CHAPTER III

RESULTS AND DISCUSSION

In Vitro Studies

The results of the in vitro tests are summerized in Table 2. All 13 brands of furosemide tablets met the USP requirement for weight variation; each of them had its own weight within the range of limit weight ($\pm$ 7.5%) (28). Assayed products showed that each brand was within the 90-110% limits. There were 5 brands that their average percent labelled amount had relatively high values of standard deviation and this made some tablets of these brands contained more than the amount of active ingredient designated. (This high standard deviation indicated that some brands had a wide variation of content of active ingredient in their tablets).

Each of 13 brands of furosemide tablets met the requirement for disintegration tests according to USP XX under uncoated-tablets. All tested tablets disintegrated within 10 minutes. Especially brand B and brand K took less than 1 minute to disintegrate in water at 37°C..

The dissolution profiles of all 13 brands of furosemide tablets in phosphate buffer pH 5.8 is illustrated in Figure 2. Many differences were observed for both rate and extent of dissolution of different brands. From the compendial monograph dissolution requirement (29), the amount of drug which is not less than 65% of the

Table 2　Physical Characteristics of 13 Commercial Brands of　Furosemide Tablets In Vitro Studies

Brand	Weight[a] (g)	% Labelled[b] Amount	Disintegration Time[c] (min)	% Dissolved[c] at 30 min	Dissolution Rate Constant[c]
A	0.162±0.004	99.47±1.32	1.8±0.27	59.83±5.11	0.022±0.003
B	0.179±0.003	100.13±0.80	0.8±0.11	74.47±0.93	0.093±0.011
C	0.162±0.005	98.58±0.89	2.4±0.76	22.81±5.65	0.009±0.001
D	0.116±0.003	100.97±2.60	8.1±0.39	7.50±0.31	0.006±0.000
E	0.205±0.006	109.84±6.88	4.9±0.67	62.14±2.22	0.030±0.002
F	0.193±0.008	95.63±2.11	8.3±1.74	64.19±6.96	0.018±0.006
G	0.161±0.003	108.02±2.69	2.9±0.34	72.88±5.34	0.044±0.006
H	0.201±0.005	95.98±3.98	1.2±0.25	60.32±6.77	0.033±0.005
I	0.162±0.002	101.42±8.28	2.6±0.50	76.54±10.29	0.076±0.054
J	0.153±0.006	110.90±2.36	1.9±0.16	27.25±3.10	0.007±0.001
K	0.164±0.003	108.11±7.87	0.9±0.17	43.62±9.50	0.018±0.005
L	0.186±0.008	101.27±9.37	1.7±0.44	92.04±6.67	0.174±0.047
M	0.205±0.004	92.24±1.84	6.2±0.86	40.48±7.11	0.030±0.009

a. values are mean ± standard deviation (n =20)

b. values are mean ± standard deviation (n =2)
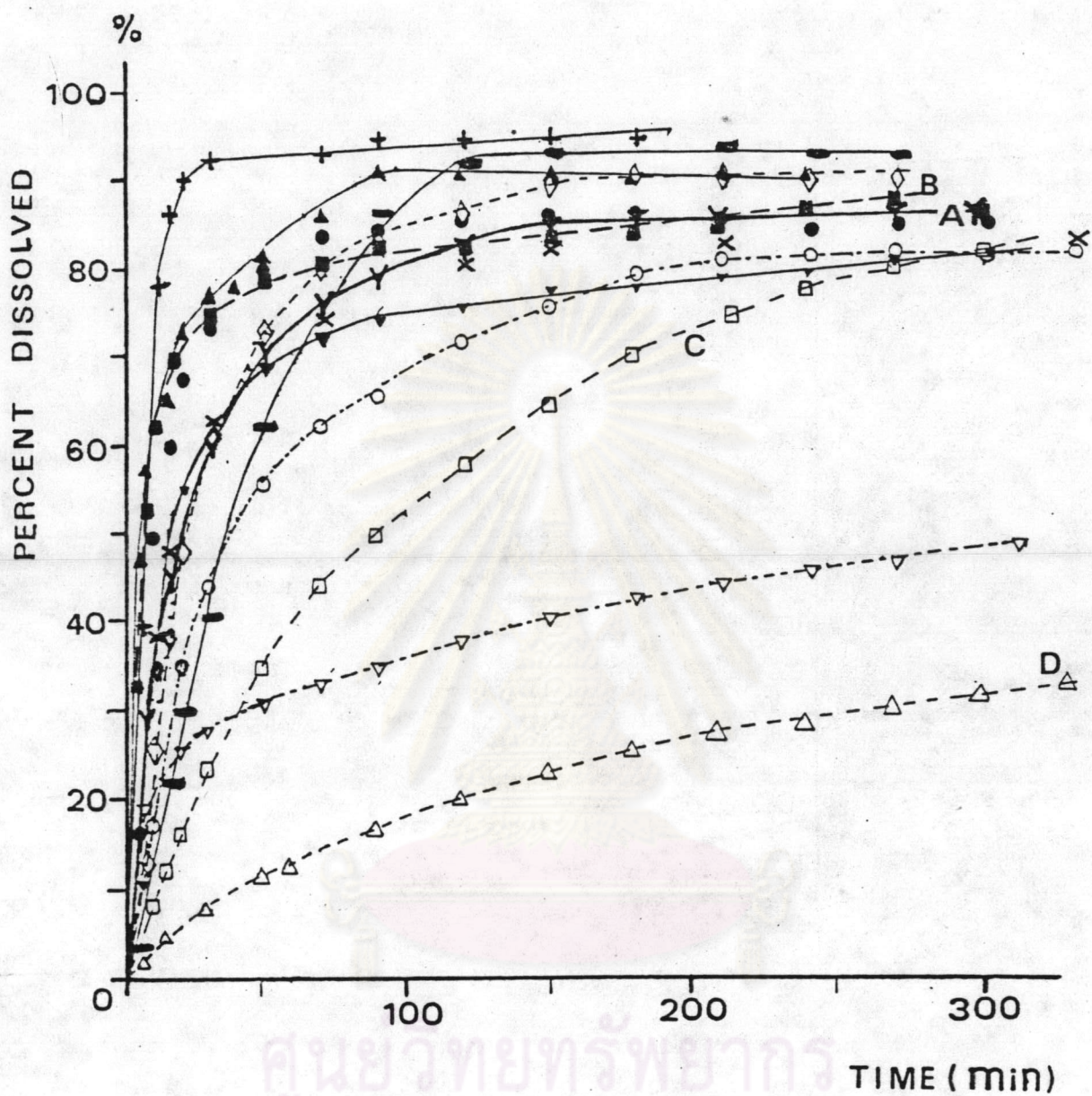
c. values are mean ± standard deviation (n =6)

Figure 2 Dissolution Profiles of 13 Brands of Furosemide Tablets in
Phosphate Buffer pH 5.8

:Brand A (Υ), Brand B (■), Brand C (□), Brand D (△), Brand E (×),
Brand F (▼), Brand G (●), Brand H (◊), Brand I (▲), Brand J (▽),
Brand K (O), Brand L (+), and Brand M (➖).

labelled amount must be dissolved in a medium after 30 minutes. As in Table 2, there were only 4 brands that passed the requirement of USP XXI. The average percent drug dissolved at 30 minutes ranged from 7.5 to 92.04. At 30 minutes, Brand L dissolved more than others. Most products except brand D and brand J, were dissolved more than 65% after 2.5 hr. From both Table 2 and Figure 2 indicate that there were wide ranges of tablets dissolution rates. These variations might be due to differences in manufacturing processes and/or sources of active ingredient and excipients used in tabletting. The interactions among constituents while tabletting might contribute to result in these variations (33). The characteristics of disintegrating of tablets might also influence the rate and extent of dissolution. As observed in dissolution test of brand D, tablet failed to disintegrate in dissolution medium after 3 hr. of sampling time, on the other hand, tablets of brand L disintegrated to coarse granule immediately after exposed to dissolution medium and to fine granule.

In Vivo Studies

1. Bioavailability Study

1.1 Assay for Furosemide in Plasma

A chromatogram from plasma containing both furosemide and internal standard is illustrated in Figure 3. Retention times for furosemide and internal standard were 3.59 and 6.03 min, respectively. Chromatographic response was readily for plasma furosemide concentrations ranged from 0.1 to 1.5 μg/ml (Appendix D, Figure 7b).
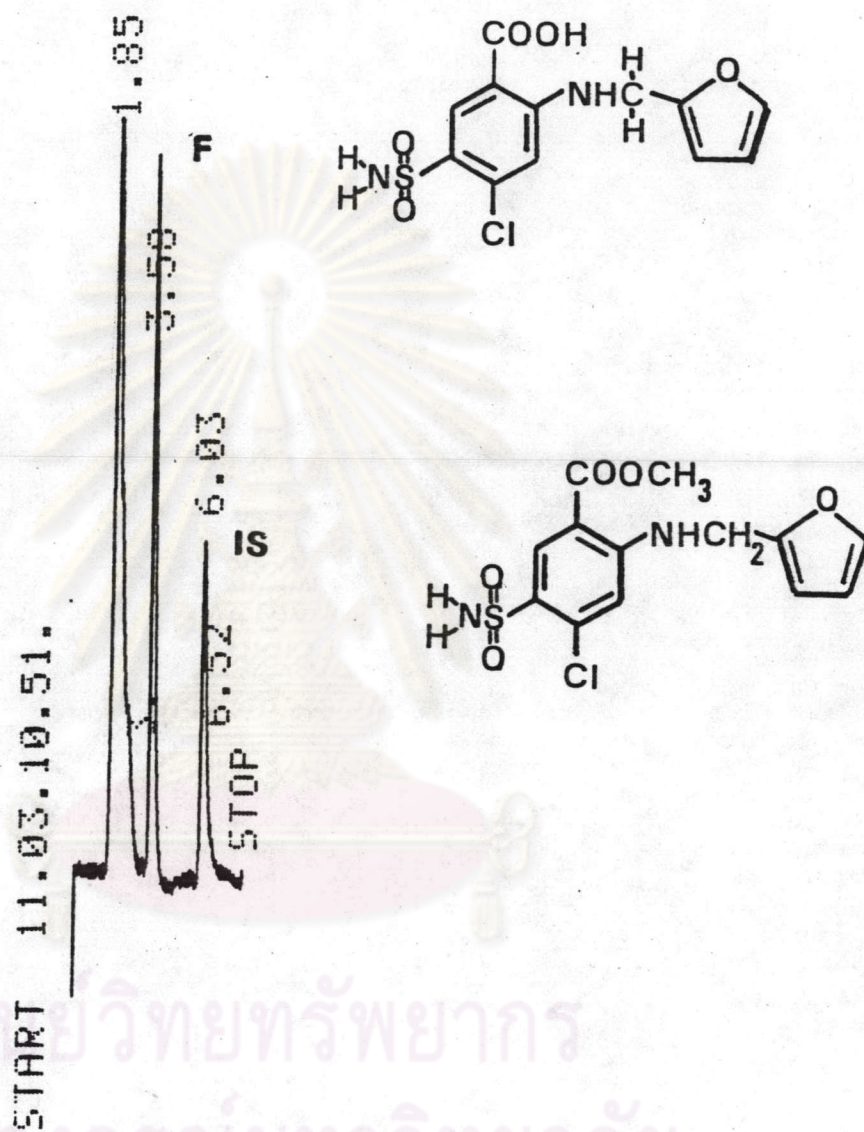
Figure 3 High Performance Liquid Chromatographic Chromatogram of

Furosemide (F) and Internal Standard (IS)[a]

[a]obtained from HPLC analysis of human serum containing

1.5 μg/ml of furosemide and ·2 μg/ml of internal standard

The sensitivity of furosemide detection in human plasma in this study was 0.1 µg/ml.

1.2 Plasma Furosemide Level

The individual furosemide concentrations in plasma for each product at each appropriate sampling time from 0 to 5 hr. are shown in Table 3. The average values are illustrated in Figure 4. Each point in the figure averaged from 8 subjects and the bars represent the standard errors. Comparison among brands are also summarized in Figure 5.

1.3 Bioavailability of Furosemide

Both the rate and the extent of drug absorbed to the general circulation indicates the bioavailability of that drug from its dosage form (34). These factors can be evaluated by determining pharmacokinetic parameters derived from plasma concentrations vs time curves. If the plasma level-time curves of two brands are superimposable, they are bioequivalence. However, bioequivalence can also be assessed by comparing the peak plasma concentrations of the drug, the times to peak concentrations and the extent of absorption which reflected by the areas under the plasma concentration-time curves.

Relative bioavailability is a relative amount of drug which compared to that of a standard or original brand. In this study, three local manufactured brands of furosemide tablets which had maximum (brand B), moderate (brand C) and minimum (brand D)

Table 3 Plasma Furosemide Concentrations(ug/ml)(Mean ± SEM) from 8 Subjects Following Oral Administration of 40 mg of 4 Different Brands

| Brand	Time(hr)	1	2	3	4	5	6	7	8	Mean	SEM
					Subject no.						
A	0.25	0.0000	0.0000	0.0000	0.0000	0.0000	‡	0.0000	0.1616	0.0231	0.0231
	0.50	0.0000	0.2088	1.1080	0.0000	0.2460	0.2149	0.0000	0.2842	0.2577	0.1287
	0.75	0.0000	0.2555	1.7318	0.0000	‡	0.2748	0.2335	‡	0.4519	0.2682
	1.00	0.0000	0.2684	1.5460	0.0000	0.7704	0.3328	0.4086	0.5590	0.4857	0.1772
	1.50	0.0639	0.5254	1.1556	0.1305	0.5210	0.4958	0.5591	1.4449	0.6120	0.1666
	2.00	0.4731	0.3185	1.0626	0.1334	0.3480	0.4104	0.2871	0.7849	0.4772	0.1067
	2.50	0.5161	0.2330	0.4808	0.1738	0.2871	0.3850	0.1362	0.4914	0.3379	0.0533
	3.00	0.3876	0.2032	0.4087	0.2223	0.3022	0.3998	0.0000	0.3631	0.2859	0.0495
	4.00	0.2025	0.1240	0.2554	0.8970	0.2529	0.3867	0.0000	0.1329[a]	0.2814	0.0977
	5.00	0.0957	0.1240	0.1409	0.5231	0.2556	0.2957	0.0000	0.1912	0.2033	0.0514
B	0.25	0.0000	0.1579	‡	‡	0.0000	0.3123	‡	‡	0.1176	0.0748
	0.50	0.0000	0.6987	0.0645	0.5248	0.0000	0.7439	0.0157	0.1295	0.2721	0.1154
	0.75	0.0000	‡	‡	0.8287	0.0000	‡	‡	0.1942	0.2557	0.1964
	1.00	0.1486	0.5909	0.4198	1.9419	0.0929	1.2262	0.0798	0.2044	0.5881	0.2358
	1.50	0.5202	0.4896	0.4113	1.2623	0.1477	0.9833	0.3023	0.2219	0.5423	0.1369
	2.00	0.6023	0.3549	0.3753	0.6592	0.3010	0.6693	0.3789	0.2942	0.4544	0.0569
	2.50	0.5205	0.3510	0.3139	0.4026	0.3529	0.4503	0.2494	0.3377	0.3723	0.0297
	3.00	0.2868	0.2667	0.2434	0.2648	0.2270	0.3398	0.2414	0.1806	0.2563	0.0164
	4.00	0.2384	0.2544	0.1455	0.2084	0.2043	0.2529	0.0919	0.1665	0.1953	0.0202
	5.00	0.1670	0.1606	0.1564	0.0000	0.1900	0.1901	0.1316	0.1689	0.1456	0.0218
C	0.25	‡	0.0000	0.2886	‡	0.0000	‡	‡	‡	0.0962	0.0962
	0.50	0.0852	0.0000	1.5374	3.0386	0.0000	0.1739	0.0089	0.0254	0.6087	0.3935
	0.75	0.1689	0.0000	‡	‡	0.1575	0.3269	‡	‡	0.1633	0.0668
	1.00	0.1883	0.0000	1.2392	2.3380	1.1263	0.4260	0.8041	0.0302	0.7690	0.2804
	1.50	0.5121	0.1391	0.8015	1.5686	0.8011	1.8690	1.2118	0.3224	0.9032	0.2140
	2.00	0.3884	0.2543	0.5307	0.9256	0.5712	1.3176	0.6750	0.9233	0.6983	0.1213
	2.50	0.2648	1.0721	0.3105	0.6608	0.3588	0.8730	0.6021	0.5676	0.5887	0.0996
	3.00	0.2258	0.5631	0.2263	0.5352	0.2009	0.7302	0.3076	0.4418	0.4039	0.0687
	4.00	0.1442	0.2791	0.1386	0.2178	0.1900	0.2493	0.1441	0.1617	0.1906	0.0188
	5.00	0.2099	0.1635	0.1108	0.1161	0.1618	0.2400	0.0790	0.0891	0.1463	0.0204
D	0.25	0.0000	‡	‡	0.0000	0.0000	‡	0.0000	0.0000	0.0000	0.0000
	0.50	0.0000	0.7601	1.1585	0.0000	0.0000	0.0226	0.0000	0.0000	0.2427	0.1609
	0.75	0.0000	‡	1.4224	0.0000	0.0000	‡	0.5437	0.0000	0.3277	0.2363
	1.00	0.0000	1.1284	1.4221	0.0000	0.0000	0.5576	1.0497	0.0000	0.5197	0.2133
	1.50	0.3933	0.9178	1.0592	0.0000	0.4257	0.5828	0.8230	0.6537	0.6069	0.1193
	2.00	0.7809	0.5889	0.6544	0.1362	1.2728	0.5368	0.4231	1.3413	0.7168	0.1451
	2.50	0.4367	0.3807	0.4281	1.1349	0.7098	0.4713	0.2614	0.7573	0.5725	0.0993
	3.00	0.2506	0.2365	0.3005	1.0028	0.3614	0.4308	0.2624	0.3855	0.4038	0.0890
	4.00	0.1799	0.1556	0.1942	0.3595	0.3174	0.2313	0.1646	0.1816	0.2230	0.0267
	5.00	0.1625	0.0000	0.1295	0.2043	0.1923	0.1232	0.1942	0.0982	0.1380	0.0239

a. sampling at 4.25 hr
‡ missing data
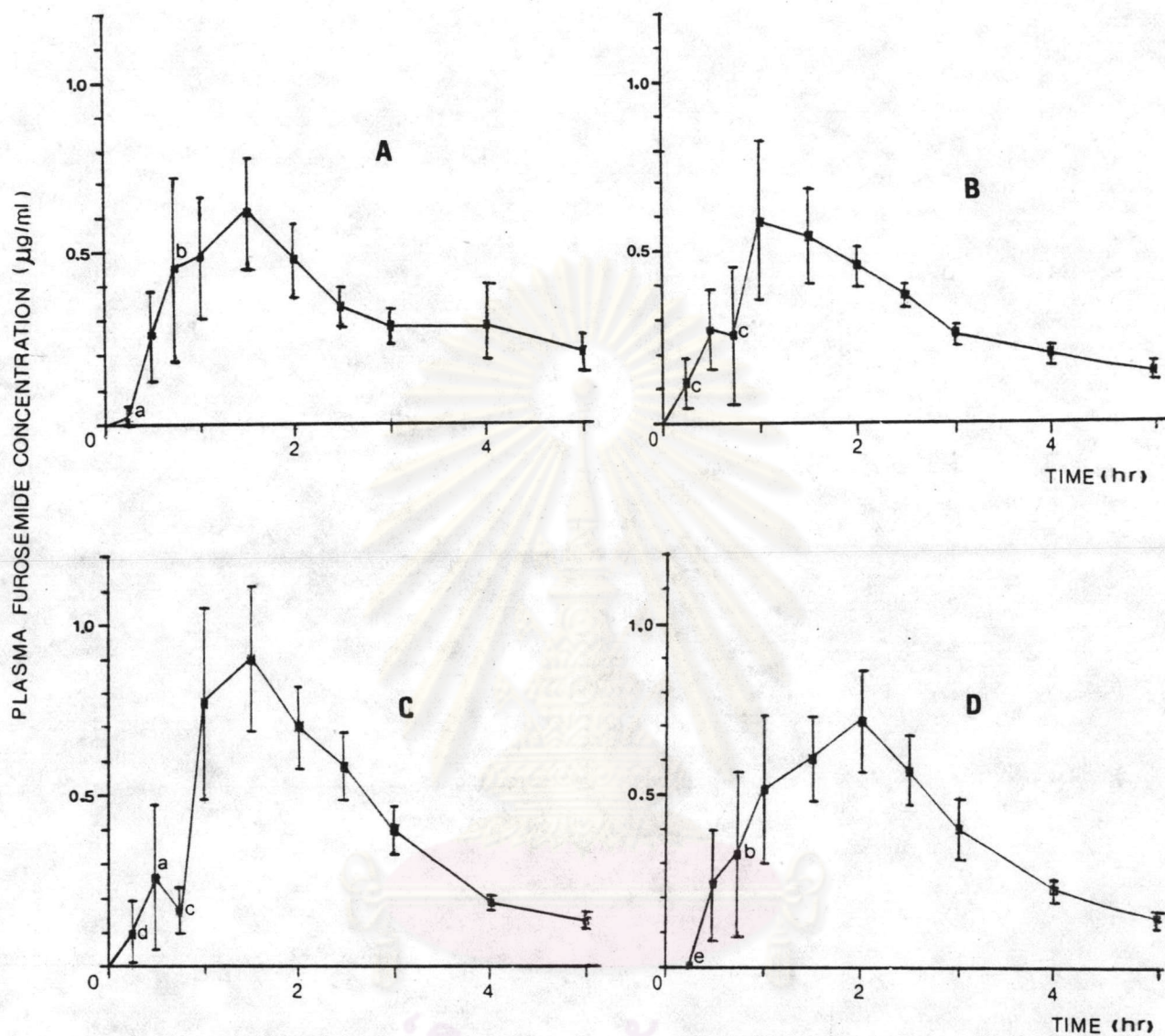
Figure 4   Plasma Furosemide Concentrations (Mean ± SEM) from 8 Subjects

Following Oral Administration of 40 mg of 4 Different Brands

(Brands A, B, C, and D)

a. average from 7 subjects

b. average from 6 subjects

c. average from 4 subjects

d. average from 3 subjects

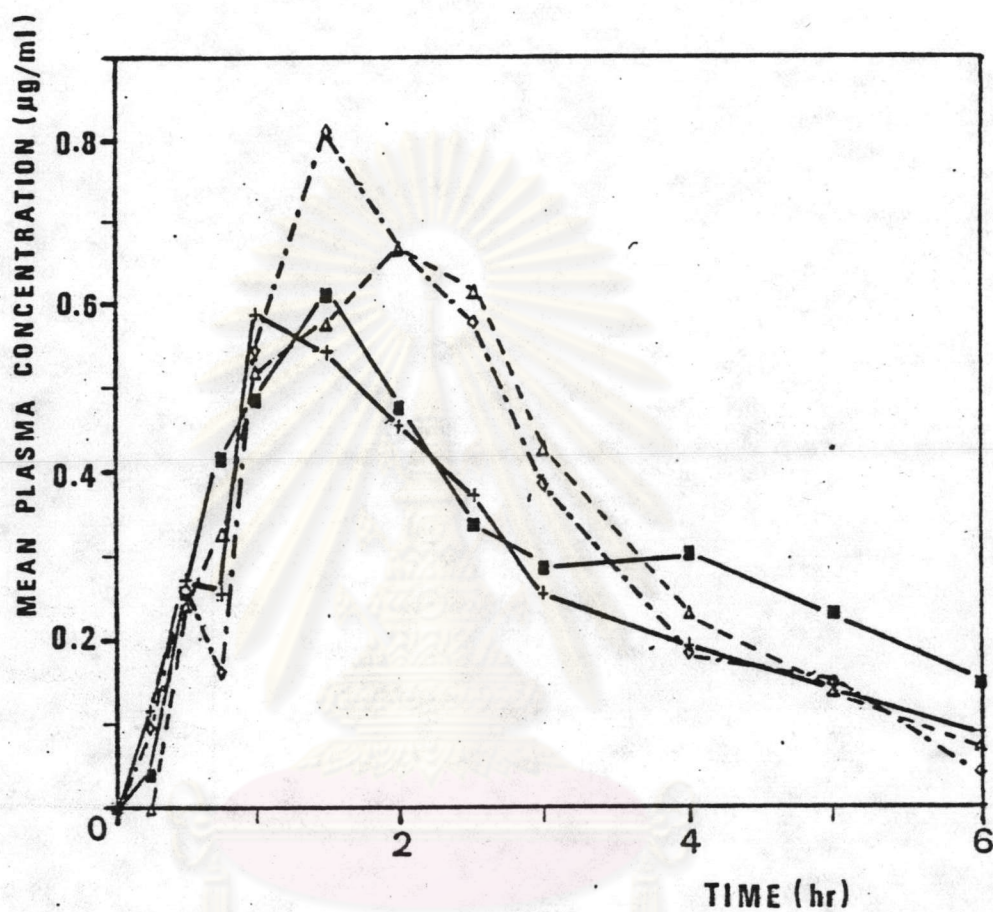**e.** average from 5 subjects

Figure 5 Comparison of Mean Plasma Furosemide Concentration-Time Curve
from 8 Subjects Following Oral Administration of 40 mg of
Brand A (■), Brand B (+), Brand C (◊), and Brand D (△).

dissolution values were compared to the original brand (brand A); parameters used to compare are the absorption rate constant (Ka), time to peak plasma concentration (t max), the peak plasma concentration (Cp_{max}) and the area under the plasma level-time curves $[AUC]_0^\infty$.

Table 4 shows the statistical comparison of the parameters obtained from plasma-level profiles of 4 different brands of furosemide tablets after oral administration to 8 healthy males. According to both Friedman's test and Wilcoxon Rank Sum Test for nonparametric statistics, there were no differences from one another in the parameters evaluated.

1.3.1 Peak Plasma Concentration

As seen in Table 3, the peak plasma levels for each of the four brands of furosemide tablet, averaging from each subject, were ranged from 0.338 to 3.039 µg/ml after a single oral 40 mg dose. It was obvious in subject 4 who received brand C that the plasma level reached 3.039 µg/ml within 30 minutes after drug administration while other cases blood levels peaked around 1 µg/ml.

The average peak plasma levels from 8 subjects after oral administration of 4 different brands of furosemide were 0.643, 0.609, 1.124 and 0.835 µg/ml respectively. There were no statistically significant difference between these values, based on Friedman's($P > 0.05$) and Wilcoxon's Test ($p > 0.0167$).

Table 4   Pharmacokinetic Parameters (Mean±SEM) for Furosemide from 8

            Subjects Following Oral Administration of 40 mg of 4 Different

            Brands of Furosemide Tablets

Brand	$Ka(hr^{-1})$	$Cp_{max}(\mu g/ml)$	$t_{max}(hr)$	$[AUC]_o^{\infty}(hr.\mu g.ml^{-1})$
A	1.71 ± 0.84	0.64 ± 0.16	2.00 ± 0.54	2.76 ± 0.62
B	1.29 ± 0.31	0.61 ± 0.14	1.64 ± 0.24	1.94 ± 0.18
C	2.02 ± 0.87	1.12 ± 0.31	1.63 ± 0.30	3.13 ± 0.49
D	1.69 ± 0.51	0.83 ± 0.12	1.85 ± 0.33	2.62 ± 0.29
Friedman's test	$X^2_{0.975} = 0.56$	$X^2_{0.975} = 4.24$	$X^2_{0.975} = 0.15$	$X^2_{0.975} = 4.95$
Wilcoxon test	NS	NS	NS	NS

Cp_{max} = mean individual peak plasma levels

t_{max} = mean individual time to peak

$[AUC]_o^{\infty}$ = mean area under the plasma concentration-time curve

SEM   = standard error of the mean

NS   = not significant difference at $p > 0.0167$

1.3.2 Time to Peak Plasma Level

In most cases, the time required to reach the peak plasma level was within 1.5 hr. but in one case, it took up to 4 hr. to reach the peak. This indicated that furosemide was rapidly absorbed after oral administration.

The average peak times were 2.00, 1.64, 1.63, and 1.85 hr. for brand A, B, C and D respectively. However, the average peak times of these 4 brands were not statistically different (p > 0.05).

1.3.3 Area Under Plasma Level-Time Curve

The average area under the plasma level-time curves estimated by PCNONLIN program are summerized in Table 4. The relative bioavailability calculated by comparing the average area under the plasma level-time curves of each brand to that of the brand-A were 70.29%, 113.41%, and 94.93% for brands B, C and D respectively. The differences among $[AUC]_0^\infty$ of each brand showed no statistical significance (p > 0.05).

From Table 4, the average absorption rate constants were also not statistically significant different among these brands. Base on the results in Table 4, the four brands of furosemide tablets showed no signficant differences in each parameter, indicating they were all bioequivalent.

2. Clinical Response Study

2.1 Urine Output

Table 5 and Figure 6 show results of clinical response. Cumulative urine volumes of 6 hr. after drug administration ranged from 1878 to 2205 ml. Brand B produced the highest diuretic effect, but from statistical evaluation urine output from these 4 brands were not significantly different.

2.2 Electrolyte Excretion

Results in Table 5 also point out that electrolyte excretion, e.g., Na^+, Cl^-, and K^+ excretion, from each brand was not remarkably different.

From both bioavailability study and clinical response study of 4 different brands of furosemide tablets indicated that there were no critical differences among brands in both the rate and extent of drug absorption and clinical response.

Table 5   Clinical Response (Mean±SEM) from 8 Subjects after Oral
Administration of 40 mg of 4 Different Brands of Furosemide
Tablets

Brand	urine output (ml)	Na^+ excretion (meq)	Cl^- excretion (meq)	K^+ excretion (meq)
A	$1878.38^a \pm 203.49$	$146.87^b \pm 8.58$	$189.61^b \pm 12.78$	$20.66^b \pm 2.19$
B	2205.00 ± 254.41	138.49 ± 17.53	174.77 ± 23.96	24.96 ± 4.20
C	2082.00 ± 230.84	159.08 ± 14.33	196.21 ± 17.82	24.86 ± 6.35
D	1905.88 ± 174.96	149.42 ± 8.31	191.88 ± 10.80	21.67 ± 3.02
Friedman's test	$X^2_{0.975} = 1.80$	$X^2_{0.975} = 5.55$	$X^2_{0.975} = 3.75$	$X^2_{0.975} = 1.05$
Wilcoxon test	NS	NS	NS	NS

a. total urine output after 6 hr.

b. total amount excreted in urine after 6 hr.

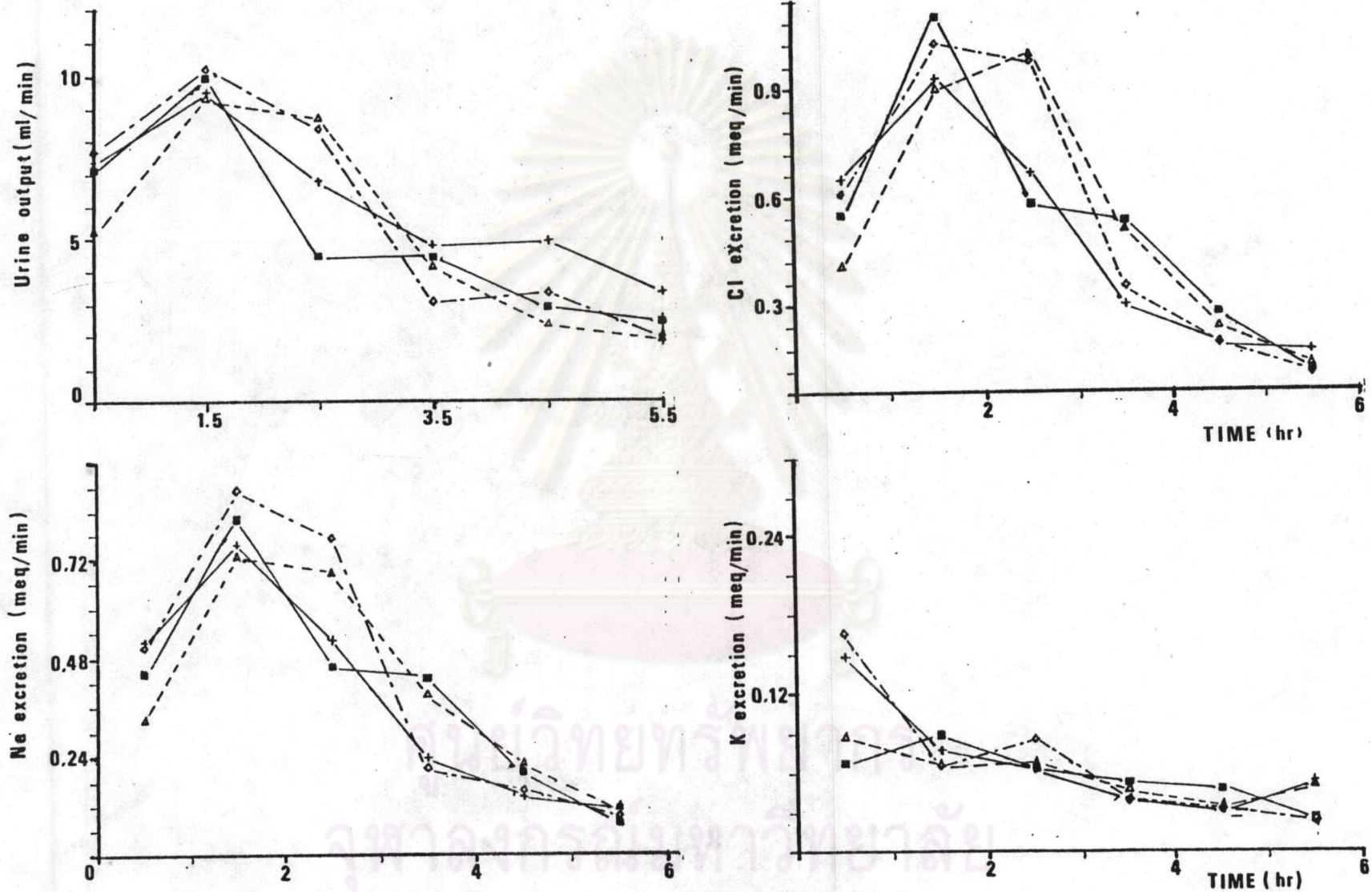NS = not significant ,p > 0.0167

Figure 6   Comparison of Clinical Response from 8 Subjects after Oral Administration of 4 Different Brands of
Furosemide Tablets   :Brand A (■), Brand B (+), Brand C (◊), and Brand D (△).

Pharamacokinetic of Furosemide Tablets

Base on the semilogarithmic plot of individual plasma concentration-time data for 8 subjects, data appeared to follow one-compartment model with or without a lag time. The 'Residual Method' (Appendix G) was employed to obtain initial estimates of the parameters needed in fitting program. An iterative nonlinear least squares fitting program, PCNONLIN, was used to fit each set of data and to obtain final estimates of the parameters. The goodness of fit was tested by comparing values of individual sum of squares of the deviations between observed data and calculated values.

The pharmacokinetic parameters estimated from each plasma data of 8 subjects after oral administration of 40 mg of 4 different brands of furosemide tablets are summarized in Table 6. Friedman's and Wilcoxon's Test showed no differences in these parameters among brands. (p > 0.0167).

The mean values of peak plasma concentration ranged from 0.609 to 1.124 μg/ml; the mean time to peak plasma level ranged from 1.625 to 1.997 hr., and the mean $[AUC]_0^\infty$ ranged from 1.94 to 3.13 hr.μg/ml.

The lag time ranged from 0.315 to 0.52 hr. and averaged 0.39 $\pm$ 0.26 hr. for all brands. The absorption rate constants were 1.71, 1.289, 2.018 and 1.688 hr.$^{-1}$ for brand A, B, C, and D respectively. The overall elimination rate constant and the plasma half-life were 0.699 hr.$^{-1}$ and 1.265 hr. The average plasma clearance was 301.72 ml/min.

Table 6   Estimated Pharmacokinetic Parameters (Mean ± SEM) for Furosemide
from 8 Subjects Following Oral Administration of 40 mg of 4
Different Brands

Paramater	A	B	C	D	Mean	SEM
t_0	0.33 ± 0.10	0.32 ± 0.08	0.40 ± 0.12	0.52 ± 0.07	0.39	0.09
K_a	1.71 ± 0.84	1.29 ± 0.31	2.02 ± 0.87	1.69 ± 0.51	1.68	0.11
T_{max}	2.00 ± 0.54	1.64 ± 0.24	1.63 ± 0.30	1.85 ± 0.33	1.78	0.36
Cp_{max}	0.64 ± 0.16	0.61 ± 0.14	1.12 ± 0.31	0.84 ± 0.12	0.80	0.20
AUC	2.76 ± 0.62	1.94 ± 0.18	3.13 ± 0.49	2.62 ± 0.29	2.61	0.43
V_d	0.58 ± 0.10	0.62 ± 0.11	0.32 ± 0.04	0.38 ± 0.04	0.48	0.04
K_{el}	0.63 ± 0.14	0.67 ± 0.11	0.76 ± 0.07	0.74 ± 0.12	0.70	0.02
$T_{1/2}$	1.76 ± 0.53	1.20 ± 0.14	1.00 ± 0.13	1.10 ± 0.16	1.27	0.30
CL	20.41 ± 12.89	21.80± 5.36	14.38 ± 6.04	15.82± 6.65	18.10	8.49

t_0 = lag time (hr)

K_a = absorption rate constant (hr^{-1})

T_{max} = time to peak level (hr)

Cp_{max}= peak plasma concentration (μg/ml)

AUC   = area under the plasma concentration-time curve (hr.μg/ml)

V_d = volume of distribution (lit/kg)

K_{el} = elimination rate constant (hr^{-1})

$T_{1/2}$ = terminal half-life (hr)

CL   = renal clerance (lit/hr)

The pharmacokinetic parameters obtained from this study were slightly different from those reported by other investigators (4, 6, 7). Chennavasin et.al. (35) observed great discrepancies in pharmacokinetics of furosemide reported from a number of investigators; this may be due to different methods of analysis of data. They found that the terminal half-life was most subject to errors of method and the plasma clearance the least. Other factors possibly effecting the differences were the subjects participated in the studies, their races, ages, weights and normal habits.

In Vitro-In Vivo Bioavailability Correlations

The relationships among and between various in vitro and in vivo parameters are in Table 7. Disintegration times and dissolution rate constants did not correlate, indicating disintegration times were not rate limiting step of furosemide dissolution. Poor correlations were also attained between in vivo parameters (Ka, tmax, Cp_{max}, and $[AUC]_0^\infty$) and in vitro parameters (disintegration time, dissolution rate, and percent drug dissolved at 30 min. in dissolution medium).

This study revealed that the bioavailability of furosemide from oral tablets was independent from its in vitro properties.

Table 7   In Vitro-In Vivo Bioavailability Correlations

Correlation	Degree of freedom[a]	Correlation Coefficient	t-value	p-value
Disintegration Times vs Dissolution Rate Constants	11	-0.22	-0.75	NS[b]
Disintegration Times vs Ka	2	0.11	0.15	NS
Disintegration Times vs Tmax	2	0.29	0.42	NS
Percent Dissolved[c] vs $[AUC]_o^\infty$	2	-0.63	-1.15	NS
Dissolution Rate Constants vs Ka	2	-0.89	-2.79	NS
Dissolution Rate Constants vs Tmax	2	0.66	1.24	NS

a. degree of freedom = number of pairs-2

b. not significant, $p > 0.05$

c. percent drug dissolved in dissolution medium at 30 min

In Vitro-In Vivo Clinical Response Correlations

The relationships between in vitro characteristics, such as disintegration times and dissolution rate constant, and in vivo clinical response, i.e., cumulative urine output and urinary sodium excretion after 6 hr, to the 4 brands of furosemide tablets were also studied. Results from Table 8 show that in vitro characteristics and in vivo clinical response did not correlate, implying that disintegration times and dissolution rate constant were not rate limiting step of diuretic response of furosemide.

This study also disclosed that clinical response of furosemide from oral tablets was independent from its in vitro characteristics.

Furthermore, Table 7 and Table 8 indicate that bioavailability and clinical response of furosemide did not depend on its in vitro characteristics.

Table 8   In Vitro-In Vivo Clinical Response Correlations

Correlation	Degree of freedom[a]	Correlation Coefficient	t-value	p-value
Disintegration Times vs urine[c]	2	-0.563	-0.963	NS[b]
Dissolution Rate[d] vs urine	2	0.754	1.621	NS
Disintegration Times vs sodium[e]	2	0.268	0.393	NS
Dissolution Rate vs sodium	2	-0.828	-2.086	NS

a. degree of freedom = number of pairs-2

b. not significant, p > 0.05

c. urine output after 6 hr (ml)

d. Dissolution Rate Constants

e. sodium excretion after 6 hr (meq)