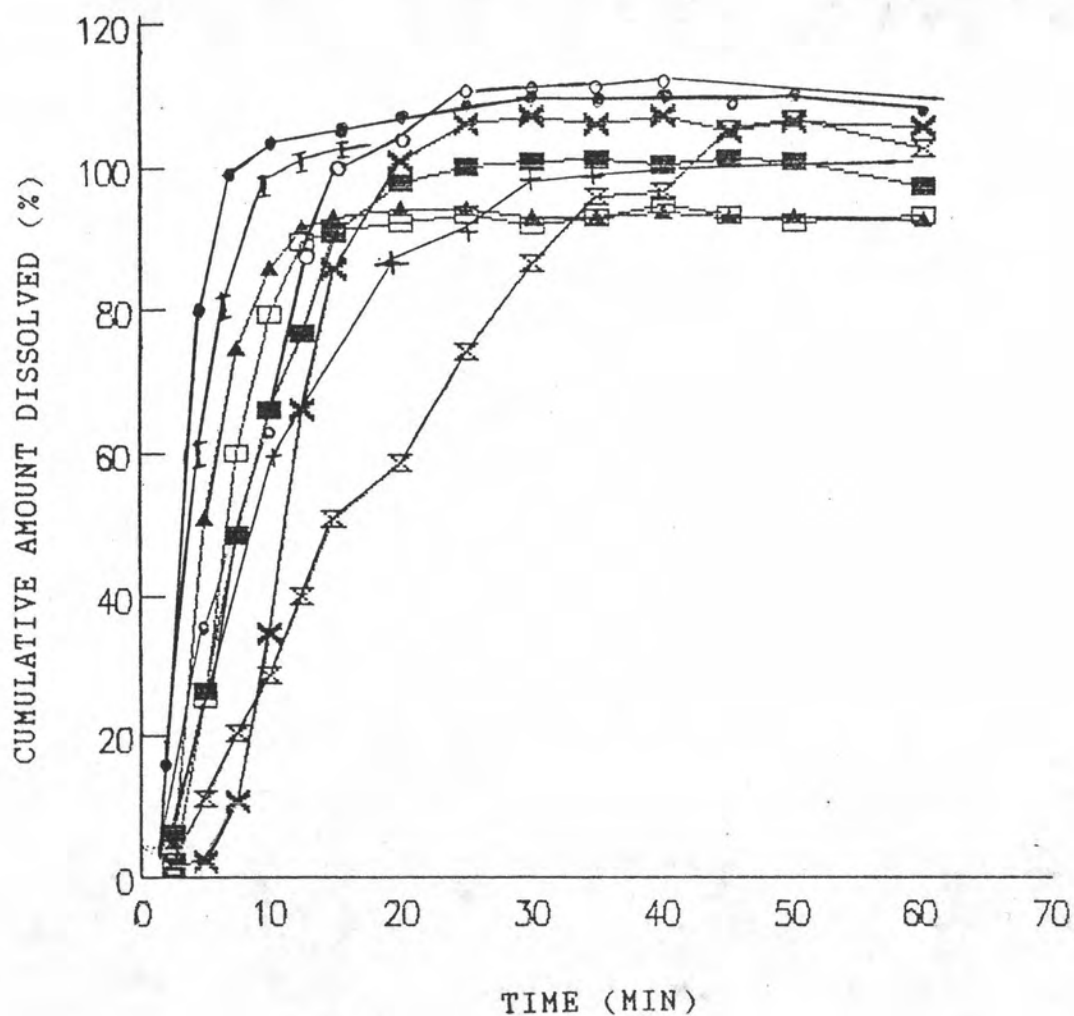
Figure 3.   Dissolution profiles of nine commercial
brands   of   ketoconazole   tablets   in
simulated gastric fluid without pepsin.

Key: A (■)   B (□)   C (▲)

            D (✕)   E (⊠)   F (●)

            G (+)   H (○)   I (I)

divided into three dissolution groups, with products C, B, F and I being relatively rapidly dissolved, product E being relatively slow dissolved, and the remainders including innovator's product having an intermediate dissolution rate in medium pH 1.2. Using t-test to analyze the differences of dissolution rate in medium pH 1.2 between the locally-made products and innovator's one, similar result was obtained that the dissolution rates of products C, B, F and I were significantly higher than that of the reference (A), no significant differences were observed between the reference (A) and products D, G and H, and product E has significantly lower rate (Table 5).

Being a dibasic drug, ketoconazole can be readily liberated and completely dissolved in acidic medium such as simulated gastric fluid used in this study (Table 2 and Figure 3) contrasting to dissolution in basic medium as shown in table 3 and figure 3 that only 1.08-2.30% of ketoconazole can be dissolved with much slower rate in intestinal fluid. Tables 6 and 8 show that these nine products can be divided into three groups having different dissolution rates in simulated intestinal fluid i.e. B, I and F have higher dissolution rates than D, C, and H and reference (A), and E and G have the lowest rate and lower than reference (A).

Disintegration performed in water also classified products tested into three groups, with products I, C, B and A being rapidly disintegrated, products E and F being

Table 3. Mean percent dissolved of ketoconazole in simulated intestinal fluid without pancreatin (pH 7.5) at each sampling time.

SAMPLING TIME (min)	MEAN PERCENT DISSOLVED[a]								
	A	B	C	D	E	F	G	H	I
5	0.00	0.04 (0.07)	0.00	0.00	0.00	0.58 (0.08)	0.00	0.00	0.56 (0.11)
10	0.00	0.53 (0.14)	0.00	0.00	0.00	0.85 (0.08)	0.00	0.00	0.96 (0.10)
15	0.05 (0.12)	0.91 (0.16)	0.21 (0.12)	0.22 (0.25)	0.00	1.20 (0.17)	0.00	0.00	1.22 (0.06)
20	0.40 (0.21)	1.14 (0.17)	0.37 (0.14)	0.43 (0.28)	0.00	1.38 (0.12)	0.06 (0.03)	0.06 (0.03)	1.39 (0.05)
25	0.68 (0.19)	1.26 (0.15)	0.50 (0.13)	0.59 (0.26)	0.00	1.57 (0.10)	0.11 (0.03)	0.26 (0.08)	1.60 (0.04)
30	0.96 (0.19)	1.38 (0.16)	0.64 (0.15)	0.73 (0.28)	0.00	1.70 (0.14)	0.08 (0.05)	0.48 (0.07)	1.70 (0.06)
45	1.15 (0.15)	1.68 (0.18)	0.86 (0.13)	1.11 (0.30)	0.00	2.03 (0.11)	0.18 (0.10)	0.92 (0.09)	2.03 (0.05)
60	1.46 (0.12)	1.82 (0.11)	0.94 (0.11)	1.40 (0.31)	0.00	2.15 (0.10)	0.24 (0.16)	1.11 (0.05)	2.14 (0.05)
90	1.71 (0.12)	1.85 (0.09)	1.14 (0.09)	1.73 (0.24)	0.00	2.30 (0.10)	0.42 (0.25)	1.43 (0.04)	2.32 (0.03)
120	1.93 (0.11)	1.96 (0.09)	1.24 (0.09)	1.98 (0.23)	0.00	2.43 (0.09)	0.64 (0.35)	1.56 (0.05)	2.33 (0.04)
150	2.03 (0.45)	1.98 (0.05)	1.30 (0.09)	2.06 (0.24)	0.00	2.48 (0.09)	0.78 (0.35)	1.62 (0.08)	2.33 (0.04)
180	2.20 (0.10)	1.99 (0.04)	1.35 (0.06)	2.04 (0.19)	0.00	2.46 (0.10)	0.97 (0.42)	1.70 (0.07)	2.31 (0.06)
210	2.20 (0.10)	1.96 (0.04)	1.42 (0.06)	2.12 (0.19)	0.13 (0.32)	2.47 (0.08)	1.03 (0.40)	1.73 (0.05)	2.29 (0.06)
240	2.21 (0.09)	1.96 (0.04)	1.54 (0.17)	2.11 (0.23)	0.34 (0.41)	2.48 (0.08)	1.21 (0.40)	1.71 (0.07)	2.30 (0.06)
360	–	–	–	–	0.55 (0.35)	–	1.30 (0.42)	–	–
480	–[b]	–	–	–	0.85 (0.40)	–	–	–	–
540	–	–	–	–	1.04 (0.40)	–	–	–	–
600	–	–	–	–	1.08 (0.40)	–	–	–	–

a   Means are based on six determinations. (S.D.)

b   No sample was collected because plateau level has been already achieved.
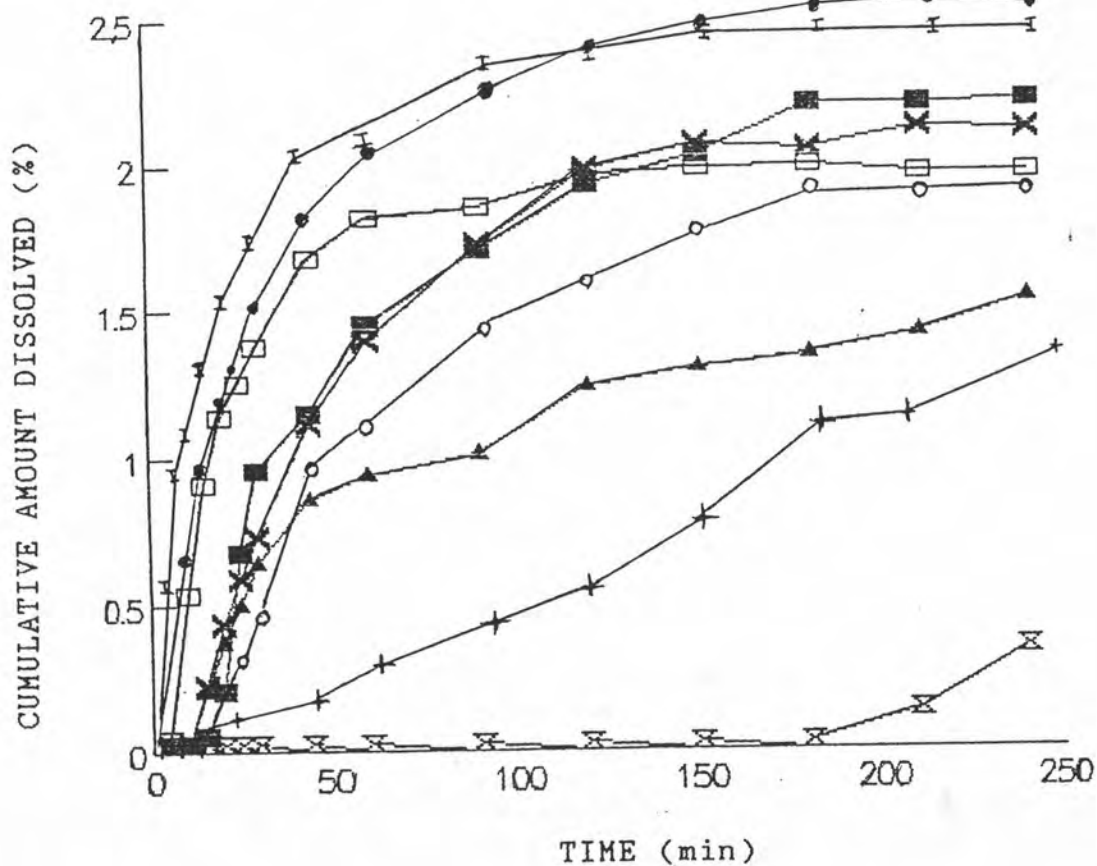
Figure 4. Dissolution profiles of nine commercial brands of ketoconazole tablets in simulated intestinal fluid without pancreatin.

Note that for brands E dissolution. was not occurred for the first 180 min.

Key: A (■)   B (❑)   C (▲)

         D (×)   E (✗)   F (●)

         G (+)   H (O)   I (I)

Table 4.   Dissolution rates of nine 200 mg ketoconazole tablets in
           simulated gastric fluid without pepsin (pH 1.20) and
           simulated intestinal fluid without pancreatin (pH 7.50)
           and disintegration time using carbon dioxide free-water as
           immersion fluid.

| PRODUCT | DISSOLUTION RATE (hr^{-1})[a] | | DISINTEGRATION TIME [a] |
	pH 1.2 (I)	pH 7.5 (II)	(MIN)(III)
A	9.70±1.24	1.59±0.15	1.92±0.14
B	19.72±5.08	2.70±0.39	1.88±0.15
C	22.00±5.70	1.41±0.34	1.22±0.23
D	10.38±1.21	1.33±0.35	5.26±1.05
E	4.57±1.26	0.19±0.08	2.32±0.30
F	18.06±5.70	2.12±0.30	2.36±0.27
G	9.63±4.32	0.24±0.12	3.68±0.57
H	13.83±5.64	1.45±0.18	9.25±1.45
I	17.71±2.49	2.41±0.36	1.21±0.11

a   Based on six determinations. (±S.D.)

b   Correlation coefficient between I and II was -0.2584 and
       t-calculated was -0.7077 ($t_{0.95,7}$ = 2.365)

c   Correlation coefficient between II and III was -0.2200 and
       t-calculated was -0.5969 ($t_{0.95,7}$ = 2.365)

Table 5. Statistical differences of dissolution rates in simulated gastric fluid without pepsin of ketoconazole tablets assessed by one-way analysis of variance and t-test (α =0.05).

SOURCE OF VARIATION	DEGREE OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F RATIO
BETWEEN PRODUCTS	8	1599.48	199.93	11.81
WITHIN PRODUCTS	45	761.41	16.92	
TOTAL	53	2360.89		

$$F_{.95} (8,45) = 2.16$$

PRODUCT	MEAN DISSOLUTION RATE ($\pm$S.D.) IN pH 1.2 (hr^{-1})[a]	t-CALCULATED[b]	SIGNIFICANT[c] LEVEL
A	9.70$\pm$1.24	-	-
B	19.72$\pm$5.08	-4.22	S
C	22.00$\pm$5.70	-5.18	S
D	10.38$\pm$1.21	-0.29	NS
E	4.57$\pm$1.26	2.16	S
F	18.06$\pm$5.70	-3.52	S
G	9.63$\pm$4.32	-0.03	NS
H	13.83$\pm$5.64	-1.74	NS
I	17.71$\pm$2.49	-3.37	S

a Based on six determinations

b A was used as a reference for comparisons.

c $t_{.95,45}$ = 2.014

   NS NON SIGNIFICANT DIFFERENT ($P > 0.05$)

   S SIGNIFICANT DIFFERENT ($P < 0.05$)

Table 6. Statistical differences of dissolution rates in simulated intestinal fluid without pancreatin of ketoconazole tablets assessed by one-way analysis of variance and t-test (α =0.05).

SOURCE OF VARIATION	DEGREE OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F RATIO
BETWEEN PRODUCTS	8	36.06	4.50	58.57
WITHIN PRODUCTS	45	3.46	0.07	
TOTAL	53	39.52		

$$F_{.95}\ (8,45)\ =\ 2.16$$

PRODUCT	MEAN DISSOLUTION RATE ($\pm$S.D.) IN pH 7.5 (hr^{-1})[a]	t-CALCULATED[b]	SIGNIFICANT[c] LEVEL
A	1.59±0.15	–	–
B	2.70±0.39	-6.93	S
C	1.41±0.34	0.62	NS
D	1.33±0.35	1.62	NS
E	0.19±0.08	8.74	S
F	2.12±0.30	-3.31	S
G	0.24±0.12	8.43	S
H	1.45±0.18	0.87	NS
I	2.41±0.36	-5.12	S

a   Based on six determinations

b   A was used as a reference for comparisons.

c $t_{.95,45}\ =\ 2.014$

NS   NON SIGNIFICANT DIFFERENT   ($P > 0.05$)

S   SIGNIFICANT DIFFERENT   ($P < 0.05$)

Table 7. Statistical differences of disintegration times (using carbon dioxide-free water as immersion fluid) of ketoconazole tablets assessed by one-way analysis of variance and t-test (α=0.05).

SOURCE OF VARIATION	DEGREE OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F RATIO
BETWEEN PRODUCTS	8	323.39	40.42	95.89
WITHIN PRODUCTS	45	18.97	0.42	
TOTAL	53	342.36		

$$F_{.95} (8,45) = 2.16$$

PRODUCT	MEAN DISINTEGRATION TIME (min±S.D.)	t-CALCULATED[b]	SIGNIFICANT[c] LEVEL
A	1.92±0.14	-	-
B	1.88±0.15	0.10	NS
C	1.22±0.23	1.87	NS
D	5.26±1.05	-8.91	S
E	2.32±0.30	-1.07	NS
F	2.36±0.27	-1.17	NS
G	3.68±0.57	-4.67	S
H	9.25±1.45	-19.55	S
I	1.21±0.11	1.89	NS

a   Based on six determinations

b   A was used as a reference for comparisons.

c $t_{.95,45} = 2.014$

   NS   NON SIGNIFICANT DIFFERENT (P > 0.05)

    S   SIGNIFICANT DIFFERENT   (P < 0.05)

Table 8.   Rank order of products in terms of dissolution
            rate in two media used and disintegration
            time.[a]

DISINTEGRATION TIMES RANGING FROM LEAST TO MOST TIME.

    I   C   B   A   E   F   G   D   H

DISSOLUTION RATE IN simulated gastric fluid without
pepsin FROM THE FASTEST TO SLOWEST DISSOLUBLE
PRODUCTS.

    C   B   F   I   H   D   A   G   E

DISSOLUTION RATE IN simulated intestinal fluid
without pancreatin FROM THE FASTEST TO SLOWEST
DISSOLUBLE PRODUCTS.

    B   I   F   A   H   C   D   G   E

_____

a   Products ranked on the basis of the multiple
       t -test . Products underlined by the same
       line are not statistical different ($P > 0.05$).

intermediately disintegrated and products G, D and H having the longest disintegration times (Table 7 and 8). As seen in table 4, no statistical correlation was found between the dissolution rate constants of ketoconazole tablets in the two media used and the disintegration times , indicating that rapidly disintegrating tablets do not necessarily guarantee high dissolution rate constants.

Five different brands of a 200-mg ketoconazole tablet with different dissolution rates in gastric and intestinal fluids were selected to submit for the *in vivo* studies which would be discussed below.

In Vivo Studies

1. Assay Validation

The typical chromatograms obtained from the analyses of the blank, the aqueous solution spiked with ketoconazole and plasma sample from a subject taking a 200 mg single dose ketoconazole tablet at 6 hr postdose were shown in figure 5. Both ketoconazole and quinidine peaks were well separated with the retention times of 5.1 min and 7.5 min, respectively, from the endogenous substance peaks. Using a signal-to-noise ratio of 3 and maximum acceptable percent C.V. of 10% as the criterion, the sensitivity for ketoconazole determination is 0.20 mcg/ml of plasma with a peak height ratio of 0.13 and 7.69 %C.V. (n=3) (Table 9). Concentrations of ketoconazole of lower than 0.20 mcg/ml result in high percent C.V. (The data was not shown).

The mean percent recoveries for quinidine and ketoconazole in the concentration range of 0.2 mcg/ml to 8 mcg/ml were 98.96 and 101.62, respectively, as shown in table 9. Results of the recovery of ketoconazole was not dependent on concentrations.

The within-run precision (n=3) and the between run precision (n=4) over the calibration range of the HPLC method employed were summarized in tables 10 and 11, respectively. The percentage of C.V. varied randomly over the concentration range.

The standard curves constructed from the spiked plasma standard were linear between the concentrations of 0.2 mcg/ml to 8.0 mcg/ml. To maintain the accuracy of the HPLC method, all samples having ketoconazole concentration more than 8.0 mcg/ml were diluted with the drug-free plasma appropriately before analysis.

2. Pharmacokinetic Studies

The means and the S.D.s of the plasma concentrations at each sampling time following administration of A, B, C, D and E are given in table 12. Figures 6 and 7 show the plot of the arithmetic means of the plasma concentrations of ketoconazole versus time obtained from the separate administration of the five brands.

Results from the CSTRIP analysis indicated that a biexponential equation (one compartment model) without a

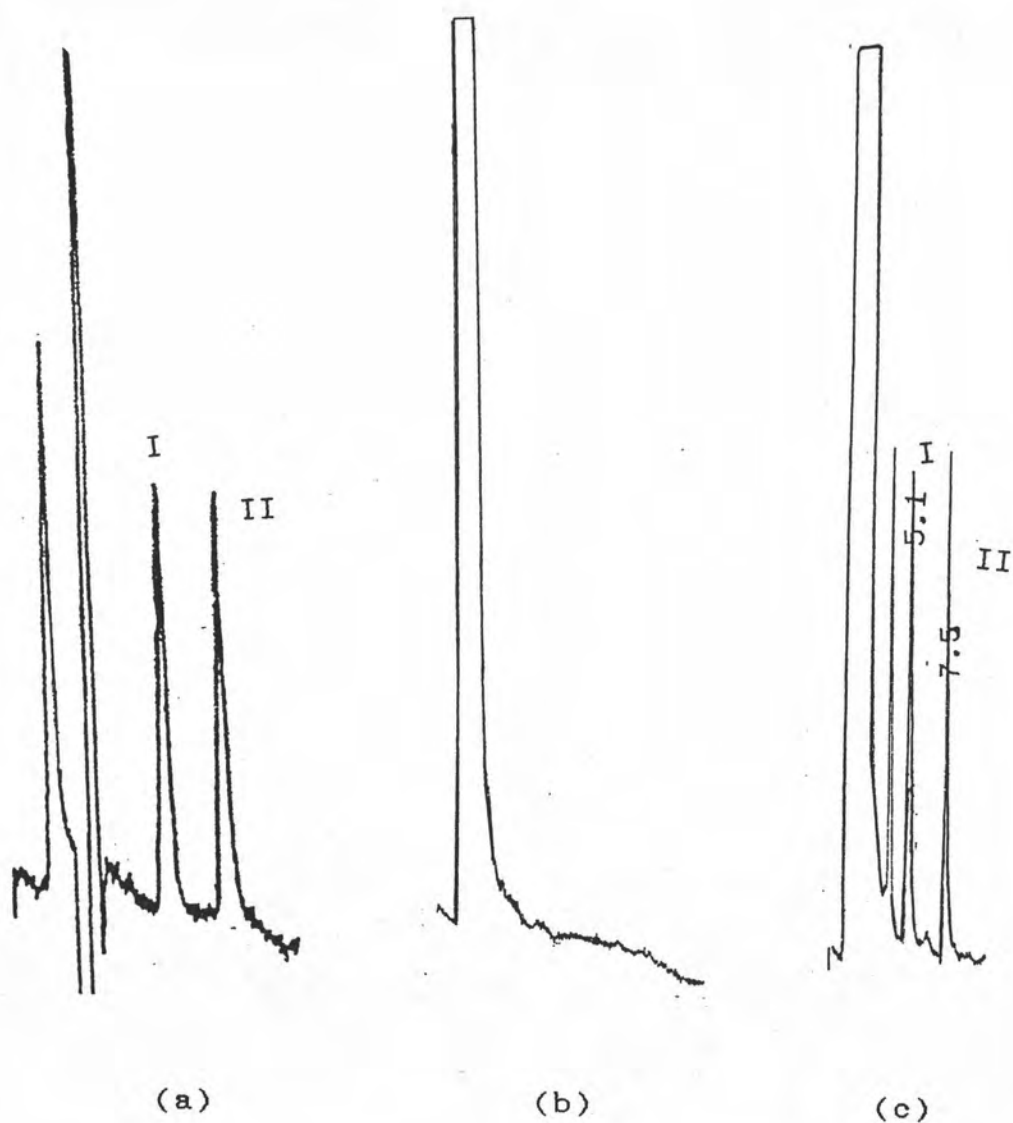(a)   (b)   (c)

Figure 5.   Chromatograms of ketoconazole and quinidine

a)   aqueous solution spiked with 2.0 mcg/ml
      of   ketoconazole (I) and quinidine (II)

b)   normal human plasma blank

c)   plasma sample obtained from a subject
      receiving a single dose 200 mg
      ketoconazole tablet at 6 hr. postdose.
      (observed concentration = 2.0165 mcg/ml)

Table 9   Recovery of ketoconazole from plasma at
            various concentrations (n=3) and quinidine
            at concentration of 4.8 mcg/ml (n=10).

CONCENTRATION (mcg/ml)	RECOVERY (mean±S.D.)	C.V.(%)
0.20	98.40±6.60	6.71
0.50	107.58±5.91	5.50
1.00	96.42±4.61	4.78
2.00	105.00±2.91	2.77
4.00	100.54±1.32	1.31
6.00	101.32±3.21	3.17
8.00	102.07±0.43	0.42
MEAN (%)	101.62±3.78	3.72
QUINIDINE (4.8 MCG/ML)	98.96±4.72	4.77

Table 10.   Within-run precision for ketoconazole
             from three replicated plasma standard
             curves obtained in the same day.

CONCENTRATION (mcg/ml)	PEAK HEIGHT RATIO (mean±S.D.)	C.V.(%)
0.20*	0.13±0.01	6.32
0.50	0.26±0.01	2.41
1.00	0.55±0.03	5.51
2.00	0.95±0.03	2.70
4.00	1.89±0.03	1.42
6.00	2.45±0.07	2.66
8.00	3.65±0.12	3.38

* When the concentrations were lower than 0.20 mcg/ml, the unacceptable % C.V. (higher than 10%) was obtained (Data are not shown).

Table   11.   Between run precision for ketoconazole
               from   four replicated   plasma standard
               curves obtained in the four different
               days.

CONCENTRATION   PEAK HEIGHT RATIO   C.V.(%)
(mcg/ml)   (mean ± S.D.)

CONCENTRATION (mcg/ml)	PEAK HEIGHT RATIO (mean ± S.D.)	C.V.(%)
0.20	0.13±0.01	7.75
0.50	0.26±0.02	7.69
1.00	0.50±0.03	6.00
2.00	0.92±0.07	7.61
4.00	1.83±0.12	6.55
6.00	2.52±0.09	3.57
8.00	3.44±0.14	4.07

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lag time was best described all of the concentration-time curves for ketoconazole. So the data were treated according to the one compartment model without lag time for obtaining the pharmacokinetic parameters as described in chapter III.

Most of the pharmacokinetic-related studies of ketoconazole previously published have employed model - independent analysis to describe the data. In all studies after the maximum plasma concentration of ketoconazole had been reached, the concentrations of ketoconazole appeared to decline monoexponentially (Baxter, 1986;Brass et al., 1982; Daneshmend et al., 1982; Daneshmend et al.,1982). However, there was one study with longer sampling period (as long as 48 hr) than any ketoconazole pharmacokinetic studies of which the result revealed that ketoconazole had biphasic elimination manner. The elimination phase of the concentration-time profile showed a fast declining initial phase (half-life about 2 hr) and a slowly elimination phase thereafter (half-life about 8 hr). The latter elimination phase was observed only when the concentration was less than 0.10 mcg/ml and usually required not less than 24 hr blood collecting time. In most of the studies including this one, the plasma samples were collected within 12 hr which should be the reason that the concentration-time profiles apparently demonstrate the one compartmental characteristics. As seen in table 13,average half-lives of the five ketoconazole products ranged from 2.28-2.8 hrs.

Table 12.   Mean plasma concentrations of ketoconazole following
brands A, B, C, D and E administrations. (Mean±S.D.)

| TIME (hr)	CONCENTRATION (mcg/ml)				
	A	B	C	D	E
0.00	0	0	0	0	0
0.50	2.39±2.01	2.48±2.56	1.58±0.73	1.33±1.17	1.89±1.25
1.00	3.96±1.88	4.10±2.23	3.12±1.37	2.90±1.88	3.05±1.32
1.50	4.45±1.11	4.14±2.16	3.38±1.29	3.45±1.29	3.78±1.16
2.00	3.95±1.15	3.73±1.69	3.69±1.38	4.12±1.64	4.16±1.16
3.00	2.86±0.96	3.17±1.58	2.68±1.10	3.38±1.31	3.20±1.20
4.00	1.88±0.77	2.26±1.20	2.19±1.06	2.47±1.06	2.12±0.98
6.00	1.18±0.59	1.23±0.63	1.21±0.59	1.43±0.64	1.18±0.67
8.00	0.67±0.27	0.70±0.34	0.81±0.40	0.94±0.39	0.64±0.42
10.0	0.45±0.21	0.35±0.18	0.57±0.32	0.56±0.31	0.41±0.23
12.0	0.26±0.14	0.16±0.12	0.32±0.18	0.35±0.20	0.24±0.23
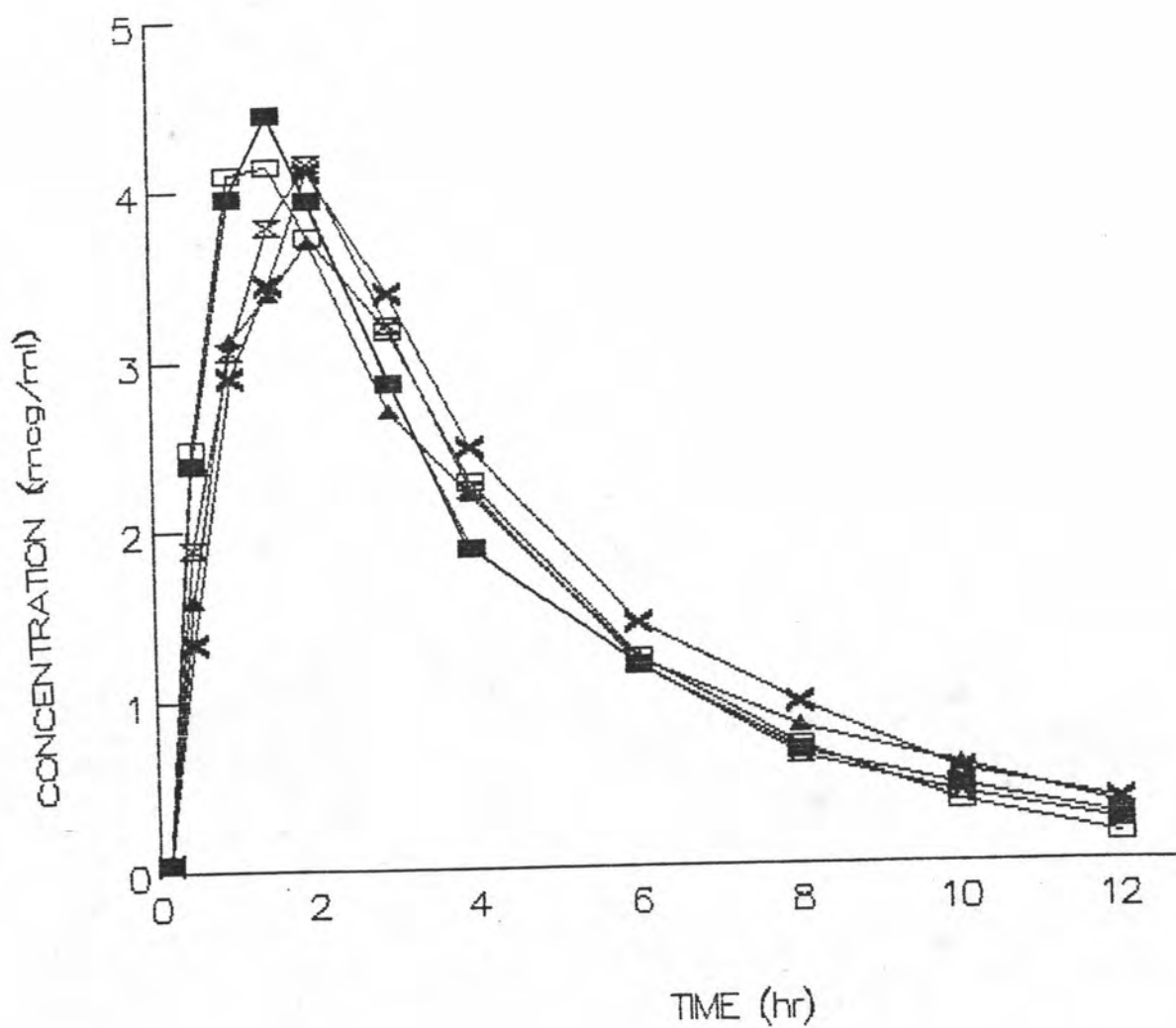
Figure 6. Mean plasma concentrations of ketoconazole in twelve healthy volunteers following administration of a single 200 mg oral doses of brands A (■) B (▢) C (▲) D (×) E (⊠)
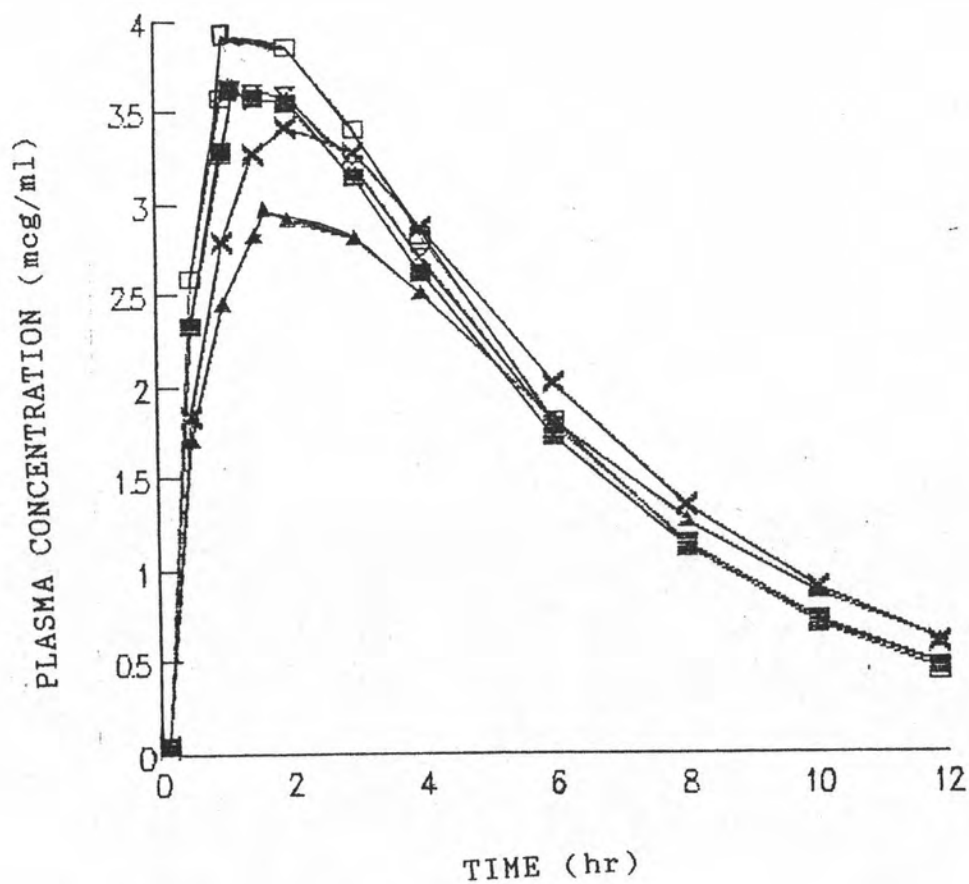
Figure 7. Mean plasma concentrations of ketoconazole over twelve hour sampling time and at time to reach the peak. Each line is based on CSTRIP-generated equation.

Keys   A (■)   B (□)   C (▲)

D (×)   E (⊠)

Collecting of blood samples over 12 hrs considered to be four or five half-lives was enough for the bioavailability assessment of ketoconazole.

Table 13 lists the mean values and S.D.s of the pharmacokinetic parameters for ketoconazole. Considerable intersubject variation were noted for all of the parameters of five brands. The intersubject variation in all of the parameters was comparable among the five brands and was similar to those obtained in all pharmacokinetic studies reported previously. (Baxter et al, 1986; Brass et al., 1982; Daneshmend et al., 1983; Daneshmend et al., 1986 and Parichart et al., 1988). Cabana(1983) has proposed that drugs having a large coefficient of variation associated with extensive first-pass metabolism should be required to undergo multiple dose steady state study comparisons. But it is worthy to note that even in the subjects undergone the multiple dosing, the steady state plasma ketoconazole concentrations and, especially, the AUC resulted in a large variation (Brass et al., 1982). Thus the multiple dose bioequivalence studies of ketoconazole may not have an advantage over the single-dose studies in the terms of intersubject variation. The mean pharmacokinetic values of ketoconazole reported by various studies (Daneshmend et al. , 1982; Daneshmend and Warnock, 1988; Heel, 1982; Mannisto et al., 1982) such as C_{max} (3.5-4.5 mcg/ml), t_{max} (1.21-2 hr) and $t_{1/2}$ (1.51-4.00 hr) are in agreement with the results in this study. The overall mean parameters of the

Table 13   Mean pharmacokinetic parameters for the five brands of ketoconazole tablets and overall mean pharmacokinetic parameters of ketoconazole. (Mean±S.D.).

	DRUG PRODUCT[a]				
PARAMETER	A	B	C	D	E
C_{max} (mcg/ml)	3.64±1.47	4.00±2.22	3.03±1.00	3.45±1.21	3.75±1.02
	Overall mean of C_{max} = 3.58±0.46 mcg/ml				
t_{max} (hr)	1.38±0.34	1.43±0.43	1.64±0.47	1.73±0.38	1.40±0.45
	Overall mean of t_{max} = 1.52±0.16 hr				
AUC_0 (mcg hr/ml)	18.75±6.72	19.49±9.91	18.65±7.55	20.37±7.31	19.07±6.48
	Overall mean of AUC_0 = 19.26±0.70 mcg hr//ml				
K_{a} (hr^{-1})	1.64±0.65	1.58±0.82	1.54±1.21	1.12±0.35	1.61±0.74
	Overall mean of K_a = 1.50±0.21 hr^{-1}				
K_{el} (hr^{-1})	0.29±0.06	0.31±0.04	0.26±0.06	0.28±0.06	0.31±0.06
	Overall mean of K_{el} = 0.29±0.02 hr^{-1}				
$t_{1/2}$ (hr)	2.49±0.51	2.30±0.41	2.80±0.68	2.60±0.65	2.28±0.51
	Overall mean of $t_{1/2}$ = 2.48±0.22 hr				

a   Appendix D shows concentration-time profiles and all parameters for 12 individual subjects and for each brand.

five brands tested in the present study were (table 13 and appendix D) 3.58 mcg/ml (0.81-6.92 mcg/ml) for C_{max}, 1.52 hr (0.56-2.20 hr) for t_{max}, 2.48 hr (1.71-4.15 hr) for $t_{1/2}$. The AUC_0^∞ reported by Daneshmend et. al. (1982) was 12.9 mcg hr/ml and was lower than 19.26 mcg hr/ml (4.6-30.6 mcg hr/ml) noted in this study. This is mainly because of the differences between the two studies in methods for AUC estimation, analytical method, the inherent high intersubject variation and the subject selection (Daneshmend et al. (1982) employed the trapezoidal rule for AUC estimation, used the microbiologic assay as the analytical methods and chose five male and three female as his subjects).

3.   Bioequivalence Study

Tables 14-19 indicate no statistical differences (P>0.05) in all pharmacokinetic parameters among the five formulations whose means were shown in table 13. The mean parameters included C_{max}, t_{max} and AUC_0^∞ which were used for the bioavailability assessment. Thus, based on the results of the ANOVA of C_{max}, t_{max} and AUC_0^∞, all four test products did not show any difference of statistical significance from the reference (A). Thus they were considered to be bioequivalent. The relative bioavailabilities which were determined by dividing the mean AUC_0^∞ of non-reference to the mean AUC of the reference were 104.00, 99.47, 108.69 and 101.76 for brands B, C, D and E. No statistical difference of any of pharmacokinetics parameters of coated

tablets (B and D) and those of uncoated tablets (A, C and E) were noted, especially in coated tablets (B) with fast dissolution rate whose C_{max}, t_{max}, AUC and K_a were close to those of reference. Between the two coated tablets (B and D), brand D with slower dissolution rate in both media appeared to have lower C_{max}, K_a and lower t_{max}, The differences statistically significant, though.

In Vitro-In Vivo Correlation

Various *in vivo* versus *in vitro* dissolution and disintegration parameters were subjected to correlative analysis and the correlation coefficients (r) were calculated. Those parameters correlated were presented in table 20 which indicated that the exist correlation among tested parameters were poor and were not sufficient to provide for a reliable prediction of ketoconazole bioavailability either from dissolution or disintegration characteristics.

Brand D was excluded from the correlative analysis between the disintegration time and the *in vivo* parameters because its disintegration time was much different from those of the others (Table 4). Without exclusion brand D from the analysis, it looked as if the analysis contained only 2 points of data which one was disintegration time of brand D and others were a group of three points of data which had disintegration times less than brand D. This results in high correlation coefficient (r) and leads to

Table 14. Statistical differences of C_{max} for the five ketoconazole formulations assessed by one-way analysis of variance and t-test ($\alpha=0.05$).

SOURCE OF VARIATION	DEGREE OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F RATIO
BETWEEN PRODUCTS	4	6.47	1.62	0.76
WITHIN PRODUCTS	55	116.66	2.12	
TOTAL	59	123.12		

$$F_{0.95}\ (4,55)\ =\ 2.55$$

PRODUCT	C_{max} (mcg/ml)	t-CALCULATED[a]	SIGNIFICANT[b] LEVEL
A	3.64±1.47	–	–
B	4.00±2.22	0.61	NS
C	3.02±1.00	-1.04	NS
D	3.45±1.21	-0.30	NS
E	3.75±1.02	0.19	NS

a   A as a reference for comparisons.

b $t_{(.95,55)}\ =\ 2.00$

 NS   NON-SIGNIFICANT ($P>0.05$)

 S   SIGNIFICANT   ($P<0.05$)

Table 15.   Statistical differences of t_{max} for five ketoconazole formulations assessed by one-way analysis of variance and t-test (α=0.05).

SOURCE OF VARIATION	DEGREE OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F RATIO
BETWEEN PRODUCTS	4	1.23	0.37	1.76
WITHIN PRODUCTS	55	9.62	0.18	
TOTAL	59	10.85		

$$F_{0.95} (4,55) = 2.55$$

PRODUCT	t_{max} (hr)	t-CALCULATED[a]	SIGNIFICANT[b] LEVEL
A	1.39±0.34	-	-
B	1.43±0.43	0.23	NS
C	1.64±0.47	1.44	NS
D	1.73±0.38	2.11	NS
E	1.40±0.45	0.06	NS

a   A as a reference for comparisons.

b $t_{(.95,55)} = 2.00$

NS   NON-SIGNIFICANT (P>0.05)

S   SIGNIFICANT   (P<0.05)

Table 16.   Statistical differences of AUC_0^∞ for five ketoconazole formulations assessed by one-way analysis of variance and t-test (α=0.05).

SOURCE OF VARIATION	DEGREE OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F RATIO
BETWEEN PRODUCTS	4	23.49	5.87	0.10
WITHIN PRODUCTS	55	3252.95	59.14	
TOTAL	59	3276.44		

$$F_{0.95} (4,55) = 2.55$$

PRODUCT	AUC_0^∞ (mcg hr/ml)	t-CALCULATED[a]	SIGNIFICANT[b] LEVEL
A	18.74±6.72	-	-
B	19.49±9.91	0.24	NS
C	18.64±7.55	-0.03	NS
D	20.37±7.31	0.52	NS
E	19.07±6.48	0.11	NS

a   A as a reference for comparisons.

b $t_{(.95,55)} = 2.00$

  NS   NON-SIGNIFICANT (P>0.05)

  S   SIGNIFICANT   (P<0.05)

Table 17. Statistical differences of K_a for five ketoconazole formulations assessed by one-way analysis of variance and t-test ($\alpha=0.05$).

SOURCE OF VARIATION	DEGREE OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F RATIO
BETWEEN PRODUCTS	4	2.20	0.55	0.85
WITHIN PRODUCTS	55	35.49	0.65	
TOTAL	59	37.69		

$$F_{0.95} (4,55) = 2.55$$

PRODUCT	K_{a} (hr^{-1})	t-CALCULATED[a]	SIGNIFICANT[b] LEVEL
A	1.64±0.65	–	NS
B	1.58±0.82	-0.18	NS
C	1.54±1.21	-0.30	NS
D	1.12±0.35	-1.58	NS
E	1.61±0.74	-0.09	NS

a   A as a reference for comparisons.

b $t_{(.95,55)} = 2.00$

   NS   NON-SIGNIFICANT ($P>0.05$)

   S   SIGNIFICANT   ($P<0.05$)

Table 18. Statistical differences of K_{el} for five ketoconazole formulations assessed by one-way analysis of variance and t-test (α=0.05).

SOURCE OF VARIATION	DEGREE OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F RATIO
BETWEEN PRODUCTS	4	0.02	0.006	1.76
WITHIN PRODUCTS	55	0.18	0.003	
TOTAL	59	0.20		

$$F_{0.95} (4,55) = 2.55$$

PRODUCT	K_{el} (hr^{-1})	t-CALCULATED[a]	SIGNIFICANT[b] LEVEL
A	0.29±0.06	–	–
B	0.31±0.04	0.86	NS
C	0.26±0.06	-1.29	NS
D	0.28±0.06	-0.42	NS
E	0.36±0.06	1.29	NS

a   A as a reference for comparisons.

b $t_{(.95,55)}$ = 2.00

  NS   NON-SIGNIFICANT (P>0.05)

  S   SIGNIFICANT (P<0.05)

Table 19.   Statistical differences of plasma half-life for five
            ketoconazoles formulation assessed by one-way analysis
            of variance and t-test (α=0.05).

SOURCE OF VARIATION	DEGREE OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F RATIO
BETWEEN PRODUCTS	4	2.10	0.52	1.66
WITHIN PRODUCTS	55	17.34	0.32	
TOTAL	59	19.44		

$$F_{0.95} (4,55) = 2.55$$

PRODUCT	$t_{1/2}$ (hr)	t-CALCULATED[a]	SIGNIFICANT[b] LEVEL
A	2.49±0.51	–	–
B	2.30±0.41	-0.83	NS
C	2.80±0.68	1.35	NS
D	2.51±0.65	0.09	NS
E	2.28±0.51	-0.92	NS

a   A as a reference for comparisons.

b $t_{(.95,55)} = 2.00$

    NS   NON-SIGNIFICANT (P>0.05)

    S   SIGNIFICANT   (P<0.05)

erroneous conclusion because the correlation coefficient for 2 points of data will clearly approach 1 or very high value.

Although the correlation coefficient as high as 0.90 was obtained for t_{max} and the disintegration time, the slope of linear regression line equaled to 0 as assessed by t-test which indicated that independent variable did not change significantly when dependent variable changed. Thus, it can be, in this case, concluded that the exist correlation among the t_{max} and the disintegration time was poor and was not statistically significant.

If the *in vivo* dissolution rate was the rate-limiting step in the absorption of ketoconazole, then this poor relationship would simply indicate that the conditions of the *in vitro* dissolution studies would not be representative of the *in vivo* dissolution condition. Otherwise the poor correlations imply that the dissolution rate is not the rate-limiting step in drug absorption. It is possible that, in stomach, the ketoconazole tablets disintegrated, liberate well. The dissolution takes place subsequently in stomach which has an acidic environment in which the basic drug such as ketoconazole should be rapidly dissolved (Carlson, 1983). But ketoconazole is not well absorbed from the stomach because most of the drug presents in an ionized form in acidic condition, so

Table 20.   Correlation of pharmacokinetic parameters and various   in
vitro parameters.

IN VITRO PARAMETER	IN VIVO PARAMETER	CORRELATION COEFFICIENT(r)	t-VALUE	SIGNIFICANT LEVEL
K_d (hr^{-1})	Cmax	-0.33	-0.60	NS
(dissolution rate	t_{max}	0.29	0.52	NS
in simulated	AUC	-0.18	-0.31	NS
gastric fluid)	Ka	0.08	0.14	NS
t_{50}(min)	Cmax	0.34	0.63	NS
(time to reach	t_{max}	-0.12	-0.22	NS
50% dissolution	AUC	0.36	0.67	NS
in simulated	Ka	-0.21	-0.37	NS
gastric fluid)				
D_{30}(%)	Cmax	-0.22	-0.38	NS
(percent dissolved	t_{max}	0.54	1.13	NS
in 30 min in	AUC	0.55	1.16	NS
simulated gastric	Ka	-0.72	-1.81	NS
fluid)				
disintegration	Cmax	0.79	1.83	NS
time (min) in	t_{max}	-0.90	-2.91	NS
water	AUC	0.48	0.79	NS
(exclude brand D)	Ka	0.76	1.64	NS

(continued)

IN VITRO PARAMETER	IN VIVO PARAMETER	CORRELATION COEFFICIENT(r)	t-VALUE	SIGNIFICANT LEVEL
K_d (hr^{-1})	C_{max}	0.27	0.49	NS
(Dissolution rate	t_{max}	-0.02	-0.04	NS
in simulated	AUC	0.14	0.24	NS
gastric fluid)	K_a	0.03	0.06	NS
D (%)	C_{max}	0.11	0.20	NS
(Maximum %	t_{max}	0.17	0.30	NS
of drug dissolved	AUC	0.35	0.66	NS
in simulated	K_a	-0.34	-0.63	NS
intestinal fluid)				
D_{30}(%)	C_{max}	0.29	0.08	NS
(percent dissolved	t_{max}	-0.04	-0.07	NS
in 30 min in	AUC	0.16	0.28	NS
simulated	K_a	0.00	0.00	NS
intestinal fluid)				

a $t_{.95,3}$ = 3.18

NS   NON-SIGNIFICANT (P>0.05)

S   SIGNIFICANT   (P<0.05)

significant amount of the drug is not absorbed, even in the brand with maximum dissolution rate in the simulated gastric fluid without pepsin. Until the stomach empties and the drug enters the duodenum where it is very quickly absorbed according to its conversion to an unionized form because of the small intestine condition.

Hence, it is reasonable to conclude that the ketoconazole tablets, even the one with the slowest dissolution or the slowest disintegration in this study, can be provide a significant amount of drug from its formulation before passing into the small intestine, the absorptive site. Consequently, a correlation between the bioavailability as described by C_{max}, t_{max} AUC and K_a and f the *in vitro* dissolution rate and disintegration parameters are not significant nor did the different between bioavailability among the five brands with different dissolution and disintegration characteristics.