CHAPTER II

MATERIALS AND METHODS

1. Materials

Test Products

Six commercial brands of praziquantel, 600-mg film-coated tablets, were provided by local manufacturers and/or distributors. The letters (A,B,C,D,E and F) were given to represent the brand names of products. Information of test products were accessible in Appendix B.

Reagents

1. Standard praziquantel powder (Bayer A.G., Leverkusen)

2. Internal standard, 2-cycloheptyl carbonyl-4-oxo-1,2,3,6,7, 11b-hexahydro-4-H-pyrazino [2,1-a] isoquinoline, powder (E.Merck, Damstadt)

3. Working standard praziquantel powder, potency 101.37 % (Biolab Laboratory) Lot no. 1102 MC

4. Acetonitrile HPLC grade (E.Merck, Damstadt) Lot no. 31086094

5. Ethyl acetate HPLC grade (E.Merck, Damstadt) Lot no. 2396293

6. Monobasic potassium phosphate (Carlo-erba, Italy) Lot no. 471686

7. Sodium hydroxide AR (Riedel-dehaen A.G., Germany) Lot no. 814709

8. Concentrated hydrochloric acid AR (E.Merck, Damstadt) Lot no. K3591317

9. Ethanol absolute AR (E.Merck, Damstadt) Lot no. K4920183

Apparatus

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

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

3. Dissolution Apparatus (72RL, Hanson Reserch 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. Rotoevaporator (Rotovapor RE120, Buchi Lab. Tech. A.G., Switzerland)

7. Digital Computor (Columbia Model 4b22-co, Pota products, Inc., U.S.A.)

II. Method

A. In Vitro Studies

Six brands of praziquantel, 600-mg film-coated tablets, were evaluated using the official and non-official tests of U.S.P. and/or B.P. for film-coated tablets. The tests included :

1. Uniformity of Weight B.P. 1973 (39)

20 tablets of each of the six brands of praziquantel tablets were sampled and accurately weighed tablet by tablet. The average weight and standard deviation were calculated.

2. Standard for Content of Active Ingredient in Tablet (40)

The amount of praziquantel in tablet was determined by the method of Yuan (41) Which was described as follows :

20 tablets were weighed and powdered. To a quantity of the powder equivalent to 0.5 g. of praziquantel, 50 ml of absolute ethanol was added and shaked vigorously for 10 minutes. The mixture was diluted to 100 ml with absolute ethanol, mixed and filtered. 10 ml of filtrate was then diluted with absolute ethanol. The absorbance of the resulted solution was measured using a spectrophotometer at 264 nm.

The actual content of praziquantel in tablet was quantified utilizing a standard curve (Appendix C).

3. Disintegration Test

The disintegration tests for six brands of praziquantel tablets were determined according to the B.P. 1980 method for coated tablets (40).

Procedure :

Individual tablet was introduced into each of the six tubes of the basket. A disk was then added to each tube, and the apparatus was operated using water maintained at 37 ± 1 °C as the immersion fluid. The tablets pass the test if all six have disinte-

grated within one hour. (If any of the tablets have not disintegrated, the test was repeated on a further six tablets. Water in the beaker was replaced with 0.1 M hydrochloric acid. The tablets then pass the test if all six tablets have disintegrated in the acid medium within one hour.) The mean disintegration time of each brands was calculated.

4. Dissolution Test

Although praziquantel had no compendial monograph dissolution requirement, The U.S.P. Dissolution Apparatus II (paddle) (42) was used to establish and compare dissolution profiles. Two types of dissolution media were used. (preparation of dissolution media see Appendix D)

a. Simulated Gastric Fluid without enzyme (pH 1.2 ± 0.1)

b. Simulated Intestinal Fluid without enzyme (pH 7.5 ± 0.1)

Dissolution rates of praziquantel from six commercial tablets were studied according to the following procedure (42) :

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 vessel, the apparatus was then immediately operated and maintained steering speed at the rate of 100 ± 5 rpm. Three milliliters of samples were taken at 5,10,15,20,25,30,35,40,45,50,55,60,75 and 90 minutes intervals. The same quantity of dissolution medium was added immediately after each sampling to keep the volume of dissolution medium constant during the course of the test. The absorbance of samples were measured using a UV-spectrophotometer at 264 nm (41).

The amount of the drug dissolved at sampting time intervals was calculated from the standard curve.

Standard Curve

Solutions with known amounts of praziquantel in each dissolution media were prepared and analyzed using a UV-spectrophotometer at 264 nm (42). Absorbances obtained versus known concentrations were fitted to a straight line using linear regression (43).

In Vitro Evaluation

Physical characteristicsof six commercial brands of praziquantel tablet were examined and evaluated to determine whether which brand passed the general standard U.S.P. and/or B.P. requirement for film-coated tablet. Analysis of variance and student't-test were performed to assess the differences between the original and local brands for the disintegration times and dissolution values. This is accomplished using a computerized statistical program ABSTAT (Appendix E).(44)

B. In Vivo Studies

Test products

Four commercial brands of praziquantel tablets with differences in in vitro dissolution characteristics were selected. One was the foreign manufactured brand in which to be assigned as the reference standard against the other three local manufactured brands with maximum dissolution values in simulated gastric fluid without enzyme (I), maximum dissolution values in simulated intestinal fluid without enzyme (II) and minimum dissolution values in both simulated gastric fluid without enzyme (I) and simulated intestinal fluid without enzyme (II).

Subjects

Eight healthy male volunteers with 18 to 23 years of ages and within 10 % of their ideal body weights participated in this study. A medical history,completely physical examination and standard laboratory screen for individual subject were performed prior to the study to ensure the absence of any significant hepatic, renal disturbance and/or the gastrointestinal tract disorder (Appendix F). The method of the study was fully explained to all subjects and all gave their written consent before entering the study. They were permitted to take no medication for at least one week preceding the study and during the experimental period.

Drug Administration

The drugs were given orally in a single dose of praziquantel 40 mg/Kg. The doses were administered under supervision with 200 ml of water 30 minutes after the subjects ingested an identical standard breakfast*

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*A plate (300g) of rice, an omelete and a bowl of vegetable soup.

Experimental Design

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

Table 1 Treatment Schedule

Subject No.	Dosage		Week			
	g/person	mg/kg	1	2	3	4
1	1.95	40.97	B	C	D	A
2	2.10	39.62	D	A	B	C
3	2.10	39.11	B	C	D	A
4	2.25	39.13	A	B	C	D
5	2.40	41.10	C	D	A	B
6	2.40	40.68	A	B	C	D
7	2.40	40.27	D	A	B	C
8	2.40	39.67	A	B	C	D
		40.07 ± 0.80[a]				

a. Dosage : Arithm. mean ± Standard deviation.

b. Each A,B,C and D represents the brand name of praziquantel tablets

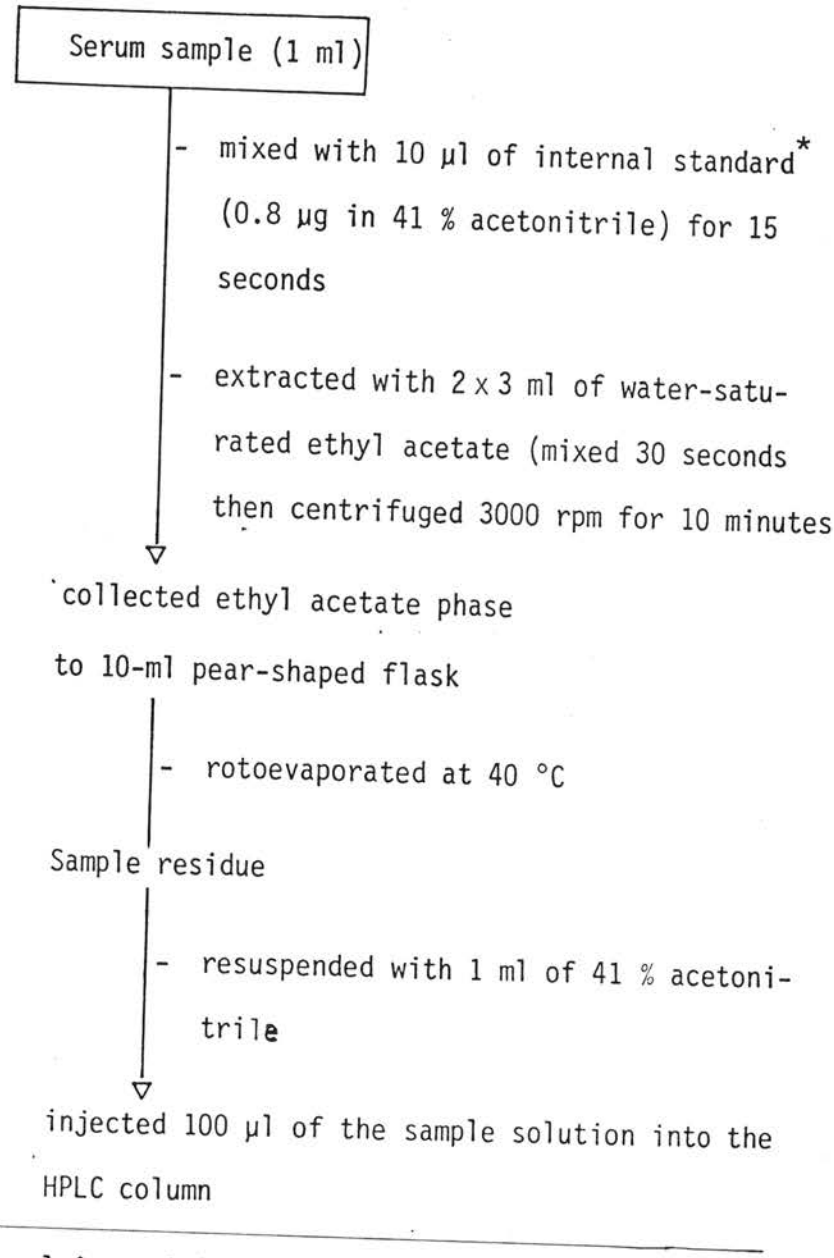
Sample Collection

Blood samples (4-5 ml) were drawn from the antecubital vein prior to dosing and at 0.5,1.0,1.5,2.0,3.0,4.0,5.0,and 8.0 hours after drug administration. They were allowed to clot at room temperature

for 1-2 hours. After centrifugation (2500 rpm for 15 minutes) the serum samples were collected and kept at -20°C until subsequent analysis.

Determination of Praziquantel in Serum

Concentrations of praziquantel in serum samples were determined using modified high-performance liquid chromatographic method described by Xiao *et al* (45). The procedure was developed as follows:



* 2-cycloheptyl carbonyl-4-oxo-1,2,3,6,7,11-b-hexahydro-4H-pyrazino [2,1-a] isoquinoline

Operating Condition

Apparatus	:	HLPC LC-3A, Shimadzn, Japan.
Column	:	μ Bondapak C_{18}, stainless steel column, Waters Associates Pty Ltd., U.S.A. pre-column 5 cm x 2.0 nm i.d. analysis-column 30 cm x 3.9 nm i.d.
Mobile phase	:	41 % acetonitrile in deionized water
UV detector	:	210 nm.
Flow rate	:	1.5 ml/min
Attenuation	:	2° mV/full scale
Pressure	:	70-80 kg/cm^3
Temperature	:	ambient
Speed chartd	:	4 mm/min
Injected volume	:	100 μl

The praziquantel concentration in serum samples were quantified employing the standard curve. (Appendix C)

Standard Curve

Known amounts (0.05,0.1,0.5,1.0,1.5,2.0,and 3.0 μg) of standard praziquantel and 0.8 μg of internal standard were added to 1 ml of pooled human serum. These sample were analysed following the same

procedure as described previously (45). The ratio of area under the peak of praziquantel and internal standard obtained versus the known praziquantel concentrations were fitted to a straight line using linear regression (43), (Appendix C).

Pharmacokinetic Analysis

Individual serum praziquantel profile from each treatment was analyzed according to a one-compartment open model with first-order absorption and elimination with lag time using the PCNONLIN nonlinear estimation program (46), (see Appendix G).

Statistical Evaluation of Bioavailability Results

The comparative bioavailability of the four brands of praziquantel tablets were evaluated using the following parameters : (a) the peak height serum concentration ($C_{p\ max}$) , (b) the time of the peak serum concentration (t_{max}), and (c) the area under the serum concentration -time curve $[AUC]_o^\infty$ A one - way analysis of variance and student't-test were used to analyzed for differences among and between the original brand and the selected local brands. This is accomplished using a computerized statistical program ABSTAT (44), (Appendix E).