CHAPTER III

EXPERIMENTAL

Materials

All chemicals were analytical or HPLC grade and were used as received.

Ranitidine HCl, Batch No. XR 048 9601, Glaxo Pharmaceutical, Thailand.

Procaine HCl, Control No. 183119, WHO, Center for Chemical Reference Substance, Sweden.

Anhydrous sodium dihydrogen phosphate, Lot No. K 2205 1045 530, Merck, Germany.

Sodium monohydrogen phosphate, Lot No. 338 A714396, Merck, Germany.

Acetonitrile, Lot No. K32822, Beaker, USA.

Methanol, Lot No. 3041 KTTE, Mallinckrodt, USA.

Triethanolamine, Lot No. 30911881, Fluka, Switzerland.

Butylated hydroxytoluene (BHT), Product No. 28067, Chemical Ltd., England.

Alpha tocopherol, Lot No. 53H0444, Sigma, USA.

Sodium bisulfite, Lot No. 7448 KDEJ, Malinckrodt, USA.

Ascorbic acid, Batch No. N 507362, Roche, Switzerland.

Ethylenediaminetetraacetic acid (EDTA), Lot No. 261365, Fluka , Switzerland.

Citric acid monohydrate, Lot No. K20379444, Merck, Germany.

Cremophore EL®, Lot No. 3218, BASF, Germany.

Tween 20®, Lot No. 91H0685, Sigma Chemical Co., USA.

Nitrogen gas, Cylinder No. 888725, Thai Industrial Gas Public Company Ltd., Thailand.

Equipments

Analytical balance, Sartorius GMPH, Germany.

pH meter, Model SA 520, Orion, USA.

Hot air oven, TY. ULM700 Schutzort Din 40050-IP20, Memmert®, Germany.

Cool daylight lamps, 13 W, SL Prismatic, Philips, Poland.

Digital iluminance meter, Model TES 1332, TES Electrical Electronic, Taiwan.

Ultrasonic bath, Bransonic 221, Branson, Smithkline company, USA.

A holder filter, Part No. 80357, Water, USA.

High performance Liquid Chromatography (HPLC) equipped with:

a tunable absorbance detector, water 484 Model M 484, Serial No. 484-PRA 902, USA,

a constant flow pump, Water 510 HPLC pump, Millipore, USA,

an autoinjector, Water 712 WISP, Serial No. 712-007617, Millipore, USA,

an integrator, Water 745B Data Models, Serial No. 7BE/400 678, USA, and

an irregular HPLC cartridge column phase separation μ-bondapack (250 x 46) 10μ, Serial No. T602272 49, Water, USA.

Methods

1. Preparations and Storage Conditions of Ranitidine HCl Solutions

1.1 Effect of Light and Oxygen

1.1.1 Preparation of Ranitidine HCl Solutions

A solution of pH 8 phosphate buffer was prepared as follows. Five hundred and eighty milligrams of anhydrous sodium dihydrogen phosphate and fifteen grams of monosodium hydrogen phosphate were weighed in a 1000-ml volumetric flask. Purified water was used to dissolved the compounds and adjusted to final volume.

Ranitidine HCl solution was prepared by dissolving ranitidine HCl in pH 8 phosphate buffer solution to obtain the final concentration of 25 mg/ml. The solution pH was adjusted to 8 by using 0.1 N sodium hydroxide.

1.1.2 Storage Conditions

1.1.2.1 Storage in the Presence of Light and Oxygen

One and a half milliliters of ranitidine HCl solution in pH 8 phosphate buffer were pipetted by using a micropipet and transferred to 2-ml transparent borosilicate glass vials. The vials were closed with closer rubbers and covered with aluminum caps using a hand clipper. The vials containing ranitidine HCl solution were placed in a black plastic box in a hot air oven controlled temperature at 49 °C. There were eight black plastic boxes in the oven. A 13 W artificial daylight lamp was positioned at the top of each box. The black plastic boxes were used to avoid disturbance of luminescence from the other lamps. The vials were positioned in the boxes to yield the light luminescence of 1600 lux. measured by lux meter.

1.1.2.2 Storage in the Presence of Light but Absence of Oxygen

One and a half milliliters of ranitidine HCl solution in pH 8 phosphate buffer were pipetted by using a micropipet and transferred to 2-ml transparent borosilicate glass vials. The air above drug solution in each vial was flushed with nitrogen gas for ten seconds and the vial was immediately closed with a closer rubber. Aluminum caps were put on the top of the vials and clipped by using a hand clipper. Then, the vials were stored in a black plastic box in the same maner as 1.1.2.1.

1.1.2.3 Storage in the Presence of Oxygen but Absence of Light

One and a half milliliters of ranitidine HCl solution in pH 8 phosphate buffer were pipetted by using a micropipet and transferred to 2-ml transparent borosilicate glass vials. The vials were closed with closer rubbers and

covered with aluminum caps using a hand clipper. The vials were placed in small groups. Each group having four vials was put in a small opaque box stored in the hot air oven controlled temperature at 49 °C.

1.1.2.4 Storage in the Absence of Light and Oxygen

One and a half milliliters of ranitidine HCl solution in pH 8 phosphate buffer were pipetted by using a micropipet and transferred to 2-ml transparent borosilicate glass vials. The air above drug solution in each vial was flushed with nitrogen gas for ten seconds and the vial was immediately closed with a closer rubber. Aluminum caps were put on the top of the vials and clipped by using a hand clipper. The vials were placed in small groups. Each group having four vials was put in a small opaque box stored in the hot air oven controlled temperature at 49 ° C.

1.2 Effect of Antioxidants

1.2.1 Effect of Free Radical Inhibitors

1.2.1.1 Effect of BHT

Two ranitidine HCl solutions containing 0.001 and 0.005 % w/v BHT, respectively, in pH 8 phosphate buffer to yield the final concentration of 25 mg/ml were prepared. Cremophore EL® was added to the sulution to obtain the final concentration of 0.5 %. A solution of 25 mg/ml ranitidine HCl containing 0.5 % Cremophore EL® was also prepared. The solutions were filled in the vials and stored in the hot air oven providing the four storage conditions previously described in 1.1.2.

1.2.1.2 Effect of Alpha Tocopherol

Two ranitidine HCl solutions containing 0.001 and 0.02 % w/v alpha tocopherol, respectively, in pH 8 phosphate buffer to yield the final concentration of 25 mg/ml were prepared. Tween 20® was added to the sulution to obtain the final concentration of 0.5 %. A solution of 25 mg/ml ranitidine HCl containing 0.5 % Tween 20® was also prepared. The solutions were filled in the vials

and stored in the hot air oven providing the four storage conditions previously described in 1.1.2.

1.2.2 Effect of Oxygen Scavenger

1.2.2.1 Effect of Sodium Bisulfite

Two ranitidine HCl solutions containing 0.01 and 0.1 % w/v sodium bisulfite, respectively, in pH 8 phosphate buffer to yield the final concentration of 25 mg/ml were prepared. The drug solutions were filled in the vials and stored in the hot air oven providing the four storage conditions previously described in 1.1.2.

1.2.2.2 Effect of Ascorbic Acid

Two ranitidine HCl solutions containing 0.01 and 0.1 % w/v ascorbic acid, respectively, in pH 8 phosphate buffer to yield the final concentration of 25 mg/ml were prepared. The drug solutions were filled in the vials and stored in the hot air oven providing the four storage conditions previously described in 1.1.2.

1.2.3 Effect of Chelating Agents

1.2.3.1 Effect of EDTA

Two ranitidine HCl solutions containing 0.01 and 0.075 % w/v EDTA, respectively, in pH 8 phosphate buffer to yield the final concentration of 25 mg/ml were prepared. The drug solutions were filled in the vials and stored in the hot air oven providing the four storage conditions previously described in 1.1.2.

1.2.3.2 Effect of Citric Acid

Two ranitidine HCl solutions containing 0.3 and 2.0 % w/v citric acid, respectively, in pH 8 phosphate buffer to yield the final concentration of 25 mg/ml were prepared. The drug solutions were filled in the vials and stored in the hot air oven providing the four storage conditions previously described in 1.1.2.

2. Assay of Ranitidine HCl

The high pressure liquid chromatographic (HPLC) technique of Gupta (Gupta, 1988) was modified for analysis of ranitidine HCl remaining in the presence of its degradation products.

2.1 Chromatographic Conditions

The appropriately modified high pressure liquid chromatographic conditions for analysis of ranitidine HCl remaining are as follows:

Column	: irregular cartridge μ-bondapack column (300x46), 10μ.
Mobile phase	: a mixture of 28 % v/v acetonitrile and 72% v/v 0.02 M pH 6.2 sodium dihydrogen phosphate buffer.
Detector wavelength	: 290 nm.
Flow rate	: 1.5 ml/min.
Attenuation	: 64.
Chart speed	: 0.1 cm/min.
Injection volume	: 20 microliters.
Internal standard	: 8 μg/ml procaine HCl.
Retention times	: Ranitidine HCl, 6.00-7.00 min.
	: Procaine HCl, 9.00-10.00 min.

The 0.02 M pH 6.2 sodium dihydrogen phosphate buffer was prepared by weighing of 2.76 grams of anhydrous sodium dihydrogen phosphate in a 1000-ml volumetric flask. It was dissolved and the final volume was adjusted using distilled water. The pH was adjusted to 6.2 using triethanolamine.

2.2 Standard Solutions

A stock solution of internal standard was prepared by accurately weighing of twenty milligrams of procaine HCl in a 100-ml volumetric flask. Purified water was added to dissolve the drug and adjust the final volume.

A Stock solution of ranitidine HCl was prepared as follows. Forty milligrams of ranitidine HCl was accurately weighed in a 100-ml volumetric flask. Purified water was used to dissolve the drug and adjust the final volume.

Standard solutions of ranitidine HCl were prepared by pipetting 1, 1, 1, 2, 3 and 5 ml of ranitidine HCl stock solution and transferring to 100, 50, 25, 25, 50 and 50 ml volumetric flasks, respectively. Then, 4, 2, 1, 1, 2 and 2 ml of the procaine HCl stock solution were added to the previous 100, 50, 25, 25, 50 and 50 ml volumetric flasks, respectively. The solutions were adjusted to the volume with purified water so that the final concentrations of ranitidine HCl were 4, 8, 16, 32, 24 and 40 μg/ml, respectively and that of procaine HCl was 8 μg/ml. Three sets of standard solutions were prepared.

2.3 Validation of the High Pressures Liquid Chromatographic Method

2.3.1 Specificity of the Method

Six injections of each standard solution of ranitidine HCl and the solutions containing 0.5% w/v Cremophor EL®, 0.5 %w/v Tween 20®, 0.005 %w/v BHT, 0.02 %w/v alpha tocopherol, 0.1 %w/v sodium bisulfite, 0.1 %w/v ascorbic acid, 0.075 %w/v EDTA, and 2.0 %w/v citric acid were prepared. The resolutions which described the sensitivity of the method were calculated.

2.3.2 Stability Indication

The solution of 25 mg/ml of ranitidine HCl and the solutions of each antioxidant or surfactant used in 2.3.1 in the same concentration were prepared. The drug, antioxidant and surfactant were forced to decompose by exposing the solutions under 13 W artificial daylight lamp of which the luminescence was about 1600 lux at 49° C for 2 months. Ten microliters of each decomposed solution was pipetted and transferred to a 10-ml volumetric flask. Each solution was adjusted to the volume with

purified water. Then, six injections of each solutions were performed. The resolutions which described the stability indication of the method were calculated.

2.3.3 Linearity Correlation

After the six standard solutions of ranitidine HCl had been injected, the relationship of peak area ratios of ranitidine HCl to its internal standard versus ranitidine HCl concentrations was evaluated. The correlation coefficient (r) of the regression lihe were determined.

2.3.4 Precision of the Method

2.3.4.1 Within Run Precision

Three sets of the six standard solutions of ranitidine HCl were injected within one day. The coefficients of variation, % CV, of ranitidine HCl to its internal standard peak area ratios from the three sets of standard solutions having the same concentration were calculated.

2.3.4.2 Between Run Precision

Three sets of standard solutions of ranitidine HCl were prepared and injected on different days. The coefficients of variation, % CV, of ranitidine HCl to its internal standard peak area ratios from the three sets of standard solutions having the same concentration were calculated.

2.3.5 Accuracy of the Method

Three sets of standard solutions of ranitidine HCl were prepared and injected. Plots of ranitidine HCl to procaine HCl peak area ratios versus ranitidine HCl concentrations were performed; the slope and intercept were calculated. Then, the percentages of analytical recovery of ranitidine HCl were calculated by dividing the concentrations fitted from the calibration curve by the known concentrations prepared.