CHAPTER I

INTRODUCTION

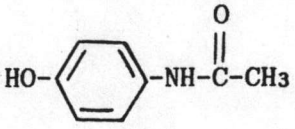
Numerous compound which are important from a pharmaceutical standpoint contain an amide function,yet there have been few quantitative studies concerning their hydrolytic stability. Paracetamol is a widely used analgesic and antipyretic drug with gentle gastrointestinal properties. As a result of its rapid success and popularity as an alternative medication, many preparation of paracetamol, both alone (tablet, syrup etc.) and combinations have received considerable attention.

Although there are many locally manufactured products of paracetamol in Thailand , the stability aspect of these preparations have not been studied. This information would be essential for the calculation of shelf-life for these preparations.

Chemistry

Paracetamol (Acetaminophen, N-acetyl-p-aminophenol)

Chemical structure (Scheme 1)



Molecular weight 151.16

(Scheme 1)

Paracetamol was first synthesised by Morse in 1878 (2). It was first used in medicine by Von Mering in 1893. Paracetamol has analgesic and antipyretic effects similar to that significantly from those of aspirin. Paracetamol reduces analgesic by elevation of the pain threshold; the site and mechanism of the analgesic effect is unclear. Paracetamol reduces fever by a direct action on the hypothalamic heat-regulating centers, resulting in increased dissipation of body heat (via vasodilation and sweating). Inhibition of the action of endogenous pyrogen on heat regulating centers is nearly as potent as aspirin in inhibiting prostaglandin synthetase in the CNS but the ability of paracetamol to inhibit prostaglandin synthetase peripherally is minimal. This may account for drug's lack of clinically significant antiinflammatory or antirheumatic effects. Generally, the antipyretic and analgesic effects of paracetamol and aspirin are comparable. Aspirin is cleary superior to paracetamol for treating pain of inflammatory origin. Paracetamol does not inhibit platelet aggregation, affect prothrombin response or produce G.I. ulceration.

Paracetamol is rapidly absorbed after oral administration with peak level obtain within 30-60 minutes, and the half-life in plasma is 2 to 4 hours. Usual analgesic doses produce total serum concentration of 5 to 20 mcg/ml. Variations in the amount of paracetamol absorbed occur depending on the vehicle used and the route of administration. Following therapeutic doses, 90 to 100% of the drug may be recovered in the urine within the first day. However, practically no paracetamol is excreted unchanged, and the bulk is excreted after hepatic conjugation with glucuronic acid (about 60%), sulfuric acid (about 35%) or cysteine (about 3%). The

plasma half-life of paracetamol is considered to be the best indicator of serious toxicity i.e. liver damage. The conventional oral dosage is 325 to 650 mg every 4 hours for adults and older children. For young children, the single dose is 60 to 120 mg, depending upon age and weight; total daily dosage should not exceed 1.2 g. Paracetamol should not be administrated for more than 10 days or to young children except upon advice of a physician (3, 4).

Paracetamol is a weak acid. Its saturated aqueous solution has a pH of 5.3 to 6.5 at 25°C (5). The pKa values have been quoted between 9.0 and 9.5 (6). It's solubility

1 : 70 water

1 : 7 alcohol

1 : 50 chloroform

1 : 9 propylene glycol

1 : 20 boiling water

1 : 13 acetone

1 : 40 glycerol

insoluble in ether

Paracetamol is soluble in alkaline hydroxide solutions (7).

The ultraviolet spectra of paracetamol in water, acidified water, neutral methanol, acidified methanol and methanol containing sodium hydroxide were obtained using Cary, 219 spectrophotometer and were shown in Figures 1 and 2.

The infrared spectrum of paracetamol in KBr disc was recorded on a Perkin-Elmer Spectrophotometer model 580B,was shown in Figure 3. And The ^{1}H-NMR spectrum of paracetamol was shown in Figure 4 (It was dissolved in acetone-d_2. Also TMS was the internal standard). Figures 5 and 6 represented ^{13}C Nuclear Magnetic

Resonance (^{13}C NMR) Spectra of paracetamol.

Stability of Paracetamol

- Stability of Solid Paracetamol to Heat

Dry, pure paracetamol is very stable at temperature up to at least 45°C. When paracetamol is exposed to humid conditions such that hydrolysis to p-aminophenol takes place, then further oxidative degradation of the p-aminophenol occurs characterised by a gradual colour change through pink to brown and eventually to black (8). This involves the breakdown of the p-aminophenol to quinonimine and related compounds (9).

- Stability of Solutions of Paracetamol

Paracetamol is slightly light sensitive in solution (7) and may degrade by a mechanism involving pre-dissociation of the N-C bond as in the case of acetanilide (10, 11).

The degradation of paracetamol in aqueous solution appears to be both acid base catalysed reactions (1). It is first order with respect to the concentration of paracetamol and first order with respect to the hydrogen and hydroxyl ion concentration. Since spon-taneous hydrolysis was found to be negligible, the rate equation may be written as

$$\text{rate} = K_{H^+}(H^+)(\text{NAPAP}) + K_{OH^-}(OH^-)(\text{NAPAP}) \qquad \text{(Eq. 1)}$$

where K_{H^+} = rate constant for the specific acid catalysed

K_{OH^-} = rate constant for the specific base catalysed

(NAPAP) = concentration of paracetamol

If the reaction is carried out in an acidic solution, hydrogen ions are then the only effective catalyst and the rate equation is reduced to

$$\text{rate} = K_{H^+}(H^+)^n(\text{NAPAP})^m \qquad \text{(Eq. 2)}$$

where n = the order with respect to hydrogen ion

m = the order with respect to paracetamol

Similarly in an alkaline solution.

$$\text{rate} = K_{OH^-}(OH)^n(\text{NAPAP})^m \qquad \text{(Eq. 3)}$$

where n = the order with respect to hydroxide ion

Since hydrogen and hydroxyl ions concentration were maintained constant, equation 2 and 3 may be written as

$$\text{rate} = K'_{H^+}(\text{NAPAP})^m \qquad \text{(Eq. 4)}$$

and

$$\text{rate} = K'_{OH^-}(\text{NAPAP})^m \qquad \text{(Eq. 5)}$$

where

$$K'_{H^+} = K_{H^+}(H^+)^n \qquad \text{(Eq. 6)}$$

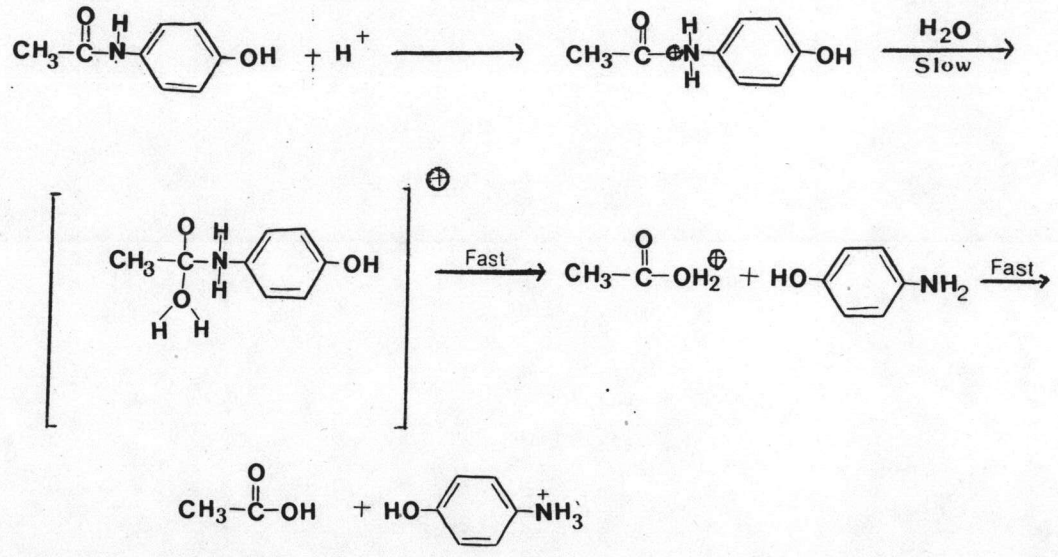
and

$$K'_{OH^-} = K_{OH^-}(OH^-)^n \qquad \text{(Eq. 7)}$$

At constant pH the reaction is pseudo first-order, with the apparent first-order rate constant (K') (1).

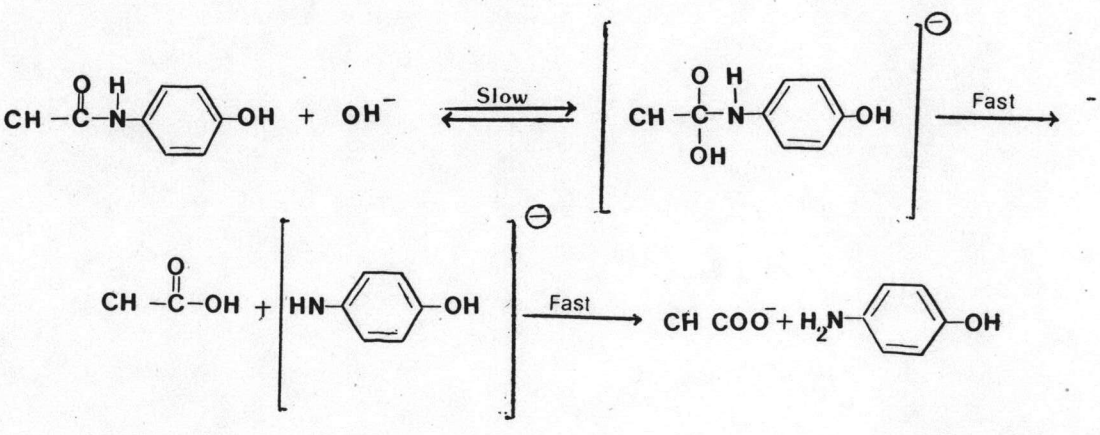
The Mechanism of Hydrolysis (1)

The acid hydrolysis takes the path (Scheme 2)



(Scheme 2)

The basic hydrolysis follows the path (Scheme 3)



(Scheme 3)

pH - Rate Profile .

Figure 7 is the logarithm of specific rate constant (K), pH plot for the hydrolysis of paracetamol at 25°C (1).

This pH-rate profile shows specific acid and specific base catalysis. The hydrolysis of paracetamol is minimum in the pH range of 5 to 7. The maximum stability occurs at about pH6. At pH6 and 25°C, the rate constant is 1.005×10^{-9} S^{-1} corresponding to a half-life of 21.8 years (1).

Analytical Method Involved in Quantitative Determination of Paracetamol

The wide spread use of paracetamol as an antipyretic and analgesic agent have created a demand for a rapidly reliable method for its determination. Various methods have been employed for the quantitative determination of drugs which contained paracetamol but the accuracy of these methods have always been in question. These methods are described below.

1. Gravimetric Method

Poethke and Kohne (12) described the quantitative precipitation of paracetamol with 1-fluoro-2,4-dinitrobenzene in a sodium p-(2, 4-dinitrophenoxy) acetanilide. This method gave good precision but it took a long time for precipitation and it was not sufficiently sensitive.

2. Titrating Method

Inamdar, Sabos, Kamdar and Sanghavi (13) determined paracetamol by hydrolysis of paracetamol with dilute hydrochloric acid then titrating with 0.1 M sodium nitrite until the first permanent grayish blue appeared using mix indicator (1:1 of methylene

blue (0.15%) and tropeoline-00 (0.1%)). This method is simple but it used hydrolysis to p-aminophenol with hydrochloric acid and heat before another steps. Therefore free p-aminophenol (hydrolysed form) consumed nitrous acid too.

Paracetamol may be determined by titrating with 0.1 N $Ce(SO_4)_2$ in ethanolic hydrochloric medium by employing Ce^{4+} to quantitative oxidised paracetamol (14, 15).

According to Thai Pharmacopoeia paracetamol was analysed by being hydrolysed with diluted sulfuric acid and boiled under a reflux condenser for 1 hour. Following by titration of titrate solution mixture of paracetamol hydrolysed form, ice, 2M. hydrochloric acid and ferroin sulfate TS. with 0.1 M ammonium cerium (IV) sulfate VS until a yellow colour is obtained (16).

Laurent (17) also used dimethylformamide solution to titrat paracetamol visually to a thymol blue end point employing 0.1 N Me_4NOH (in benzene methanol titrant).

Agarwal and Walash (18) described non-aqueous titration for analysis of the mixture of salicylamide and paracetamol. Drugs mixture were dissolved in dimethylformamide and titrated potentiometrically with 0.1 N lithium methoxide (in benzene methanol). A similar method can use 0.1 N sodium methoxide (in benzene-methanol) as a titrant (19). However these methods are not specific.

3. Polarographic Method

Brockelt (20) described a cathode polarographic method for the determination of paracetamol after nitration with 5 N Nitric acid. The solution containing nitrated paracetamol is treated with

potassium hydroxide and phosphoric acid to give a solution pH of 5.8 and examined polarographically ($E^1/_2$ Versus S.C.E.-0.38 V.).

Sheare *et al* (21) found that with the use of a glassy carbon electrode, paracetamol could be determined polarographically with a peak potential of about +0.5 Volts. versus S.C.E. This method is capable of selective determination paracetamol in the presense of p-aminophenol ($E^1/_2$ versus S.C.E.+0.2 V.) and thus may be used as a stability-indicating assay. The water content of the acetate-acetic acid methanol supporting electrolyte significantly alters the measured peak current for a given concentration of paracetamol and thus this method has to be limited.

4. U.V. Spectrophotometric Method

According to British Pharmacopoeia method, paracetamol tablets were determined by treating paracetamol with sodium hydroxide solution to form phenolate then measure its extinction at the λ_{max} about 275 nm and use E = 715 for calculate the content of paracetamol (22). The other method, USP, paracetamol in pure form and effervescent oral tablet were determined by measuring absorbance at λ_{max} 244, 243 nm, respectively (23). However these methods are accirate for determined paracetamol alone.

Elsayed and co-worker (24) described a differential spectrophotometric method (Δ A method) and Glenn's method of orthogonal function (P_2 method) for the assay of paracetamol in many preparations. P_2 method, Δ A method can determine concentration of an unknown solution of paracetamol from the use of the least squares method :

$$A = -0.0031 + 42.28\ C$$

where A = absorbance of alkaline solution at 276 nm, using aqueous solution of same concentration as blank

C = concentration of paracetamol

ΔA method measure absorbance of alkaline solution at 276 nm. The P_2 method use equation.

$$P_2 = [(+5)A_{240} + (-1)\ A_{242} + (-4)A_{244} + (-4)A_{246} + (-1)A_{248} + (+5)A_{250}]/84$$

and $$P_2 \times 10^3 = -0.0137 - 323.7\ C^*$$

where P_2 = coefficient

A_{240}, A_{242},..., and A_{250} = absorbance of paracetamol at wavelength 240,242,..., and 250, respectively

C^* = concentration of paracetamol

( ) = the figures between brackets are given in the standard works on numerical analysis

84 = the normalizing factor.

These method, interference from excipeints in the formulations is thereby avoided. Accuracy of the analysis is greater than with the British Pharmacopoeiae method.

Korany, Bedair, Mahgoub and Elsayed (25) analysed solutions of paracetamol in 0.1 N hydrochloric acid by measuring their second derivative spectral response at 295 nm where the degradation products did not interfere (see Figure 8). They claimed that this method is a rapid and accurate method for determination of paracetamol and phenacetin in the presence of their degradation products. This method can be used for assessing the stability of

paracetamol. However this method requires special instrument, derivative UV spectrometry.

Shane and Kowblansky (26) determined acetylsalicylic acid,salicylamide, paracetamol and caffeine in tablets or powders by independent methods. They analysed paracetamol in preparations by UV differential spectrophotometry. Using the buffer pH 6 solution as the reference, and the solution,pH 10 in the sample position, absorbance readings of 263.5 nm.

Paracetamol has been determined spectrophotometrically in mixtures of other drug substances by several procedure involving preliminary ion-exchange or partition chromatographic separation of paracetamol.

5. Colourimetric Method

The majority of the published colourimetric methods for the determination of paracetamol are based on one of three systems. These are nitration, oxidation ,hydrolysis to p-aminophenol followed by diazotisation and phenolic coupling.

5.1 Nitration

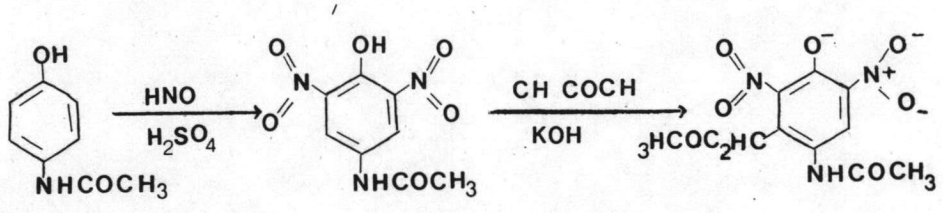
Le Perdriel *et al* (27) determined paracetamol by nitrating paracetamol with sodium nitrite and dilute hydrochloric acid to yeild 2-nitro-4-acetamidophenol. They made the final solution alkaline with sodium carbonate solution before measuring absorption at λ_{max} between 440-445 nm.

Chafetz *et al* (28,29) quantitatively determined paracetamol as 2-nitro-4-acetamidophenol, formed by the reaction of nitrous acid (10% sodium nitrite and 6 N Hydrochloric acid) and destroyed excess nitrous acid by adding 15% sulfamic acid. They

determined the absorption at maximum absorbance of wavelenght,430 nm. In this method, they found no interference from p-aminophenol.

The official method, USP XXI described quantitative analysis of paracetamol oral suspension by nitrating paracetamol with (10% sodium nitrite and 6 N hydrochloric acid) and destroying excess nitrous acid by 15% ammonium sulfamate solution. Determined the absorbance of this alkaline solution (by adding 2.5 N sodium hydroxide) at λ_{max} at 430 nm (23).

El Khur *et al* (30) determined paracetamol by nitration and subsequent complexation reaction with nitric acid and sulfuric acid. They treated aliquot of solution with acetone and potassium hydroxide solution. The absorbance of the resulting colour was measured at 355 nm (Scheme 4).



(Scheme 4)

They claimed that this method was sufficiently accurate and precise. The method was fairly sensitive and selective of good reliability owing to the stability of the complex.

5.2 Nitrosation

Inamdar and Kaji (31) utilised the nitrosation of paracetamol for its determination. Paracetamol was dissolved in methanol and treated with 10% hydrochloric acid and 10% sodium

nitrite. A finally stable yellowish derivative was measured at 395 nm.

Belal *et al* (32) determined paracetamol through nitrosation and subsequent chelation; in order to increase the selectivity of the nitrosation reaction. Paracetamol was treated with 3% sodium nitrite solution and 1 N hydrochloric acid. And susequent chelation by adding copper (II) acetate or cobolt (II) bromide, while the latter must be extract with chloroform before measuring absorption. The λ_{max} were 530 and 400 nm respectively.

5.3 Oxidation or Hydrolysis to p-aminophenol Followed by phenolic Coupling.

Sanghavi and Vishwasrao (33) described the determination of paracetamol, based on formation, after hydrolysis of paracetamol with hydrochloric acid and heat, of a complex with p-dimethylaminobenzaldehyde. This complex had maximum absorption at 444 nm.

Inamar, Gore and Bhide (34) hydrolysed paracetamol with dilute hydrochloric acid to p-aminophenol, and subsequence diazotised with sodium nitrite. N-1-naphthyl ethylenediamine dihydrochloride was added to form colour product with it. The colouring product had maximum absorption at 575 nm.

Joseph ,Vaughan (35) utilized reaction between vanilline and aromatic amine, which produced stable yellow solutions of azomethine compound in determining of paracetamol. First paracetamol was hydrolysed with 1 N hydrochloric acid, 10 minutes in boiling water. When vanillin was added to hydrolytic product, p-aminophenol, a stable intense yellow colour is produced, which

shows a permanent absorbance peak near 395 nm. This is not simplified method. Time and temperature of hydrolysis were found to be critical indicating the need for running a standard in parallel. The degradation products of paracetamol can also interfere with this method.

Davis *et al* (36) described for the colourimetric determination of paracetamol as indophenol dyes. Paracetamol was hydrolysed first to p-aminophenol by adding concentrated hydrochloric acid and then refluxing on a hot-plate. The hydrolysed paracetamol was added on acidify hypochlorite to form p-quinonechlorimide, which then undergoes a reaction with phenol. The blue indophenol dye was produced, which had maximum absorbance at wavelength at 625 nm.

Elloock and Fogg (37) developed the determination of paracetamol by mean of an indophenol reaction. After complete hydrolysis of paracetamol, the procedure involved spontaneous oxidation of a mixture of p-aminophenol and alkaline solution of phenol, to form indophenol. Alkaline solution of phenol was a mixture of trisodium orthophosphate, sodium hydroxide and phenol pH 10.5.

Muffin and Wragg (38,39) developed automate colourimetric method consist of paracetamol into manual method. Paracetamol was added to a hydrochloric acid-sodium hypochlorite mixture at pH 3.4. The excess of hypochlorite was removed with sodium arsenite. Then a solution of phenol was added and followed by borate buffer solution (pH 9.9). The optical density of the blue indophenol dye solution was measured at 625 nm.

5.4 Oxidation

Paracetamol undergoes oxidation with 2 iodylbenzoate in acid medium, producing an orange-yellow colour that attained maximum intensity for 1 minute and at λ_{max} at 444 nm. The method was claimed that it was maximum suitable for routine analysis and unaffected by the presence of salicylamde, oxyphenbutazone, analgin and a number of other exipients (40).

5.5 Coupling with Diazotised Reagents Directly.

Hassan, Mohammed *et al* (41) determined paracetamol based on coupling between the phenolic compound and diazonium salts of both sulfanilic acid and p-nitroaniline. First mix amine reagent (sulfanilic acid or p-nitroaniline) and sodium nitrite and let stand at room temperature for 10 minutes. Add methanol and mix well to eliminate excess nitrous acid. Add paracetamol, follow by 1 N sodium hydroxide to form azo dye derivative. Paracetamol couples with diazosulfanilic acid or diazo-p-nitroaniline to give red and yellow dye derivatives, respectively. Measure absorbance of red and yellow dye derivatives at λ_{max} at 281 and 425 nm, respectively.

S. Belal *et al* (42) determined paracetamol, based on its coupling with diazotizated o-nitroaniline. And Copper (II) chelation with the coupled compound made the method highly selective. Sensitivity was increased when paracetamol was carried out indirectly through the determination of the chelate's copper content.

6. Chromatographic Method

6.1 Ion Exchange Chromatographic Method.

Hunt, Rhodes and Blake (43) separated paracetamol from its decomposition product in elixir formulation by ion-exchange column chromatography. They used Dowex I-X8, 200-400 mesh (hydroxide form) as ion-exchange medium and 20% glacial acetic acid in methanol as eluate. They quantitatively determined paracetamol by titrimetry in dimethylformamide solution with sodium methoxide.

Koshy and Lach (1) studied hydrolysis of paracetamol inaqueous solutions. They separated paracetamol in column of a strong cation exchange resin in the hydrogenated form (Amberlite IR-120). After that, they hydrolysed paracetamol to p-aminophenol and coupled it with an alcoholic solution of α-naphthol in alkaline media to develope a colour product. Absorbance of finally product was determined at 590 nm.

6.2 Partition Chromatographic Method.

The United State Pharmacopoeia used partition chromatographic method for determination of paracetamol elixirs, capsules and tablets. Mix and transfer of the mixture of paracetamol aliquot and siliceous earth to the chromatographic column. Wash the column with water-saturated chloroform and discard the eluate. Elute the paracetamol with water-saturated ether. Evapolate the ether on a steam bath. Without delay, dissolve the residue in acidic methanol. Determine the absorbance of final

preparation at the wavelength of maximum absorbance, 249 nm. Calculate the quantity compare to the standard preparation (23).

6.3 Paper and Thin Layer Chromatographic Method.

A number of thin layer and paper chromatographic methods have been found suitable for the isolation and identification of paracetamol (44).

6.4 Gas Liquid Chromatographic Method.

Paracetamol pronounced elution peak tailing caused by its polar hydroxy group. So that it should be derivertised to other form before running GLC.

Koibuchi *et al* (45) acetylated the hydroxy group of paracetamol to give N,O-diacetyl-p-aminophenol which gave a good symmetrical peak after elution from a 1% DEGS column. The paracetamol was acetylated with a pyridine acitic anhydride reagent employing strictly controlled reaction conditions to suppress the formation of product of the reaction. Quantitation was affected by peak height ratio measurement using an internal standard.

Paracetamol may be readily silylated to form derivative suitable for quantitative GLC determination (44).

Table I gives details of the various GLC system described for the separation and quantitative determination of paracetamol.

6.5 High Pressure Liquid Chromatographic Method.

Burtis, Butts and Rainey (46) first described the use of HPLC method in the determination of paracetamol and its

glucuronide metabolite in urine. Their procedure employed a high pressure anion exchange chromatographic system with U.V. detector and gave very long retention times in excess of 16 hours.

HPLC methods were developed for separation, determination of paracetamol in solid and liquid form contained single or combination drugs and in body fluids.

Table 2 was showndetails of the various HPLC system described for the separation and quantitative determination of paracetamol.

Proposed Investigation

The increase popularity of paracetamol prescribed for analgesic and antipyretic cases in children has driven locally pharmaceutical manufacturers to formulate paracetamol liquid preparation. The important of drug quality is dilute due to the high competition to gain maximum market share. Some liquid paracetamol preparations have no expiration date indicated on the preparation's labelled. In fact, stability test has never been conducted in testing of liquid paracetamol preparation in Thailand. Consequently, a practical stability analysis which need less time should be the answer to drug manufacturers. After stability investigation is conducted, an exact expiration date can be accurately specified to acknowledge patients.

The concept of stability investigation is to measure quantity of the intact paracetamol in the preparation at appropriate time interval. It is essential to avoid interference from degraded product (p-aminophenol) of which the structure close to that of

paracetamol. Most chemical analysis methods of paracetamol are not practical since they require multiple steps and time consuming procedures. The prerequisite of these methods is to change paracetamol to p-aminophenol before taking subsequent steps. These inaccurate and complex procedures make most of these methods unappropiate procedures for stability testing of paracetamol.

Nowadays most pharmaceutical companies are interested in using HPLC or GLC method for drug analysis since it is a quick and accurate method. However, to use GLC in a paracetamol analysis is complicated because it has a prior requirement of derivertization of paracetamol before using GLC method. Quantitative analysis of paracetamol by HPLC ought to be a good method for testing drug stability and calculation of shelf-life. Thus, HPLC method is the proper method to use in this stability investigation of commercially available liquid paracetamol preparations in Thailand. It is necessary to choose the method which requires available equipments in most manufacturers such as pump, detector and HPLC column. The recommended HPLC methods were published in the international journals, often requires complex apparatus which were not available in Thailand. For some methods, all the equipments needed were available, but they were still impractical procedures. These methods failed to discrete peak of p-aminophenol from paracetamol even though ratio and rate of mobile phase was adjusted. Therefore, the columns interested in this study will be available in laboratory such as column C_8, C_{18} or CN. Detector should be a UV-spectrophotometer.

The study in this report used HPLC method in the analysis of paracetamol by comparing with the official method in the USP XXI. This official method is used as a control method since p-aminophenol does not interfere the quantitative analysis of paracetamol.

The samples used in this study were selected from the existing paracetamol products in Thailand. Suspension #1 and Suspension #2 were selected as samples for suspensions. In the syrup form, Syrup #1 and Syrup #2 were selected. Elixir #1 and Elixir #2 were two elixir form samples. The concept of stability test is to accelerate degradation of drug by increasing the temperature and then measuring drug left in the time series basis. The specific rate constant of degradation at different temperature can be indicated by this quantitative analysis. From this specific rate constant, Arrhenius curve can be plotted and the energy activation will be calculated. This figure can be used to calculate specific rate constant at different temperature in addition to calculate shelf-life of the particular products.

This proposed method can be used to quantitatively measure paracetamol in both solution form and tablets form. Therefore two paracetamol tablet products are selected as samples for this study.

The outline of this thesis was based on the following statements :

1. Selection of appropiate chromatographic condition of HPLC method for determining paracetamol.

2. Evaluation of each commercially liquied paracetamol preparation by accelerating stability test method by the proposed method compared with the USP method.

3. The theoretical predicted shelf-life of commercially

liquid paracetamol preparations would be compared with the direct shelf-life measurement method at room temperature condition.

4. The proposed method can be applied to a determination of paracetamol content uniformity in commercial tablets.

Table 1
G.L.C. Determination of paracetamol

Column support	Column stationary phase	Column temp.	Detector system	Internal standard	Type of determination	Ref.*
Chromosorb W (AW-DMCS) 80/100 (mesh)	10% UCC-W-982	195°C	F.I.D.	Amitryptyline-HCl	Analgesic preparation	47
Gas Chrom Q 100/120	3% OV-17	165°C	KCl-T.I.D.	Amobarbital	Pharmaceutical preparations	48
Aeropak 30 70/80 (in silanished Column)	2% FFAP	240°C	F.I.D.	Diphenylphthalate	Metabolic	49
Chromosorb G 70/80	5% Carbowax 20 M	225°C	F.I.D	External standard	Analgesic mixture	50
Chromosorb G 100/120	SE-52	220°C	F.I.D.	4-Butyryl-p-aminophenol	Qualitative clinical	51
Acid-washed and silanized Chromosorb W	3% OV-225	225°C	T.I.D.	4-Butyryl-p-aminophenol	Qualitative clinical	52

Table 1 (cont.'d)
G.L.C. Determination of paracetamol

Column support	Column stationary phase	Column temp.	Detector system	Internal standard	Type of determination	Ref.*
Supelcoport 100/120	3% SP-2250-DA	180°C	F.I.D.	2-acetamidophenol	Quantitative of clinical	53
Chromosorb A (HP)	0.33% CDMS	temp -programmed 170-225°C (10°/min.)	alkaline F.I.D.	metanolic	Qualitative of clinical	54
Gas Chrom Q 100/120	5% Apiezon L.	160°C	F.I.D.	n-Hexadecane	Pharmaceutical preparations	55

*Ref. = Reference

Table 2

High-Pressure Liquid Chromatographic Determination of Paracetamol

Instrument	Column Size and Packing	Column Temp. and Pressure	Flow rate ml/min.	Elution	Retention time	Ref.*
Du Pont Model 820 Liquid Chromatograph with Model 410 Photometer Detector (254 nm)	0.21 x 1000 cm Zipax coated with strong anion exchange resin	Temperature n.a.1200 p.s.i.g.	1.5	Buffer (Fisher Gram-Pac) pH 9.2 containing 0.005 M ammonium nitrate	ca. 2 min.	56
Varian LSC-1000 with Photometer Detector (254 nm)	0.10 x 300 cm LSF pellicular anion excharge resin	60°C 925-1000 p.s.i.g.	0.125	1.0 M. Tris Buffer (pH 9.0)	Paracetamol 649 sec. ± 1.08%	57
Waters Associates with Photometer Detector (254 nm)	4 mm x 30 cm μ-Bondapak-C 18	Ambient temp.	0.9	Acetonitrile/ 0.0085% ammonium carbonate in water (38:62 V/V)	Paracetamol 215 sec.	58

Table 2 (cont.'d)
High-Pressure Liquid Chromatographic Determination of Paracetamol

Instrument	Column Size and Packing	Column Temp. and Pressure	Flow rate ml/min.	Elution	Retention time	Ref.*
Model MB-2-300, Chromatronix, Inc. with Electrochemical detector (E = +1.00 V. versus-silver-silver chloride)	2 mm x 30 cm Zipax scx strong cation exchange	Ambient temp.	0.25	0.04 M sulfuric acid	Paracetamol about 1.8 min.	59
Waters associates Model 6000 A pump. with Model 440 Photometor detector (254 nm.)	4 mm x 30 cm μ-Bondapak-C 18 Phenyl (ion pair)	Ambient temp. 2000 p.s.i.g.	3.0	0.005 M tetra-butylammonium phosphate in distilled water containing 15% (V/V) acetonitrile pH 7.	Paracetamol about 2.1 min.	60

Table 2 (cont'd)
High-Pressure Liquid Chromatographic Determination of Paracetamol

Instrument	Column Size and Packing	Column Temp. and Pressure	Flow rate ml/min.	Elution	Retention time	Ref.*
Waters Associates model ALC 202 with Model 6000A pump and differential RI detectors	4 mm x 30 cm μ-Bondapak-C_{18}	Ambient temp. 2000 p.s.i.g.	2.0	Methanol : Water 50 : 50)	Paracetamol 100 sec.	61
Altex 100 A with Pye LC3 Photometer detector (275 nm)	4.6 mm x 25 cm OD2-2 Column (Whatman; Partisil PXS 10/25 ODS-2)	Ambient temp.	1.0	Acetonitrile-acetic acid-water (25 : 5 : 70, V/V/V)	Paracetamol about 4 min.	62
Waters ALC 204, waters Associates with SF 770 Spectro flow Monitor Photometer detector (283 nm)	μ-Bondapak-C_{18}	Ambient temp.	1.1	Methanol : aqueous solution containing 0.01 N monobasic potassium phosphate and 0.05 N potassium nitrate pH 4.5 (25:75)	Paracetamol 5.9 min.	63

Table 2 (cont'd)
High-Pressure Liquid Chromatographic Determination of Paracetamol

Instrument	Column Size and Packing	Column Temp. and Pressure	Flow rate ml/min.	Elution	Retention time	Ref.*
Rapid-HPLC Column temp. controler, Neslab Model EX-300 temperature and Model DCR-1 digital controller with Photometer detector (254 nm)	4.5 mm x 5 cm octyl column. (column was phase rearrange by initially conditioning with at least 50 ml of acetonitrile followed by an equivalent volumn of water. This conditioning was carried out at 55°C in 20 min.	40°C	2.0	Water	Paracetamol about 2 min.	64
Waters associates Model 6000 A. with Model 440 Photometer detector (280 nm)	4 mm x 30 cm μ-Bondapak-C 18	Ambient temp.	2.5	Methanol : Water : Glacial acetic acid (45 : 55 : 2) containing 0.005 M octanesulfanic acid	Paracetamol about 1.8 min.	65

Table 2 (cont'd)
High-Pressure Liquid Chromatographic Determination of Paracetamol

Instrument	Column Size and Packing	Column Temp. and Pressure	Flow rate ml/min.	Elution	Retention time	Ref.*
Spectra-Physics 3500B with Waters associates Model 440 Photometer detector (254 and 280 nm)	8 mm x 10 cm Rad-Pak A columns and contained unsilanized C_{18} packing. These were operated in a water ASSOC. radial compression module	Ambient temp.	2 min.	Methanol : Water (1 : 1)	Paracetamol about 1.8 min.	66

*Ref. = Reference.