

CHAPTER 2

MATERIALS AND METHODS

MATERIALS

A. Test Products

1. Quinine dihydrochloride injection 300mg/ml (2 ml/ampule)
Lot no. J309487 of Government Pharmaceutical Organization
2. 3 sterile intravenous solutions manufactured by Pharmacy Department, Songklanagarind Hospital
 - 5% Dextrose in water Lot no. 120691
 - 5% Dextrose in normal saline solution Lot no. 110691
 - 5% Dextrose in 0.45% saline solution Lot no. 210591
3. Normal saline solution Lot no. 0220291 was obtained from Thai Nakorn Patana because normal saline solution is not manufactured by Pharmacy Department, Songklanagarind Hospital.

B. Chemicals

1. Quinine sulphate (Fluka Chemie), Lot no.276921-388
2. Diazepam (Sigma), Lot no. 83C0516
3. Acetonitrile AR (Ridel-de Haen), Lot no. 00500
4. Methanol AR (Ridel-de Haen), Lot no. 20720
5. Sodium Pentanesulfonate (TCI), Lot no. FCY01
6. Concentrated hydrochloric acid GR (Merck), Lot no. K13848219

C. Apparatus

1. Analytical balance (Precisca 300A, Switzerland)
2. High pressure liquid chromatography (Gilson712 USA.)
3. Digital pH meter (model PHI 31, Beckman USA.)
4. Ultrasonic bath (Bransonic 221, Branson USA.)
5. Vortex mixer (Vortex Genie-2, Scientific Industries USA.)

METHODS

The study was divided into three parts.

1. Development and evaluation of stability-indicating assay for quinine.
2. Stability study of quinine at the concentration of 1.20 mg/ml in 4 IV fluids as follows,
 - normal saline solution (NSS)
 - 5% dextrose in normal saline solution (D/NSS)
 - 5% dextrose in half strength saline solution (D/1/2NSS)
 - 5% dextrose in water (D5W)
3. Stability study of quinine at the concentration of 3.60 mg/ml in 4 IV fluids described above.

PART1: Development and evaluation of stability-indicating assay for quinine.

Before initiating the quinine stability study, a stability indicating method had to be developed and the performance characteristics of this assay must be evaluated to ensure the accuracy and the precision of the method.

Assay Validation

Following the development of a chromatographic system, the suitability of this system for use as a stability indicating method was tested by accelerating the degradation of quinine. One hundred and twenty mg of quinine sulphate was dissolved in 5 ml of 1 N hydrochloric acid and the volume was adjusted to 100 ml with water. This solution (10 ml) was placed in a pyrex tube and heated by direct flame for 15 minutes. After heating, the solution was brought to the volume of 10 ml with water. Heated and unheated samples were diluted and subjected to the chromatographic system as described in PART2 and the chromatograms were inspected for the appearance of additional peaks. Quinine peak was compared between heated and unheated samples for change in retention time, and peak shape.

Following this first phase of evaluation, the reproducibility and linearity of standard curves was tested. On each day fresh standard was prepared by dissolving a known weight of quinine sulphate in 5 ml of 1 N hydrochloric acid. The solution was adjusted to 100 ml with water. From this standard, five or six aliquots were taken to construct a standard curve. Three replicates were prepared for each concentration. Within run precision was determined by analyzing of three set of these solution at the same day. Peak height ratio of quinine and diazepam, internal standard, was compared and coefficient of variation was calculated to show within run reproducibility. Between run precision was determined by comparing peak height ratios for three standard curves prepared and analyzed on three different days and Coefficient of variation of peak responses was calculated to indicate between run precision. Note: One gram of quinine sulphate is equivalent to 1.015 grams of quinine dihydrochloride

PART2. Stability study of quinine at the concentration of 1.20 mg/ml

2.1 Sample preparation: Mix 2 ml of quinine dihydrochloride injection (equivalent to quinine dihydrochloride 600 mg) into each of the following sterile IV fluid that was contained in glass bottle.

- 5% dextrose in normal saline solution (D/NSS)
- 5% dextrose in half strength saline solution (D/1/2NSS)
- 5% dextrose in water (D5W)
- normal saline solution (NSS)

All IV fluids except normal saline solution were contained in glass bottles. Normal saline solution was contained in plastic bag, so it was transferred to glass bottle before mixing quinine in order to avoid the sorption of drug by plastic container. Three separate experimental runs were carried out for each IV fluid.

2.2 Sample collection

All IV solutions were kept at room temperature under usual laboratory fluorescent lighting (mimic the condition in patient room). Samples were drawn at time zero and at 2, 4, 6, 10 and 24 hours. Each sample was assayed immediately and dublicately after collection. At each sampling time, a physical inspection of colour clarity and particles was carried out. Particulate matter inspection was performed visually against a white and black background.

2.3 Analysis of samples

Samples were subjected to the analytical method modified from Edstein's method (10) as described below.

Transfer 1 ml of sample, accurately measured, to a 10 ml volumetric flask, then add 1 ml of internal standard solution, mix and adjust to volume with methanol. Inject 20 microlitres portion of the resulting solution into chromatograph. Quinine concentrations were quantitated by comparison of the peak height ratio of the drug to the internal standard with standard curve.

Internal standard solution: Dissolve 10 mg of diazepam in methanol to achieve the solution concentration of 100 microgram/ml.

2.4 Standard curve

2.4.1 Standard curve for quinine in D5W and D/1/2NSS

A standard curve was prepared each day by weighing 120 mg of quinine sulfate. The powder was dissolved in 5 ml of 1.0 N HCL and adjust to 100 ml with water (stock solution). Four hundred, 600, 800, 1000, 1200, 1500 microlitres aliquots were taken, mixed with 1 ml of internal standard and diluted to 10 ml with methanol. These final solution represented concentrations of approximately 48, 72, 96, 120, 192 and 240 microgram/ml. Twenty microlitres of this solutions were injected to the chromatograph described below. Standard curves were generated by using the least square regression of the peak height ratio against the concentrations.

2.4.2 Standard curve for quinine in NSS and D/NSS. Procedure described in 2.4.1 was used to construct standard curve but 1 ml of normal saline solution was added to the solution before the solution was adjusted to the volume of 10 ml with methanol.

2.5 HPLC CONDITION

APPARATUS : HPLC model Gilson712, Gilson
COLUMN : C18 synchopak, stainless steel column, Gilson
USA. (25*4.6 mm) 10 micrometer particle size
MOBILE PHASE: acetonitrile:methanol:0.001 M PIC B5 (40:40:20)
FLOW RATE : 1 ml/min
UV DETECTOR : 254 nm (model 115UV)
AUFS : 0.05 mv/full scale
CHART SPEED : 0.2 cm/min
TEMPERATURE : ambient

PART3: Stability study of quinine at the concentration of 3.6 mg/ml

3.1 Sample preparation: Mix 6 ml of quinine dihydrochloride injection (equivalent to 1800 mg of quinine dihydrochloride) to each of four IV fluids studied. The samples were collected by the same procedure described in PART2 of the study.

3.2 Analysis of samples: The analytical method employed in PART2 was also used in this part of study but the concentration of internal standard was changed from 100 to 150 microgram/ml.

3.3 Standard curve

3.3.1 Standard curve for quinine in D5W AND D/1/2NSS

A standard curve was prepared each day by weighing 330 mg of quinine sulfate. The powder was dissolved in 5 ml of 1.0 N hydrochloric acid and the volume was adjusted to 100 ml with water (stock solution). Four hundred, 800, 1000, 1200, 1500 microlitres aliquots were taken, mixed with 1 ml of internal standard and diluted to 10 ml with methanol. These final solutions represented concentrations of approximately 132, 264, 330, 396 and 495 microgram/ml. Twenty microlitres of this solutions were subjected to the chromatograph described below. Standard curves were generated by using the least square regression of the peak height ratio against the concentrations.

3.3.2 Standard curve for quinine in NSS AND D/NSS.

Procedure in 3.3.1 was used but 1 ml of normal saline solution was added to the solution before the solution was adjusted to the volume of 10 ml with methanol.

3.4 HPLC CONDITION: Use the condition described in part2 but 0.1 AUFS instead of 0.05 AUFS was used throughout the study in this part.

DATA REDUCTION AND STATISTICAL ANALYSIS

Means and standard deviation were calculated for analysis completed in duplicate. These means are reported in summary tables. Reproducibility was measured by coefficient of variance (CV-standard deviation divided by the mean). Analysis of Variance was used to evaluate the effect of different intravenous fluids on the quinine concentration. The five percent level was used as the apriori cut off for statistical significance. All references to significance refers to this level.

Quinine concentrations were considered within acceptable limits if the concentration at any analysis was never less than 90 percent of the initial concentration.