CHAPTER III

EXPERIMENTAL SECTION

Materials.

All chemicals were analytical and/or pharmaceutical grade and they were used as received.

Piroxicam, Siam Pharmaceutical Co.,Ltd, Batch No: 90 280.

Nicotinamide Powder, BP 1980, USA, Lot No. NA 01/2, Srichand-United Dispensary Ltd. Partnership Thailand.

Citric Acid AR., Lot No 604 K 1739444, Merck, Germany.

Sodium Hydrogen Phosphate, Lot No. 505 M., Merck, Germany.

Potassium Dihydrogen Phosphate, Lot No. 180A 56408, Merck, Germany.

Propylene Glycol, Lot No. Pl 0612, Srichand United Dispensary Co, Ltd., Thailand.

N, N Dimethylacetamide, Analysis No. 297169 390, Fluka Chemika Switzerland.

Ethyl Lactate, Analysis No. 304564 191, Fluka Chemika Switzerland.

Dimethyl Formamide, Lot No. 2447045, Merck Germany.

Tenoxicam, Siam Pharmaceutical Co.,Ltd., Batch no:91 380.

Polyethyleneglycol 400, Lot No. PE 0562, Srichand United Dispensary Co, Ltd, Thailand.

Innovator's product (Feldene^{R}) Lot No : 180099, Pfizer GmbH, West Germany.

Equipments.

Analytical Balance, H 51 AR, Mettler.

Spectrophotometer, Spectronic 2000, Baush & Lomb, USA

Shaker, Edmund Buhler, Germany.

High Performance Liquid Chromatography (HPLC) equipped with

an absorbance detector, FLD-1, Shimadzu

an injector, Rheodyne injector

a constant flow pump, LC-3A, Shimadzu

an integrator, C-RIA, Shimadzu

a Lychro CART^{R} C 18 column (4 mm. ID x 12.5 cm.)., E. Merck.

Methods.

1. Analytical procedures of piroxicam in solution.

1.1 Calibration curve (UV spectrophotometer)

Solutions containing known amount of piroxicam (1, 2, 4, 6, 8, 9, 10 and 12 ug/ml) in 0.01 M methanolic hydrochloric acid were prepared and analyzed using the UV spectrophotometer at a wavelength of 334 nm. (Zazhi, 1989). Absorbances obtained versus known concentrations were fitted to a straight line using linear regression.

1.2 Calibration curve (high performance liquid chromatography)

Solutions containing known amount of piroxican (10.4, 20.8, 31.2, 52.0, 72.8, 93.6 and 104 ug/ml) and internal standard tenoxicam (25 ug/ml) in 0.01 M methanolic hydrochloric acid were prepared and analyzed using high performance liquid chromatography which was composed of a constant flow pump (Shimadzu, LC-3A), a varible wavelength UV absorption detector (Shimadzu, FLD-1), and an integrator (Shimadzu, C-R1A). Injections were made with a 20 u1 constant-volume injector valve. The chromatograph was operated at a flow rate of 1 ml./min and the eluent was monitored spectrophotometrically at 361 nm. The column was Lychro CART^{R} C 18 column (4 nm. ID x 12.5

cm.). The mobile phase was a mixture of 40% v/v methanol and 60% v/v 0.085 M monopotassium phosphate buffer (pH 5.6). The peak area ratio of piroxicam to that of internal standard obtained versus known concentration were fitted to a straight line using linear regression.

2. Solubility of piroxicam

In order to formulate piroxicam injection in the solution dosage form and since piroxicam could not soluble in water, so the study of piroxicam in cosolvent and complexing agent was performed to increase its solubility as the method following.

2.1 Solubility of piroxicam using cosolvents

Binary solvent mixtures were prepared by mixing water and non aqueous solvents. Non agueous solvents selected were dimethylformamide, propylene glycol, polyethylene glycol 400, N, N-dimethylacetamide, ethyl lactate. (Spiegel and Noseworthy, 1963) The composition of each solvent in the mixture was varied from 10-90% v/v

Solubilities of piroxicam were determined by equilibrating excess drug with the pure solvent and/or a mixture in 20 ml screw-capped glass tubes which were rotated at 120 rpm. in a constant temperature water bath maintained at 30 $\pm$ 0.5°C. The tubes were equilibrated for a period of 48 hours. This time was adequate enough to ensure

saturation. After equilibrium was obtained, the tubes were removed. The supernatants were filtered through 0.45 um filter paper and the filtrates were analyzed for piroxicam content by uv spectrophotometer.

2.2 Solubility of piroxicam using complexing agent.

Solutions containing nicotinamide with concentration of 50, 100, 150, 200, 250, 300, 350 and 450 mg/ml. were prepared. Solubilities were determined by equilibrating excess drug with the solution in 20 ml screw-capped tubes which were rotated at 120 r.p.m. in a constant temperature water bath maintained at 30 $\pm$ 0.5°C. The tubes were equilibrated for 48 hours to ensure saturation. After equilibrium was obtained. The tubes were removed and the supernatant were filtered through 0.45 um filter paper. The filtrates were then analyzed for piroxicam content using HPLC.

2.3 Solubility of piroxicam in cosolvents and complexing agent.

Three concentrations of nicotinamide (150, 200 and 250 mg/ml) solution in binary solvents (propylene glycol and water) were prepared by varying the composition of propylene glycol from 20-60% v/v.

Solubility of piroxicam in these solutions

were determined by equilibrating excess drug with the solutions and repeating the method as described in items 2.1 and 2.2. piroxicam content was analyzed by HPLC.

2.4 Solubility of piroxicam in cosolvents and complexing agent in various pHs of buffer systems

Solution of 250 mg/ml nicotinamide in binary solvents (40% propylene glycol) at pH ranged from 5-8 were prepared. The two buffer systems selected were phosphate buffer and Mc Ilvaine buffer. They were adjusted to pH required by using pH-meter.

Excess quantities of piroxicam were then equilibrated following the same method as described before as items 2.1 and 2.2. The samples were analyzed for piroxicam content by HPLC.

3. Formulation

3.1 Eight solutions of piroxicam were prepared and filled in ampoules of 2-ml size using the formula.

Rx

Piroxicam 40 mg
Cosolvent qs
Nicotinamide qs
Additives qs
Water for injection qs to 2 ml

Each formula was made up using ingredient as listed in Table 4.

Table 4 : Some additives added in piroxicam solutions.

Rx / Ingredient	1	2	3	4	5	6	7	8
Piroxicam	+	+	+	+	+	+	+	+
Nicotinamide	+	+	+	+	+	+	+	+
Cosolvent	+	+	+	+	+	+	+	+
Buffer pH 7	–	–	–	–	+	+	+	+
Buffer pH 7.5	+	+	+	+	–	–	–	–
Antioxidant[a]	–	+	–	+	–	–	+	+
Antimicrobial[b]	–	–	+	+	–	+	–	+

[a] Antioxidant = Sodium sulfite 0.15%

[b] Antimicrobial = Benzyl alcohol 2%

All solutions were steriled by filtering through 0.22 um filters. The headspace of the ampoules were filled with nitrogen gas prior to sealing. Leak test was done by vacuum method.

3.2 Analysis of active ingredient in the formulation.

A 0.025 ml of formulated piroxicam injection and equal quantity of an innovated product were transfered to a 10 ml volumetric flask and brought up to the final volume with 0.01 M methanolic hydrochloric acid with internal standard (Tenoxicam 25 ug/ml). The solutions were then analyzed using HPLC following the same condition as item 1.2. The concentration of piroxicam were quantified utilizing the calibration curve. The percent labeled amount of piroxicam in the formulation was then calculated using an equation;

$$\% \text{ Label Amount} = \frac{\text{actually conc.} \times 100}{\text{labelling conc.}}$$

4. Stability study.

Samples of all formulated formulas from item 3 and sample from an innovator's product were placed in incubators maintained at 45°C, 55°C,and 65°C and the samples were placed at refrigerated temperature and room temperature for about 2 months. All samples were then observed as follows :

4.1 Observation on physical changes.

Changes of physical appearances of piroxicam injection were observed as follows.

4.1.1 pH. The pH of both the formulated formulas and innovator's product which were kept at room temperature were measured using pH meter at days 7, 14, 21, 28, 35, 42, 49, 56 and 63.

4.1.2 Crystal formation. Samples of both the formulated formulas and innovator's product which were kept at room temperature or refrigerated temperature were inspected visually for the crystal formation at days 7, 14, 21, 28 35, 42, 49, 56 and 63.

4.1.3 Color. All samples stored at room temperature were inspected visually for color changed over the time of study.

4.2 Chemical stability.

4.2.1 Analysis of the remain amount of piroxicamin injection.

A 0.025 ml of the formulated piroxicam injection and equal quantities of an innovator's product, keeping at 45°C, 55°C, 65°C and room temperature, were transferred to a 10 ml volumetric flask, and diluted to

volume with 0.01 M methanolic hydrochloric acid with internal standard (Tenoxicam 25 ug/ml). The solutions were analyzed using HPLC following the procedure described in item 1.2 at days 0, 7, 14, 21, 35, 56 and 63. The concentrations of piroxicam remained in injection were quantified utilizing the calibration curve.

4.2.2 The data of the concentration of remained piroxicam versus time was calculated based on zero order and first order kinetic reaction. The log of rate constants obtained in various temperature of each formula was plotted against 1/T (Arrhenius plot). The shelf-lives were calculated using the rate constants obtained from the Arrhenius plot.

5. Evaluation.

The shelf-lives from various formulas of each pH were evaluated by using one-way ANOVA to select the best formula. The statistic t-test was then used to compare for difference between the best formula from pH 7 and that from pH 7.5. Finally, the shelf-lives of the best formulated formula and innovator's product were compared.