# CHAPTER III

# EXPERIMENTAL

## Materials:

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

Ranitidine HCl, Batch No. 5-22974, Glaxo pharmaceutical, Singapore.

Caffeine UN-No. 1544, E. Merck.

Phosphoric acid, E. Merck.

Sodium dihydrogen phosphate, E. Merck.

Sodium monohydrogen phosphate, E. Merck.

Sodium phosphate, E. Merck.

Acetonitrile, Lab scan.

Methanol, E. Merck.

Triethanolamine, E. Merck .

## Equipments:

High Performance Liquid Chromatography (HPLC) equipped with:

a tunable absorbance detector, Water 484 Model M 484, Serial No. 484-PRA 902, USP,

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

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

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

a spherisorb HPLC cartridge column phase separation S5 ODS 2 (250x46 mm) 5μ, Phase Seperations Inc, USA.

pH meter, Model SA 520, Orion, USA.

Analytical balance, Sartorius GMPH, Germany.

UV spectrophotometer, Model U 2,000, Serial No. 0402-040, Hitashi, Germany.

Hot air oven, Memmert$^{R}$, Germany.

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

**Methods:**

**1. Chemical Stability Study of Ranitidine HCl Solutions.**

1.1 Effect of Specific Acid/Base Catalysis.

Phosphate buffers having pH's of 1 to 12 were prepared and their details are presented in Table 1. The ionic strength of all buffer solutions were kept constant at 0.5 using NaCl. Ranitidine HCl was dissolved in the buffer solutions to possess the final concentration of 25 mg/ml. The drug solutions were filled in two-ml amber glass ampules. The ampules were then sealed and stored in a hot air oven at 70$^{0}$C. Four ampules were sampled for analysis of ranitidine HCl remaining at appropriate time intervals. The ampules were stored and analyzed for drug concentration.

Appropriate rate constants were calculated from the data obtained and a corresponding rate-pH profile was performed.

1.2 Effect of General Acid/Base Catalysis.

**Table 1:** Composition of pH 1-12 phosphate buffers at an ionic strength of 0.5.

| pH * | Buffer composition ( in $10^{-2}$ x Molar ) | | | | | measured pH |
|---|---|---|---|---|---|---|
| | $H_3PO_4$ | $NaH_2PO_4$ | $NaHPO_4$ | $NaPO_4$ | NaCl | |
| 1 | 9.09 | 0.91 | - | - | 40.00 | 1.36 |
| 2 | 2.50 | 2.53 | - | - | 45.00 | 2.08 |
| 3 | 0.91 | 9.09 | - | - | 40.00 | 2.70 |
| 4 | 0.39 | 39.61 | - | - | 10.00 | 3.96 |
| 5 | - | 38.96 | 1.04 | - | 7.92 | 4.80 |
| 6 | - | 23.71 | 6.29 | - | 7.42 | 5.89 |
| 7 | - | 5.47 | 14.53 | - | 0.94 | 7.12 |
| 8 | - | 0.58 | 15.42 | - | 32.00 | 7.93 |
| 9 | - | 0.06 | 15.94 | - | 2.12 | 8.93 |
| 10 | - | - | 14.60 | 0.40 | 3.80 | 9.94 |
| 11 | - | - | 10.50 | 2.54 | 0.60 | 11.06 |
| 12 | - | - | 2.62 | 6.38 | 3.86 | 11.95 |

* Calculated by $pH = pK_a + \frac{\log[salt]}{[acid]} - \frac{0.51(2n-1)\mu^{1/2}}{1+\mu^{1/2}}$ at $T = 25^{\circ}C$

where n is the stage of the ionization.

Phosphate buffers having pH's of 4, 5 and 6 and concentrations of 0.10 to 0.40 M were prepared and their details are presented in Table 2. The ionic strength of all buffer solutions were kept constant at 0.5 using NaCl. Ranitidine HCl was dissolved in the buffer solutions to possess the final concentration of 25 mg/ml. The drug solutions were filled in two-ml amber glass ampules. The ampules were then sealed and stored in a hot air oven at $70^{0}$C. Four ampules were sampled for analysis of ranitidine HCl remainings at appropriate time intervals.

Appropriate rate constants were calculated from the data obtained. The relationship between the observed rate constants and the buffer concentration was then evaluated.

1.3 Effect of Ionic Strength.

Phosphate buffers having pH's of 5 and 12 were prepared. Their ionic strengths were adjusted to 0.2, 0.5, 0.8, 1.0, and 1.2 using sodium chloride as shown in Table 3. Ranitidine HCl was dissolved in the buffer solutions and adjusted the volume to the final concentration of 25 mg/ml. The drug solutions were filled in two-ml amber glass ampules. The ampules were sealed and stored in a hot air oven at $70^{0}$C. Four ampules were sampled for analysis of ranitidine HCl remaining at appropriate time intervals.

Appropriate rate constants were determined from the data obtained. The relationship between the observed rate constants and the ionic strength was then evaluated.

1.4 Effect of Solvent Polarity.

**Table 2:** Composition of pH 4 ,5 and 6 phosphate buffers having total concentrations of 0.1- 0.4 M.

| pH * | Buffer composition ($10^2$ x molar) | | | | | measured |
|---|---|---|---|---|---|---|
| | $HPO_3$ 4 | $NaHPO_2$ 4 | $NaHPO_2$ 4 | total conc | NaCl | pH |
| | 0.10 | 9.90 | -- | 10 | 40.00 | 4.02 |
| 4 | 0.20 | 19.80 | - | 20 | 30.00 | 4.00 |
| | 0.39 | 39.61 | - | 40 | 10.00 | 3.96 |
| | - | 9.72 | 0.26 | 10 | 39.70 | 4.83 |
| 5 | - | 19.50 | 0.52 | 20 | 29.21 | 4.82 |
| | - | 38.96 | 1.04 | 40 | 7.92 | 4.80 |
| | - | 7.90 | 2.10 | 10 | 36.90 | 5.85 |
| 6 | - | 15.80 | 4.20 | 20 | 23.70 | 5.88 |
| | - | 23.71 | 6.29 | 30 | 7.42 | 5.89 |

* Calculated by $pH = pK_a + \frac{\log[salt]}{[acid]} - \frac{0.51(2n-1)\mu^{1/2}}{1+\mu^{1/2}}$ at $T = 25^{\circ}C$

where n is the stage of the ionization.

**Table 3:** Composition of pH 5 and 12 phosphate buffers having ionic strength values of 0.2 , 0.5 ,0.8 , 1.0 and 1.2.

| pH * | Buffer composition (in $10^2$ x molar) | | | | | measured |
|---|---|---|---|---|---|---|
| | ionic strength | $NaHPO_4$ | $NaHPO_4$ | $NaPO_4$ | NaCl | pH |
| 5 | 0.20 | 19.50 | 0.52 | - | - | 5.07 |
| | 0.50 | 19.50 | 0.52 | - | 29.21 | 4.82 |
| | 0.80 | 19.50 | 0.52 | - | 59.00 | 4.71 |
| | 1.00 | 19.50 | 0.52 | - | 79.00 | 4.61 |
| | 1.20 | 19.50 | 0.52 | - | 99.00 | 4.48 |
| 12 | 0.20 | - | 1.20 | 2.80 | - | 11.66 |
| | 0.50 | - | 1.20 | 2.80 | 40.80 | 11.54 |
| | 0.80 | - | 1.20 | 2.80 | 59.60 | 11.41 |
| | 1.00 | - | 1.20 | 2.80 | 79.60 | 11.25 |
| | 1.20 | - | 1.20 | 2.80 | 99.90 | 11.22 |

* Calculated by $pH = pK_a + \frac{\log[salt]}{[acid]} - \frac{0.51(2n-1)\mu^{1/2}}{1+\mu^{1/2}}$ at $T = 25^{\circ}C$

where n is the stage of the ionization.

Five methanol-water mixtures containing 20, 40, 60, 80 and 100% w/w methanol were prepared. Ranitidine HCl was dissolved in the cosolvents and adjusted the volume to the final concentration of 25 mg/ml. The drug solutions were filled in two-ml amber glass ampules. The ampules were sealed and stored in a hot air oven at 70 $^{0}$C. Four ampules were sampled for analysis of ranitidine HCl remaining at appropriate time intervals.

Appropriate rate constants were determined from the data obtained. The relationship between the observed rate constants and dielectric constant values was then evaluated.

**2. HPLC Assay.**

2.1 HPLC Conditions for Ranitidine HCl Analysis.

The high pressure liquid chromatography (HPLC) technique was used for analysis of ranitidine HCl. The system consisted of a constant flow pump, a variable wavelenght UV detector, an integrator and a fixed volume sample injector with a 20 microliter loop. The appropriate conditions for analyzing Ranitidine HCl remaining by HPLC tednique are the followings.

| | |
|---|---|
| Column | : spherisorb S5 ODS2 (250x4.6 mm), 5 micron. |
| Mobile phase | : a mixture of 13% v/v acetonitrile and 87% v/v pH 6.2, 0.02 M sodium dihydrogen phosphate buffer. |
| Detector wavelength | : 262 nm. |
| Flow rate | : 1.5 ml/min. |
| Attenuation | : 4. |
| Chart speed | : 0.1 cm/min. |

| | |
|---|---|
| Injection volume | : 20 microliter. |
| Internal standard | : 0.4 mcg/mlcaffeine. |
| Retention time | : Ranitidine HCl, 12.45-13.65 min. |
| | : Caffeine, 5.41-6.50 min. |

The pH 6.2, 0.02 M sodium dihydrogen phosphate buffer was prepared by weighing 2.76 grams of anhydrous sodium dihydrogen phosphate in 1,000 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 ten milligrams of caffeine in a 100-ml volumetric flask. Purified water was added. The solution was swirled until caffeine dissolved completely. The final volume was then adjusted.

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 dissolved the drug and adjusted the volume.

Standard solutions were prepared by pippetting 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, 0.4, 0.2, 0.1, 0.1, 0.2 and 0.2 ml of the caffeine stock solution were added to the 100, 50, 25, 25, 50 and 50 ml volumetric flasks, respectively. The solutions were adjusted to volume with purified water so that the final concentrations of ranitidine HCl were 4, 8, 16, 32, 24 and 40 mcg/ml, respectively and that of caffeine was 0.4 mcg/ml.

## 2.3 Preparation of Sample Solutions for HPLC Analysis.

The stored ampules were placed at room temperature until the drug solutions were cool. Ten microliters of the drug solution were then pipetted using a micropipet and transferred to 10-ml volumetric flasks containing 40 microliters of 100 mcg/ml internal standard. The solutions were adjusted to volume with purified water and ready for HPLC analysis.