CHAPTER III

MATERIALS AND METHODS

I. Materials

Test Products

Nine commercial brands of 250 mg. naproxen tablet were randomly bought from drug stores in Bangkok. The letters A, B, C, D, E, F, G, H, and I were given to represent the brand names of the products. Informations of test products were reported in Appendix A.

Reagents

1. Reference standard naproxen powder, potency 99.9% (Biolab Co., Ltd.) Lot no. NAP 092.

2. Reference standard phenylbutazone powder, potency 100.1% (Dr. Esteve Lab) Lot. no. 67.417

3. Working standard naproxen powder potency 100.54% (Biolab Co., Ltd.) Lot. no. 84/174C

4. Methanol AR grade (E. Merck, Damstadt) Lot. no. K 4184709

5. Sodium Chloride AR grade (Vidhyasom. Co., Ltd.) Lot. no. 000527

6. Concentrated hydrochloric acid (E. Merck, Damstadt) Lot. no. K 00188817

7. Monobasic potassium phosphate (E. Merck, Damstadt) Lot. no. 253273

8. Sodium hydroxide AR grade (E. Merck, Damstadt) Lot. no. C 605098

9. Acetonitrile AR grade (Fisher Sciencific, U.S.A.) Lot. no. 855916

Apparatus

1. Analytical Balance (August Sauter KG D-7470, West Germany)

2. Tablet Hardness Tester (Strong-Cobb Units, Schleuniger)

3. Disintegration Tester (GC-21, Hanson Research Corp., Northridge, Calif., U.S.A.)

4. Dissolution Apparatus (72 RL, Hanson Research Corp., Northridge, Calif., U.S.A.)

5. Spectrophotometer (Spectronic 2000, Bausch & Lomb, N.Y., U.S.A.)

6. Digital pH meter (PBS 730, El-Hama Instruments)

7. Vortex mixer (Vortex-Genie, Scienfific Industries, Inc. Bohemia, N.Y., U.S.A.)

8. Refrigerated Centrifuge (Sigma 302K, Sigma Lab. Centrifuges Gmbtt, Germany)

9. High Pressure Liquid Chromatography (LC-3A, Shimadzu, Japan)

II. Methods

A. In Vitro Studies

Nine commercial brands of 250 mg. naproxen tablets were evaluated using both official and non-official tests as stated in tne pharmacopoeia. The tests were :

1. Uniformity of Weight (24-25)

20 tablets of 250 mg. naproxen tablets from each brand were sampled and accurately weighed tablet by tablet. The average and standard deviation of weight were calculated.

2. Content of Active Ingredient

The amount of naproxen in tablet was determined according to the USP XXI (26) method which was described as follow :

20 tablets of each brand were weighed and finely powdered. A portion of the powder, equivalent to about 250 mg. of naproxen was accurately weighed, and transfered to a 250 ml. volumetric flask. About 180 ml. of methanol was added and shaken for 1 hour, then methanol was adjusted to volume. The solution was mixed and filtered through filter paper. A 5 ml. of filtrate was withdrawn and diluted with methanol to 100 ml. The dilute solution and standard solution, 50 µg/ml of naproxen in methanol, were concomitantly determined for their absorbances at 331 nm., in a spectrophotometer, using methanol as a blank.

The quantity of naproxen (in mg.) in the portion of tablets was calculated from the formula 5C (Au/As), in which C was the concentration, in µg/ml., of standard solution, and Au and As were the absorbances of the test solution and the standard solution, respectively.

3. Hardness

Six tablets of each brand of naproxen tablets were sampled to test for their hardness using Tablet Hardness Tester. The average of hardness and standard deviation were calculated.

4. Disintegration Test

The disintegration test of naproxen tablets were determined according to the USP XXI (27) method. The procedure was described as follow :

A tablet was introduced into each of the tubes of the basket and disk was placed over the tablet. The apparatus was operated using water as a medium and maintained the temperature at 37 ± 2 °C. The time that the apparatus started to move until the tablet passed through the basket was the disintegration time, then the average and standard deviation of disintegration time of each brand were calculated.

5. Dissolution Test

Since the dissolution test method for naproxen tablet was not available in the pharmacopoeia, the USP Dissolution Apparatus Type II was used to establish and compare the dissolution profiles of naproxen tablet. Two types of dissolution media were used (Appendix B).

a. Simulated gastric fluid (pH 1.2 ± 0.1)

b. Simulated intestinal fluid (pH 7.5 ± 0.1)

The procedure for dissolution test of naproxen tablet was assigned as follow (27) :

Nine hundred milliliters of dissolution medium was placed in the vessel and equilibrated to 37 ± 0.5 °C. A tablet was introduced into each of the six vessels, then the apparatus was immediately operated and maintained stirring speed at 50 rpm. Five milliliters of samples were withdrawn at 5, 10, 15, 20, 25, 30, 45, 60, 80, 100, 120, 150, 180, 210 and 240 minutes. The same equilibrated quantity of dissolution medium was added immediately after each sampling to keep the volume of dissolution medium constant throughout the test. The absorbances of samples was measured using spectrophotometer at 331 nm. The amount of the drug dissolved at various time intervals was quantified using the standard curve.

Standard curve

Standard solutions with appropriate concentration of naproxen in each dissolution media were prepared and analyzed using spectrophotometer at 331 nm. Absorbances obtained versus known concentrations were fitted to a straight line using linear regression (Appendix C).

In Vitro Evaluation

Physical characteristics of nine commercial brands of naproxen tablets were examined and evaluated to determine whether which brand passed the general standard of B.P. and/or U.S.P. requirement.

A one-way analysis of variance and t-test (39) were used to assess the differences between the original and local brands for the tablet hardness, the disintegration time, and the dissolution data. For the correlation between the tablethardness or the disintegration time, and the dissolution rate constant in both simulated gastric fluid and simulated intestinal fluid, the correlation coefficient test was performed.

B. In Vivo Studies

Test products

Five commercial brands of naproxen tablets were selected for in vivo studies. The criteria were :

1. The original brand of naproxen tablet which was assigned as the reference standard against the local brands.

2. Naproxen tablet manufactured by The Government Pharmaceutical Organization.

3. The local brand with maximum dissolution rate constant in simulated gastric fluid.

4. The local brand with maximum dissolution rate constant in simulated intestinal fluid.

5. The local brand with minimum dissolution rate constant in both simulated gastric fluid and simulated intestinal fluid.

Subjects

Eight healthy male volunteers with 19 to 23 years of age participated in this study. All subjects received physical and clinical laboratory examinations and written consent forms were recorded prior to the study to assure the absence of hepatic and renal disturbance, the gastrointestinal tract disorder, asthma and had no hypersensitive to aspirin and/or aspirin-related groups. The method and the condition were clearly explained to all subjects. They were allowed to take no medication for at least one week preceding the study and throughout the entire experimental period.

Drug Administration

A single oral dose of 250 mg naproxen tablet was given to each overnight fasted volunteer. The volunteer continued to fast until 2 hours after administration and then a normal diet was permitted.

Experimental Design

The study was conducted in a randomized crossover design. Each subject received the drug in a randomized order with a two-week washout period between each administration as shown in Table 1.

Table 1 Treatment Schedule

Subject No.	week 1	3	5	7	9
1	C	D	E	A	B
2	B	C	D	E	A
3	E	A	B	C	D
4	A	B	C	D	E
5	D	E	A	B	C
6	B	C	D	E	A
7	A	B	C	D	E
8	C	D	E	A	B

Sample Collection

4-5 ml. of blood samples were drawn from the antecubital vein prior to dosing and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0 and 24.0 hours after drug administration. The samples were kept in heparinized tubes and centrifuged at 3000 rpm for 5 minutes. The plasma was separated and then kept at -10 °C until subsequent analysis.

Determination of Naproxen in Plasma

Concentrations of naproxen in plasma samples were detected using high performance liquid chromatographic method modifying from Høylandskjaer and Aarbakke (29). The procedure was developed as follows :

0.5 ml. of plasma sample

- added 1 ml. of acetonitrile and 1 ml. of internal standard (50 µg/ml. in acetonitrile)
- mixed 10 seconds then centrifuged at 3,000 rpm. for 15 minutes

injected 10 µl of supernatant into the HPLC column.

Operating Condition

Apparatus	HPLC (LC-3A), Shimadzu, Japan
Analytical column	µ-Bondapak C_{18} I.D. 3.9 mm. × 30.0 cm. particle size 10 µ
Mobile phase	acetonitrile : 0.05 M. potassium dihydrogen phosphate-buffer pH 3.70 (47:53)
Internal standard	phenylbutazone
UV detector	272 nm.
Flow rate	1.5 ml/min
Attenuation	2^1 mV/full scale
Pressure	90 $Kg/cm.^2$
Temperature	ambient

The naproxen concentration in plasma samples were quantified employing a standard curve (Appendix C).

Standard Curve

Known amounts of standard naproxen and 50 µg of internal standard were added to 0.5 ml. of plasma to make the concentration of 5, 10, 20, 30, 40, 50, 60, 70, 80 µg/ml. These samples were analysed following the same procedure as previously described.

Pharmacokinetic Analysis

Individual plasma naproxen profile from each treatment was analyzed according to noncompartmental method (Appendix E). The following parameters were calculated.

a. The area under the plasma concentration-time curve, AUC_0^∞, and the area under the first moment curve, $AUMC_0^\infty$

b. The elimination rate constant, Kel.

c. The mean residence time after oral and intravenous administration, MRT_{oral} and MRT_{iv}, respectively.

d. The mean absorption time, MAT

e. The first order absorption rate constant, Ka

f. The plasma half-life, $t_{\frac{1}{2}}$

Bioavailability and Statistical Analysis

The comparative bioavailability of the four commercial brands of naproxen tablets with the original product were evaluated using the following parameters :

a. The area under the plasma concentration time curve, AUC_o^{∞}

b. The peak plasma concentration, Cp_{max}.

c. The time to peak plasma level, T_{max}.

d. The first order absorption rate constant, Ka.

A one-way analysis of variance (ANOVA) and Student's t-test were performed to test the difference among the five brands of naproxen tablets.

In Vitro - In Vivo Correlation Study

The relationship between in vitro and in vivo parameters was analyzed using correlation coefficient test and Student's t-test were then performed to test whether this correlation was statistically significant. The in vitro parameters used to study were disintegration time, and dissolution rate both in simulated gastric fluid and in simulated intestinal fluid whereas the in vivo parameters were the absorption rate constant (Ka), the amount of naproxen absorbed (AUC_o^{∞}) and the parameters which related to the bioavailability of the drug such as the peak plasma concentration (Cp_{max}), and the time to peak plasma level (T_{max}).