CHAPTER III

MATERIALS AND METHODS

Materials

A. Test Products

1. Tablets : Five commercially available ranitidine tablets, 150 mg film coated tablets, were evaluated. One was the innovator's product which was assigned as the reference standard against the other four local manufactured brands.

2. Injection : The innovator's product of ranitidine injection, 50 mg/2 ml, was used in this study.

The letters A, B, C, D, E and I were given to represent the brand names of each product. Other information of these products were accessible in Appendix A.

B. Reagents

1. Standard ranitidine hydrochloride powder 99.5% (Uquifa) Lot 121/86

2. Internal Standard : procainamide hydrochloride powder (E.R. Squibb and Sons U.S.A.) Lot. 78438

3. Acetonitrile HPLC grade (Merck) Lot 42063964

4. Heparin 5,000 i.u./ml (Leo Pharmaceutical Products, Ballerup-Denmark) Lot A 87 A

5. Diethyl ether, GR grade (Merck) Lot 448 K 5060421

6. Chloroform, GR grade (Merck) Lot 801 K 03034045

7. Isopropanol, GR grade (Merck) Lot V 6 F B 1203666

8. Sodium hydroxide AR. (Merck) Lot 64 Z C 605098

9. Sodium metabisulfite GPR. (BDH Chemicals Ltd.) Lot 45332406

10. 85% ortho-phosphoric acid GR grade (Merck) Lot K 2771873

11. Concentrated Hydrochloric acid 37%, GR grade (Merck) Lot 746 K 02257817

12. Monobasic potassium phosphate GR (Merck) Lot 721 A 253273

13. Sodium chloride GR (Vidhyasom) Lot 000630

14. Triethylamine for synthesis (Merck) Lot 3194027

15. Methanol AR. (Merck) Lot 708 K 4184709

C. Apparatus

1. Analytical Balance (Mettler H 51 AR)

2. Disintegration Tester (Manesty machines Ltd., Liverpool 24, England)

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

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

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

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

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

8. Water bath (Memmert, Edelstahl Rost Frei)

9. Digital Computer (IBM Compatible 16 Bit, Micro Source)

Method

A. In Vitro Studies

Five brands of ranitidine, 150 mg film-coated tablets, were evaluated using the official and non-official test of U.S.P. and/or B.P. for film-coated tablets.

The test included :

1. Uniformity of Weight B.P. 1973 (26)

Twenty tablets of each of the five brands of ranitidine tablets were sampled and accurately weighed tablet by tablet. The average weight and standard deviation were calculated.

2. Assay for Content of Active Ingredient

2.1 Ranitidine Tablets :-

The amount of ranitidine in tablet was determined by the method of Hohnjec M.(14) as follows :

Not less than 20 ranitidine tablets were weighed and finely powdered. A portion of the powder equivalent to 10 mg of ranitidine hydrochloride was quantitatively transferred into a 250 ml volumetric flask. A 100 ml of water was added and the resulting suspension automatically was shaken for 20 minutes. The mixture was made up to volume and mixed well. Aliquot portion of 20 ml was centrifuged at 4000 rpm. for 10 minutes. A 10 ml of the clear supernatant was pipetted into a 100 ml volumetric flask and made up to volume. The absorbance of the resulted solution was measured using the wavelength of maximum absorbance at 313 nm., against water as the blank and compared to that of an appropriate standard solution. (Appendix B)

2.2 Ranitidine Injection :-

A 2.0 ml of ranitidine injection (50 mg) was pipetted into a 100 ml volumetric flask and made up to volume with water. A portion of 2.0 ml of the dilute solution was

adjusted to 100 ml with water. The absorbance of this solution was measured at 313 nm. against water as the blank and compared to that of an appropriate standard solution (Appendix B)

3. Disintegration Test :-

The disintegration tests for five brands of ranitidine tablets were studied according to the B.P. 1980 method for coated tablets (27)

Procedure : 1 tablet of the drug was placed in each of the six tubes of the basket, then a plastic ring was added to each tube. The apparatus was operated using water maintained at 37 $\pm$ 1 °C as the immersion fluid. The tablets passed the test if all six had disintegrated completely within one hour. If any of the tablets failed to disintegrate, the test was repeated on a further 6 tablets and substituted water for 0.1 N hydrochloric acid maintained at 37 $\pm$ 1 °C as the immersion fluid. The tablets then passed the test if all six tablets, in the acid medium, had disintegrated within one hour.

The mean disintegration time of each brand and standard deviation were then calculated.

4. Dissolution Test

The dissolution test for five brands of ranitidine tablets were determined by the rotating basket method of the USP XXI (28) using simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.5) as the medium. (Composition of dissolution media see Appendix C)

Procedure : 1 tablet of the drug was placed in each of the six baskets, then immersed the basket into each vessel containing nine hundred millilitres of dissolution medium at 37 $\pm$ 0.5 $^{\circ}$ C. The apparatus was then immediately operated and maintained stirring the basket at 100 $\pm$ 2 rpm. Five millilitres of samples were taken at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes intervals. Then immediately added the same quantity of dissolution medium after each sampling to keep the volume of the dissolution medium constant during the experiment. The amount of drug dissolved was determined by UV spectrophotometer at 228 nm, in comparison with a standard curve.

Standard Curve : Standard solution of ranitidine with concentrations of 1, 2, 3, 4, 6, 8, 10, 15 and 20 mcg/ml in simulated gastric fluid and simulated intestinal fluid were determined in a UV spectrophotometer at 228 nm. Absorbances obtained versus known concentrations were fitted to a straight line using linear regression (Appendix B).

5. Evaluation of the In Vitro Studies

The characteristics of all five brands of ranitidine tablets were examined and evaluated using general standard of USP. and/or BP. A one way analysis of variance and t-test were used for comparing the differences between the innovator's product and others for the disintegration times and the dissolution rates.

B. In Vivo Studies

1. Products

All brands of ranitidine tablets commercially available in Thailand and innovator's ranitidine injection were used in this study.

2. Subjects

Twelve male volunteers with a mean age of 21.8 $\pm$ 1.3 yr. (range 20 to 24) and a mean weight of 62.7 $\pm$ 7.1 kg (range 50 to 77) participated in this study. All subjects were healthy based on history, clinical examination and pre-entry hematologic and biochemical tests. Demographic data are presented in Appendix D. The methods of the study

were fully explained to all subjects. Informed written consent was obtained from all subjects. They were taking no medication for at least one week prior to and throughout the study.

3. Dose and Drug Administration

Ranitidine HCl was given as an intravenous (IV) bolus or two tablets with 200 ml water orally in a single dose equivalent to 50 mg and 300 mg ranitidine base, respectively. All subjects received each dose in the morning after overnight fast. No food or drink (other than water) was permitted until 2 hours after dosing.

4. Experimental Design

The study was conducted in a crossover experiment, with at least 1 week between doses as shown in Table 1.

Table 1 Dosing Schedule

Subject No.	Week					
	1	2	3	4	5	6
1	A	B	C	D	E	I
2	B	C	D	E	I	A
3	C	D	E	I	A	B
4	D	E	I	A	B	C
5	E	I	A	B	C	D
6	I	A	B	C	D	E
7	A	B	C	D	E	I
8	B	C	D	E	I	A
9	C	D	E	I	A	B
10	D	E	I	A	B	C
11	E	I	A	B	C	D
12	I	A	B	C	D	E

A,B,C,D and E : represent the brand names of ranitidine tablets

I : represents the brand name of ranitidine injection

5. Sample Collection

5.1 Oral study : Blood samples (5 ml) were drawn from the antecubital vein before and at 0.5, 1, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0 and 10.0 hours after dose.

5.2 Parenteral study : Blood samples (5 ml) were taken before and at 5, 10, 15, 20, 30, 45 min and 1, 2, 3, 5 and 7 hours after dose.

All blood samples were collected in heparinized tubes After centrifugation (2500 rpm. for 10 minutes), the plasma was then separated and stored at -20 °C until subsequent assay.

6. Determination of Ranitidine in Plasma

Plasma ranitidine concentrations were determined by high-performance liquid chromatography using a modification of the method described by Mihaly et. al. and Boutagy et. al. (29, 30, 31). The procedure was developed as follows :

Plasma sample 1 ml

- added 0.08 ml of Internal standard (100 µg./ml of procainamide hydrochloride in 0.09% Sodium metabisulfite solution)
- added 5 mol/L Sodium hydroxide 0.2 ml
- mixed 30 sec.
- extracted with a mixture of ether, chloroform and isopropanol (2:1:1) (5 ml) by vortexing for 2 min. and centrifuged at 4000 rpm. for 15 min.

transferred the organic top layer to a second tube

- evaporated under a gentle stream of nitrogen at 45 °C

residue

- reconstituted in 0.2 ml of the chromatographic mobile phase

inject 150 µl of reconstituted sample into the HPLC

HPLC Condition for Ranitidine Analysis in Plasma

Apparatus	:	HPLC LC-3A, Shimadzu, Japan
Column	:	μ-Bondapak C_{18}, Stainless steel column, Water Associates Pty-Ltd., U.S.A. pre-column 5 cm x 2.0 mn i.d. analytical column 30 cm. x 3.9 mn i.d.
Mobile phase	:	0.9% triethylamine in water adjust to pH 3.2 with phosphoric acid and 6.5% acetonitrile (V/V)
UV detector	:	330 nm
Flow rate	:	1.5 ml/min
Attenuation	:	2^1 mv/full scale
Column temperature	:	ambient
Pressure	:	120 kg/cm^2
Retention time	:	Ranitidine 4.4 minutes Procainamide 3.0 minutes

The ranitidine concentration in plasma samples were calculated from the standard curve (Appendix B).

Standard Curve :

Certain amount (0.05, 0.1, 0.2, 0.3, 0.5, 0.8, 1.2, 2.0 mcg) of standard ranitidine were added to 1 ml of pooled drug free plasma. These samples were analyzed following the

same procedure as described previously (29-31). The ratio of the peak height of ranitidine to internal standard obtained versus the known ranitidine concentrations were fitted to a straight line using linear regression (Appendix B).

7. Evaluation of the In Vivo Studies

The kinetic of ranitidine was assumed to be linear therefore, individual plasma ranitidine profile from each treatment was analyzed according to noncompartment estimating program (16, 17, 22, 32, 33).

The absolute and relative bioavailabilities were calculated using the following equations :-

$$F_{ab} = \frac{[AUC]_0^{\infty}{}_{oral}}{[AUC]_0^{\infty}{}_{intravenous}} \times \frac{Dose_{intravenous}}{Dose_{oral}} \qquad Eq...1$$

$$F_{rel} = \frac{[AUC]_0^{\infty}{}_{oral\ (test)}}{[AUC]_0^{\infty}{}_{oral\ (reference)}} \times \frac{Dose_{oral\ (test)}}{Dose_{oral\ (reference)}} \qquad Eq...2$$

Area under the concentration-time curves, [AUC] were calculated from the first sampling time to the latest one by using the trapezoidal rule and then extrapolated to infinity. The calculation can be shown as follows :

$$[AUC]_0^{\infty} = [AUC]_0^{t} + C^*/Kel \qquad Eq....3$$

where, C^* is the last measured concentration, Kel is the elimination rate constant (Appendix E).

The comparative bioavailability of the 5 brands of ranitidine tablets were evaluated using the following parameters : (a) the peak plasma concentration (Cp_{max}), (b) the time of the peak plasma concentration (t_{max}), (c) the area under the plasma concentration-time curve [AUC] and (d) the first order absorption rate constant (Ka). The peak plasma concentrations and the times of the peak plasma concentration were observed from the concentration-time profiles. The areas under the plasma concentration-time curve were calculated as described previously. The absorption rate constants were calculated using equations 12 and 13 as described in Appendix E. A one way analysis of variance and t-test were used to assess the bioequivalent differences among various brands and between each others.