CHAPTER III

RESEARCH/METHODOLOGY

Materials

1. Sodium acetate trihydrate (Lot No. TA914567 203, Merck KGaA, Darmastadt, Germany)
2. Glacial acetic acid (Lot No. 02-08 0025, Lab-Scan Ltd. Ireland)
3. Distilled water for HPLC grade.
4. Aspirin Referent Standard (Lot No. A3160, Sigma Chemical Co., St. Louise USA)
5. Salicylic acid standard (Lot No. S6271, Sigma-ALDRICH CHEMIE Gmbh, Darmastadt, Germany)
6. Alcohol (Lot No. K31369783 245, Merck KGaA, Darmastadt, Germany)
7. Hydrochloride acid (Lot No. K23776817 710, Merck KGaA, Darmastadt, Germany)
8. pH paper (Lot No. CE 1371-1, Panpeha®, Riedel-deHaen, RdH Laborchemikalien GmbH & Co. KG, Germany)
9. Sodium 1-heptanesulfonate (Lot No. 51833, Fluka Chemie GmbH, Switzerland)
10. Acetonitrile (Lot No. 01 05 0091, Lab-scan Ltd., Ireland, UK)
11. Formic acid (Lot No. AOC0227, APS, Asian Pacific Specialty Chemicals Ltd., Australia)
12. Filter paper No.1 (Lot No. A752298, Whatman®, Whatman international Ltd Maidstone, England)
13. 96 brand names of the uncoated aspirin tablets were collected from 96 drugstores in seven districts in Phnom Penh city, Cambodia, between October and November 2002.

Equipments

1. pH meter (WTW® 539 TÜN Product Service, Geprufte Sicherheit, Germany)
2. Friabilator (Vankel®, Model: 45-2200, New Jersey, USA)
3. Disintegrator (Erweka® ZT3, Type ZT3-1 Nr. 69831, Erweka® GmbH, Germany)
4. Disssolution apparatus (Vankel VK 7000, Model: 10-1500, New Jersey, USA)
5. HPLC instrument, equipped with pump (ConstaMetric® 3200, USA), column (Alltech®, 5μm, 4.6mm x 250mm packed with C18, Alltech Associates, Inc., USA), injector (Model: 7125NS, Rheodyne®, USA), UV detector (SpectroMonitor® 3200, USA), recorder (Computer, peak/word 95)
6. Glass desicator
7. Centrifuge (Beckman Type J2-MC Rotor JA-10, Beckman, USA)
8. Analytical balance (Mettler Toledo, Type AB 204, Switzerland)
9. Hardness tester (Erweka TBH 30, GmbH Heusenstamm, Germany)
10. UV spectrophotometer (Beckman DU 650, USA)
11. Refrigerator (Model: XLR416ABA, Puffer Hubbard, Harris Manufacturing Co., USA).

Methods

Uncoated aspirin tablets regardless brand name used in this study were collected from drugstores between October and November 2002. Both legal and illegal drugstores were randomly selected proportionately from the all districts in Phnom Penh city to be included in the sample. All samples were investigated for their qualities including labeled amount, weight variation, hardness, friability, disintegration, and dissolution. The study was divided into two phases. Phase one was data collection and phase two was quality determination.

1. Data Collection

Cluster sampling method (Scheme 1) was used in the study. The population was 800 drugstores from seven districts; four districts in city and three districts in out-skirt areas, in Phnom Penh, Cambodia. Sample is a random sample of 96 legal and illegal (76 and 20) drugstores, i.e., about 10% of the population. The 100 uncoated aspirin tablets for each brand name were collected from each drugstore and then randomly selected only one brand name from each drugstore for quality analyses. Moreover, the information about drugstores and the descriptive conduction of data collection were described as follow:

According to the list of drugstores from Department of Drug and Food (DDF) of Ministry of Health year 2001, there were approximately 400 legal drugstores in Phnom Penh city. A legal drugstore means a drugstore which is given the license by Ministry of Health. In addition, according to the official information obtained from Ministry of Health in the year 2000 [90], there were approximately 400 illegal drugstores in the Phnom Penh city. An illegal drugstore means a drugstore which was set up without permission by Ministry of Health. In total, there were approximately 800 drugstores selling medicines to Phnom Penh population. For this reason, the samples were collected from illegal drugstores concurrently with the legal drugstores. As described above, in this study the sample size was determined to be 10% of the population because only the descriptive statistic was used. The total sample size was 96 from 800 drugstores. Number of legal drugstores in Phnom Penh was shown in Table 1 and the total proportion numbers of all drugstores of this study were shown in Table 2.

Table 1. Number of legal drugstores in Phnom Penh

District	Total number of legal drugstores								Number of legal drugstores 10% of total							
	D1	D2	D3	D4	D5	D6	D7	Total	D1	D2	D3	D4	D5	D6	D7	Total
Dru. A	89	49	55	34	14	4	2	247	9	5	6	4	2	1	1	28
Dru. B	16	4	6	7	13	18	2	66	2	1	1	1	2	2	1	11
Dru. C	11	2	11	6	21	26	10	87	1	1	1	1	2	3	1	10
Total	116	55	72	47	48	48	14	400	12	7	8	6	6	6	3	48

Remarks

D : District

Dru. A : Drugstore A

Dru. B : Drugstore B

Dru. C : Drugstore C

District in the city

District 1: Chamkar Mon District

District 2: 7 Makara District

District 3: Daun Penh District

District 4: Tuol Kok District

District in the out-skirt of city

District 5: Mean Chey District

District 6: Russey Keo District

District 7: Dang Korv District

Drugstore A means a drugstore which is responsible by a pharmacist,

Drugstore B means a drugstore which is responsible by a pharmacist assistance,

Drugstore C means a drugstore which is responsible by a retired nurse.

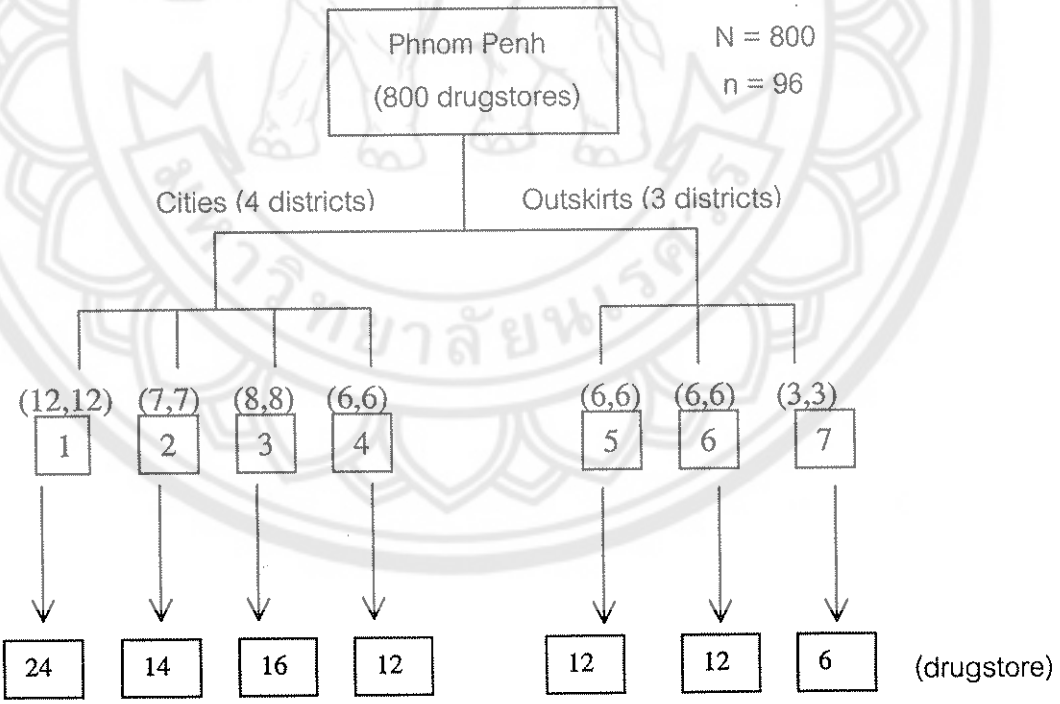
Table 2. Total proportional numbers of all drugstores in Phnom Penh

District	D1	D2	D3	D4	D5	D6	D7	Total
Legal drugstore	12	7	8	6	6	6	3	48
Illegal drugstore (approximately)	12	7	8	6	6	6	3	48
Total	24	14	16	12	12	12	6	96

Remark

D: District

Sampling procedure:



Scheme 1. Cluster sampling in Phnom Penh city

Data collection procedure:

The investigator visited the drugstores and asked for 100 tablets of the uncoated aspirin, regardless of the brand names. The strength of acetylsalicylic acid in the uncoated aspirin tablet is 325 mg or 300 mg per tablet. The investigator also observed various characteristic of the stores and the sellers such as the condition of each drugstore. After leaving the drugstore, the investigator recorded the information in the report form (Appendix C) and transferred aspirin sample into a plastic bottle, sealed and attached the label. The plastic bottle was then kept in a big plastic bag filled with desiccant (silica gel). The schedule of data collection is shown in Appendix D.

2. Quality Determination

Quality of collected aspirin tablets was tested in six aspects: labeled amount, weight variation, hardness, friability, disintegration, and dissolution. Except for hardness testing, other tests were analyzed in compliance with USP XX and USP XXV. Each sample was required to meet standard of each method to be considered of acceptable quality. The standard requirement of each method included labeled amount (90.0 to 110.0% of active ingredient), weight variation (not more than 5 of percentage difference), hardness (not less than 4kg or 39.2 N), friability (not more than 1%), disintegration (not more than 15 minutes), and dissolution (not less than 80% in 30 minutes). In addition, any sample that failed in percent label amount or in disintegration time test would not be subject to dissolution test.

2.1. Labeled Amount or Drug Content

The contents of twenty the uncoated aspirin tablets were ground into powder, as fine as possible, and were weighed accurately. The powder contents were transferred an accurately weighed quantity of 100mg of the contents to 50-ml volumetric flask. Twenty milliliters of the diluting solution was added to the flask, and mixed. A portion of this solution was filtered through a suitable filter of 1 μm pore size, and transferred 1.0 ml of filtrate into 10-ml flask and diluted with 9.0 ml of diluting solution. The last obtained solution was used as the assay preparation, and also use for the test for limit of salicylic

acid. The samples were assayed by the procedure which followed the method of USP XXV.

The HPLC (High performance liquid chromatography) condition as follows.

Column : 4.6mm x 250mm packed with C18

Mobile phase : Sodium 1-heptanesulfonate in water and acetonitrile (8 : 2 by volume) adjusted pH to 3.4 with glacial acetic acid.

Flow rate : 1ml/min

Detector : UV detector at 280 nm

Injection volume : 20 µl

Temperature : ambient

2.2 Weight Variation of Tablet

Weight variation was investigated according to the method described in USP XX. The twenty uncoated aspirin tablets of collected samples were weighed individually using analytical balance. The average weight, standard deviation and percentage of relative standard deviation were then calculated.

2.3 Hardness of Tablet

The tablet hardness was measured as follow. From each sample, ten tablets were randomly drawn and their hardness was determined using the hardness tester (Erweka, Germany). The breaking strength values are shown in Newton (N). Means and the standard deviations were calculated.

2.4 Friability of Tablet

Friability was investigated according to the method described in USP XXV. Twenty preweighed tablets or at least six grams were placed in a friabilitor (Vankel®, USA) rotated at 25 rpm for 4 minutes. Loss of their weights with respect to the initial value was calculated as percent friability.

$$\%Friability = \frac{Weight\ before\ test - weight\ after\ test}{Weight\ before\ test} \times 100 \qquad \text{.....(11)}$$

2.5 Disintegration Test

The disintegration time of the uncoated aspirin tablet was determined individually in distilled water using disintegration test apparatus (Erweka®, Germany). The method was based on USP XXV. Water maintained at 37 ± 2°C was used as the test fluid. The results were reported as mean values and standard deviation of six tablets.

2.6 Dissolution Test

The dissolution of the collected uncoated aspirin tablets was carried out following the method described in USP XXV. The conditions of dissolution testing were as follow.

Medium : 500 ml of 0.05 *M* acetate buffer solution having the pH of 4.50 ± 0.05 at temperature of 37 ± 0.5°C

Apparatus 1: 50 rpm

Time : 30 minutes

Procedure : Determined the dissolved amount of aspirin using UV spectrophotometer at maximum wavelength of absorption of 265 nm, immediately after sampling at 30 minutes. The dissolution rate of drug release from tablet at this specified time was calculated as percents of the labeled amount.

Tolerances : Not less than 80% (Q) of the labeled amount of aspirin is dissolved in 30 minutes.

3. Statistical Analysis

The data were compared by ANOVA. They were analyzed using SPSS version 10.0. The level of significance was set 0.05.