CHAPTER III

RESULTS AND DISCUSSION

Beer's Law Plot of Paracetamol Solution

For quantitative analysis, the linearity of absorbance with concentration is one of the requirements. Since the absorbance is proportional to the amount of drugs, then the relationship between absorbance and concentration of drugs should be adherence to Beer's law. Under the suitable conditions, the linearity of absorbance with the concentration of paracetamol was found. The calibration curve was prepared by ploting absorbance versus concentration of paracetamol, of the final solution as shown in Table 3 and Figure 9. The curve showed a good correlation (r=1.0). The result USP method obeyed Beer's Law.

Determination Amount of Intact Paracetamol in Commercially Available Liquid Paracetamol Preparations in Thailand By USP Method.

For studying stability of commercial paracetamol products, the USP method was used to determine percent amount of intact paracetamol in product. The results were shown in Tables 5-10.

The accelerated stability testing method has been introduced to shorten duration of degradation by increasing temperature. This technique is based an Arrhenius relationship (Eq.11) where the degradation rate is dependent on temperature. The incubated paracetamol products at 70°, 60°, 55°, and 40°C, gradually changed their colour from yellow to brown between investigating stability.

At 70°C colour of paracetamol products changed fastest in contrast to its slow change at 40°C. The experimental data of percent paracetamol remaining at any time and at difference temperature was concerned that the USP method for paracetamol in that dosage form is not appropriate for stability indicating paracetamol product. Because percent amount of intact paracetamol in products should be decreased when they stored more duration. But the increased amount of remaining paracetamol in some interval times was happened. Therefore, something might interfere USP method.

Effect of Colour of Incubated Syrup on Determinating of Amount Remaining Paracetamol in Liquid Preparation by USP Method.

The official USP analytical method of paracetamol suspension was recommended for determining content of paracetamol. Even though there was no comment about this method. Then the comparison of this method for analysing of paracetamol which used incubated syrup and refrigerated syrup as vehicles was studied. The results were shown in Table 4.

The mean percentage value for paracetamol in undecomposed syrup preparation was 99.25% with 0.11% of coefficient of variation by official USP method and for paracetamol in decomposed syrup preparation was 104.19% with 0.17% of coefficient of variation by the same method. Therefore, this method is not appropriate for stability indicating paracetamol product. Colour change of paracetamol product caused from syrup, vehicle, suspending agent, etc. when product had been stored for a long time or stored at high temperature, affected the accuracy of USP method.

Determination of the Linearity of the Ratio of Peak Height Paracetamol Divided the Peak Height of Internal Standard (Sulpyrin) with Concentration of Paracetamol Solution.

Under the suitable condition of HPLC method, the linearity of the ratio of the peak height with concentration of paracetamol was found. The calibration curve was prepared by ploting ratio of the peak height versus concentration of paracetamol of the final solution, as shown in Table 11 and Figure 10. The plot showed good linearity over the desired concentration range (r = 1.0). Therefore HPLC method obeyed to Beer's Law.

Determination Remaining Paracetamol Amount in Available Commercial Liquid Paracetamol Preparations in Thailand by HPLC Method.

Optimization of resolution of paracetamol and its degradation product (p-aminophenol) in paracetamol preparation can be done by variation in solvent composition combinding absolute methanol with changing concentrations of phosphate solution and pH of phosphate buffer solution. The stationary phase was limited to octylsilane chemically bonded to a porous silica support. So that the proposed method used absolute methanol 15% and phosphate buffer solution pH 4.0 85% as mobile phase and solvent for dissolving paracetamol and sulpyrin. UV-spectrum of paracetamol was shown in Figure 11. It had maximum absorption at wavelenght 250, 280 nm. The HPLC method used UV-detector equipped with optical path at 254 nm. If quantitative of p-aminophenol needed to be analysed, wavelength of optical path should be used at 280 nm because of its higher sensitive. UV-spectrum of p-aminophenol was shown in Figure 11.

The typical chromatograms of the HPLP method was shown in Figure 12. P-aminophenol, paracetamol and sulpyrin had retention times 1.4, 2.1 and 3.6 minutes respectively. The peak height of each peak on the chromatogram was determined with electronic integrator. The ratio of paracetamol peak height to the peak height of internal standard (sulpyrin) was calculated for each chromatogram. Since ratio of paracetamol peak height were directly proportional to concentration, amount of remaining paracetamol in commerical paracetamol products were determined. The results were shown in Tables 12-17.

Determination of Specific Rate Constant of Commercially Available Liquid Paracetamol Preparations Degradation and Activation Energy.

Concentration (or ln concentration), time plot for samples paracetamol were shown in Figures 13-18.

The specific rate constant (K) of paracetamol degradation in liquid preparation was obtained from slope of the linear regression line of the concentration-time profile for suspension formula, and slope of the linear regression time of the natural logarithm concentration-time profile for syrup and elixir formulas. The 95% confidence limit of the slope was also calculated as shown in Tables 18-23 for each sample. The specific rate constants (K) were used to indicate the stability of paracetamol in the investigated commercial products. The products, which specific rate constant was lower, was more stable.

In this study the actual normal degradation rate of products at room temperature (average 31°C) wasn't calculated. Because it will take a long time for investigating accurate rate of products.

Instead, stability of products at room temperature were predicted from the Arrhenius relationship. The predicted degradation rate at room temperature (20°, 25° ,30°, and 35°C) were obtained from extrapolation of Arrhenius plot. The degradation rate was considered the lower value as being the more stable. The result indicated that paracetamol Suspension #1 (pH 5.50) was stable more than paracetamol Suspension #2 (pH 4.60). The stability of products decreased in the following pattern : paracetamol Elixir #1 (pH 3.60) > paracetamol Syrup #1 (pH 4.88) > paracetamol Syrup #2 (pH 6.00) > paracetamol Elixir #2 (pH 3.00) at 30°C.

The commercial paracetamol products investigated in this study had difference pH and viscosity. From the previous study, the maximum stability of paracetamol solution occured at about pH 6. And the hydrolysis of paracetamol was minimum in the pH range 5 to 7. However pH was not the most important stabilizer. Theoretically, paracetamol Syrup #2 with pH 6.00 should be the most stable product. But from this study paracetamol Syrup #1 with pH 4.88 was more stable than paracetamol Syrup #2. Therefore, the other pharmaceutical adjuvance included vehicle had influenced on the stability of paracetamol.

The Arrhenius plots of commercial products investigated preparation were shown in Figures 19-24. Their activation energies were calculated and presented in Table 24. All values were in the range 11-17 kcal $mole^{-1}$.

The studied commercial products had difference activation energy. It would suggest that pH and adjuvance in preparation had affected on the heat of activation. The heat of activation is useful for estimation the reaction rate constant at any temperatures

from rate obtained at one elevated temperature (Eq.11). This method will provide some advantage in reducing the number of experiments required for prediction of product stability if substance in preparation is similar. It is also useful in routine quality control to assure constant batch-to-batch products.

Determination of Shelf-Lives of Commercially Available Liquid Paracetamol Preparations.

The interpretation of E_a of any commercial products investigated from Arrhenius plot, and the Eq.10 were used to be calculated for specific rate constant at room temperature (20°, 25°, 30°, and 35°C). The specific rate constant were presented in Table 26.

From Eq.14, the shelf-life of paracetamol suspension could be calculated. (D_o = 100%)

From Eq.15, the shelf-lives of paracetamol syrup and elixir could be calculated.

Therefore, all shelf-lives of paracetamol samples are investigated by predictation which were shown in Table 26.

The shelf-lives (t_{90}) of paracetamol in all products calculated from predicted rate. The shelf-life at 20°C was the longest and the shelf-life at 35°C was the shortest, paracetamol Suspension #1 had the longest shelf-life while paracetamol Elixir #2 had the shortest shelf-life.

The shelf-lives of products at 30°C, listed in decreasing order, were as following: paracetamol Suspension #1 > paracetamol Suspension #2 > paracetamol Elixir #1 > paracetamol Syrup #1 > paracetamol Syrup #2 > paracetamol Elixir #2 at temperature 30°C.

From the calculation, shelf-life at 30°C, paracetamol Elixir #2 had the shelf-life of 1.3 years, while paracetamol Suspension #1 and paracetamol Syrup #1 have the longest shelf-life of 3.5 years. It could be concluded that shelf-lives of liquid paracetamol preparations available in Thailand are obviously distinct. Consumers may misunderstand that drug should be expired 5 years after manufactured date which may not cause problem of consuming expired drug. Consequently, it is necessary to clearly specific expiration date labelled on each package. From this experiment, paracetamol Elixir #2 has the shortest shelf-life. Furthermore, paracetamol preparation should have expiration date indicated on its package. In addition, expiration date should be clearly specified for the stock management.

Determination Percent Labelled Amount of Paracetamol in Commercially Available Paracetamol Tablet in Thailand by HPLC Method

Table 27 was result from single 500 mg paracetamol tablet analysed. These values represented the mean of at least triplicate injection per sample. The overall single tablets variation were from 100.00% to 100.97% paracetamol with average value of 100.49% for product A and from 98.73% to 99.27% paracetamol with average value of 99.00% for product B. The result obtained from this method was accurate precise.

So that HPLC method also represents a quick and simple means of determination paracetamol in paracetamol tablets.