CHAPTER II

EXPERIMENTATION

1. INSTRUMENTS AND MATERIALS

1.1. INSTRUMENTS

1.1.1 Gas Chromatograph HP 5890A with Flame Ionization Detector

1.1.2 Integrator HP 3396A

1.1.3 HP-20M Column (Carbowax 20M) 10 m x 0.53 mm x 1.33 μm film thickness.

1.1.4 Shimadzu UV 180 Spectrophotometer

1.1.5 Shimadzu IR 440 Spectrophotometer

1.1.6 Jeol FX 90 Q NMR Spectrometer

1.1.7 Presica 300A Analytical Balance

1.1.8 Mettler Pan Balance

1.1.9 Buchi Melting Point Apparatus

1.2. MATERIALS

1.2.1 Paracetamol* BP.

1.2.2 Paracetamol in various preparations**

1.2.3 Acetic anhydride***-Riedel-de Haen

1.2.4 Pyridine****-E. Merck

1.2.5 Diethyl ether-E. Merck

1.2.6 Chloroform-E. Merck

1.2.7 Hydrochloric acid-E. Merck

1.2.8 Sodium hydroxide-E. Merck

1.2.9 Sodium carbonate-BDH

1.2.10 Sodium bicarbonate-E. Merck

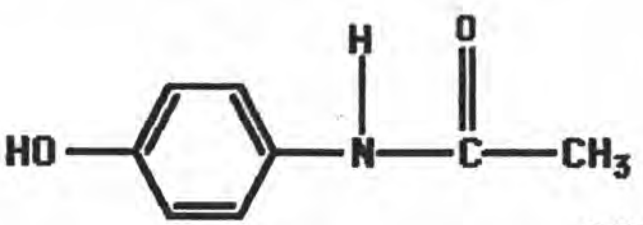
1.2.11 Siliceous earth

1.2.12 Benzophenone as internal standard-BDH

1.2.13 Silicone Oil

1.2.14 Human serum-Research Institute for Health Sciences, Chiang Mai University

*The chemical name of paracetamol is 4'-hydroxyacetanilide, N-acetyl-p-aminophenol, p-acetaminophenol, p-hydroxyacetanilide, p-acetamidophenol and p-acetylaminophenol. Its structure is shown below :-



$C_8H_9NO_2$

MW =151.16

Paracetamol (54) is a white, odorless, crystalline powder with a slightly bitter taste. Its melting point range between 168°c and 172°c. It is sparingly soluble in water and chloroform, insoluble in ether but readily soluble in alcohol and glycerol. It is freely soluble in solutions of alkali hydroxides and pyridine.

** Paracetamol in various formulations, i.e.

Syrup No.1 : in each 5 ml contains	paracetamol 120 mg
Syrup No.2 : in each 5 ml contains	paracetamol 120 mg
Syrup No.3 : in each 5 ml contains	paracetamol 120 mg
	phenylpropanolamine HCl 5 mg
	phenylephrine HCl 2.5 mg
	chlorpheniramine maleate 1 mg
Syrup No.4 : in each 5 ml contains	paracetamol 120 mg
	pseudoephedrine HCl 7.5 mg
	chlorpheniramine maleate 1 mg
Syrup No.5 : in each 5 ml contains	paracetamol 120 mg
	phenylpropanolamine HCl 5 mg
Tablet No.1 : in each tablet contains	paracetamol 500 mg
Tablet No.2 : in each tablet contains	paracetamol 500 mg
Tablet No.3 : in each tablet contains	paracetamol 300 mg
	phenylpropanolamine HCl 12.5 mg
	chlorpheniramine maleate 1 mg
Tablet No.4 : in each tablet contains	paracetamol 325 mg
	pseudoephedrine HCl 15 mg
	chlorpheniramine maleate 2 mg
Tablet No.5 : in each tablet contains	paracetamol 300 mg
	phenylephrine HCl 5 mg
	chlorpheniramine maleate 4 mg
	caffeine 30 mg
Injection No.1 : in each 2 ml contains	paracetamol 300 mg
Injection No.2 : in each 2 ml contains	paracetamol 300 mg
	lidocaine HCl 20 mg

*** Acetic anhydride (54) is known chemically as acetic oxide or acetyl oxide. It's molecular formula is $(CH_3CO)_2O$ and has the molecular weight of 102.09. It is a very refractive liquid, has a strong acetic odor, readily combustible and therefore fire hazard. Its melting point is -73°c but boiled at 139°c. It is slowly soluble in water, forming acetic acid. Forming ethyl acetate when it is in alcohol. It is soluble in chloroform and ether .

**** Pyridine (54) has the molecular weight of 79.10 and its structure is as below :-

It is flammable, colourless liquid and has characteristic disagreeable odour with sharp taste. Its boiling point lies between 115-116°c and melts at -42°c. It volatiles with stream. Miscibles with water, alcohol, ether, petrolium ether, oils, and many other organic liquids. It is a good solvent for many organic and inorganic compound. Pyridine itself is a weak base, forms salts with strong acid. The pH of 0.2M pyridine in water is 8.5.

Note : Chemical No.1 was BP. grade, No.2 to 5 were analytical grade and No. 6 to 10 were reagent grade.

2. PREPARATION OF BENZOPHENONE SOLUTIONS AS INTERNAL STANDARDS

2.1 BENZOPHENONE SOLUTION-1 AS INTERNAL STANDARD FOR TABLET, SYRUP AND INJECTION

6.0 g of benzophenone was accurately weighed in a 50-ml volumetric flask. Pyridine was added and swirled until benzophenone was completely dissolved. Added to volume with more pyridine so that the final concentration of benzophenone in this solution was 120 mg/ml.

2.2 BENZOPHENONE SOLUTION-2 AS INTERNAL STANDARD FOR HUMAN SERUM

75 mg of benzophenone was accurately weighed in a 10-ml volumetric flask. Pyridine was added and the mixture was swirled until benzophenone was completely dissolved. Added to volume with more pyridine. One ml of this solution was transferred to another 10-ml volumetric flask, and was further diluted with pyridine so that the final concentration of benzophenone was 0.75 mg/ml.

3. DERIVATIZATION REACTION OF PARACETAMOL (ACETYLATION)

3.1 PREPARATION OF PARACETAMOL DERIVATIVES

3.1.1 O-Monoacetyl-p-acetaminophenol (MAAP)

A mixture of 10 ml of pyridine with 10 ml of acetic anhydride and 12.0 g of paracetamol BP was made in a pear-shape flask. As soon as the mixture had been made, the flask was fixed to one end of the condenser

while a moisture trapper was attached to the other. The solution was allowed to reflux on a steam-bath for a period of 4 hours. Afterwhich, a white precipitate forms fairy readily when mixture was allowed to cool to room temperature. The precipitate was collected by filtration, pyridine and acetic anhydride residues were removed by repeated washing with 100 ml of cold water. The crystalline precipitate obtained was resuspended in 40 ml of hot water and was heated up almost to boiling. A small amount of methanol was added until complete dissolution occurred. The solution was thereafter allowed to cool slowly to the room temperature to obtain products.

3.1.2 N,O-Diacetyl-p-acetaminophenol (DAAP)

A mixture of 5.0 ml of pyridine and 10.0 ml of acetic anhydride with 2.0 g of paracetamol was made in a pear-shape flask. As soon as the mixture has been made, the flask was fixed to one end of the condenser while a moisture trapper was attached to the other. The solution was allowed to reflux in oil bath at 220°c for a period of 3 hours to ensure that reaction had completed. Afterwhich, a portion of this mixture was spotted on a preparative TLC plate (20cm x 20cm) pre-coated with silica gel 60 GF 254 (1.25 mm thick). Separation was achieved with hexane-ethyl acetate (1:1) as mobile phase. After the plate had dried, migratory products were identified under long-wavelength ultraviolet light, and subsequently removed from the plate to a collecting flask. Chloroform was added to dissolved product from silica gel. Mixture was filtered to remove silica gel, and chloroform was evaporated under a gentle stream of air to dryness to obtain the product.

3.2 IDENTIFICATION OF PARACETAMOL AND ITS DERIVATIVES

Paracetamol and products obtained in 3.1.1 and 3.1.2 above were identified by their melting points, thin layer chromatography, ultraviolet-visible and infrared spectrophotometry, gas-liquid chromatography, and ^{1}H-nuclear magnetic resonance spectrometry.

3.2.1 Measurement of Melting Point

Paracetamol and its derivatives were grounded into fine powder and transferred to capillary tubes so that approximately 1 cm column of the packed powder was obtained. These capillary packed tubes were then subjected to the measurement of melting point.

3.2.2 Thin Layer Chromatography (TLC)

Both monoacetyl- and diacetyl-p-acetaminophenol were dissolved in chloroform, but paracetamol itself in methanol. Each of these three solutions was chromatographed, on a thin layer of silica gel 60 GF 254 spread uniformly on a glass plate, in a glass tank containing hexane and ethyl acetate system (1:1) until the solvent front has travelled about 10 cm from the origin. Paracetamol and monoacetyl-p-acetaminophenol could be located with help of short wavelength ultraviolet light, but the diacetyl-p-acetaminophenol product, on the other hand, needs longer wavelength of the ultraviolet light before it becomes visible.

3.2.3 Ultraviolet Spectrophotometry (UV-Spectrophotometry)

Absorption spectra were scanned on Shimadzu Spectro-photometer using 1-cm quartz cells. Paracetamol and its derivatives were

dissolved in chloroform and scanned for UV absorption spectra from 160 to 350 nm against chloroform blank.

3.2.4 Infrared Spectrophotometry (IR Spectrophotometry)

Infrared spectrophotometry of paracetamol and its derivatives were taken in KBr discs on a Shimadzu IR 440 Spectrophotometer at the Scientific and Technological Research Equipment Center of Chulalongkorn University.

3.2.5 Proton Nuclear Magnetic resonance spectrometry

(^{1}H-NMR spectrometry)

The ^{1}H-NMR spectra of paracetamol and its derivatives were recorded on a Jeol FX 90Q NMR spectrometer with frequency of 90 MHz at the Scientific and Technological Research Equipment Center of Chulalongkorn University. Deuterated dimethylsulfoxide was used as solvent for paracetamol and deuterated chloroform for its derivatives.

3.2.6 Gas-Liquid Chromatography (GLC)

Paracetamol derivatives were identified by injected into the gas-liquid chromatograph compared to the pure derivative products. Using oven temperature programming from 140°c (for one minute) to 200°c with the rate of 20°c/minute. The detector's and injector's temperature was 250°c. The carrier gas (nitrogen) flow with pressure of 50 psi. The integrator was set at the attenuation 2^9 and the chart speed was set at 0.3 cm/min.

4. STUDIES OF FACTORS AFFECTING DERIVATIZATION REACTION (Acetylation)

4.1 EFFECT OF THE TEMPERATURE

Nine similar solutions, each was made up of 4 ml of pyridine 4 ml of acetic anhydride and 300 mg of paracetamol BP were prepared. Each flask was allowed to reflux for a period of 5 hours in the same condenser system as detailed in section 3.1.2. The incubating temperature was varied by subjecting one solution each to temperature of 5°c, at room ambient temperature at 29°c, at 60°c, at 90°c, at 120°c, at 160°c, at 200°c, at 235°c and at 260°c. After reaction has been completed, the reaction mixtures were transferred to 10-ml volumetric flasks then one ml of benzophenone solution-1 (section 2.1) was added to each of these flasks. The volume was adjusted with acetic anhydride and 0.2 µl of each mixture prepared and incubated was injected into gas-liquid chromatograph with the condition as detailed in section 3.2.6. Peak area ratio between N,O-diacetyl-p-acetaminophenol (DAAP) and benzophenone on one hand, and between O-monoacetyl-p-acetaminophenol (MAAP) and benzophenone on the other, could be directly related to the amount of N,O-diacetyl- and O-monoacetyl-p-acetaminophenol formed, respectively.

4.2 EFFECT OF INCUBATION TIME

Three hundred milligrams of paracetamol was allowed to acetylated with 4 ml each of acetic anhydride and pyridine in the condenser system as detailed in section 3.1.2 at 220°c for varying periods. One solution each was incubated for 30, 60, 90, 120, 150, 180, 210, 270 and 300 minutes, respectively. The reaction mixtures were transferred to 10-ml volumetric flasks and 1 ml of benzophenone solution-1 (section 2.1) was added to each of these flasks. The

volume was adjusted with acetic anhydride then 0.2 µl of each mixture prepared and incubated was injected into the GLC with the condition as outlined in section 3.2.6.

4.3 EFFECT OF AMOUNT AND COMPOSITION OF ACETYLATING REAGENTS

The effect of varying volumes of acetylating reagents (pyridine and acetic anhydride) was studied, i.e. by employing a fixed weight of 300 mg of paracetamol in a varying volumes and there by ratios between pyridine and acetic anhydride between 2:4, 4:4, 4:8, 4:12, 4:16, 8:8, 12:12, 16:16, 8:4, 12:4, and 16:4 respectively. As before, each acetylating reaction was allowed to take place for 3 hours at 220°c in a condenser system as detailed in section 3.1.2. The reaction mixtures were transferred to 10-ml volumetric flasks and one ml of benzophenone solution-1 (section 2.1) was added to each of these flasks. The volume was adjusted with acetic anhydride then 0.2 µl of each mixture prepared and incubated was injected into the GLC with the condition as outlined in section 3.2.6.

4.4 EFFECT OF VARYING CONCENTRATION OF PARACETAMOL

Each value of peak area ratio between DAAP/benzophenone was related to the corresponding amount of paracetamol BP present in or added to the incubating flask, i.e. 20, 40, 60, 80, 100, 200, 300, 400, 500, 600, 800, 1000 and 1500 mg, at the start of acetylation procedure. Each of 4 ml of pyridine and acetic anhydride was added into each flask and the reaction was allowed to take place for a period of 3 hours at 220°c in condenser system as detailed in section 3.1.2. The reaction mixtures were transferred to 10-ml volumetric flasks and one ml of benzophenone solution-1 (section 2.1) was added to each of these flasks. The volume was adjusted with acetic anhydride then 0.2 µl of each

mixture prepared and incubated was injected into the GLC with the condition as outlined in section 3.2.6.

5. QUANTITATIVE DETERMINATION OF PARACETAMOL IN SYRUP PREPARATIONS

5.1 ACETYLATION - GLC METHOD

Five preparations of syrup, i.e. syrup number 1 to 5, utilized in this experiment. Each preparation was aliquotted six times, and each was subjected to acetylating procedure. Each acetylating sample was then subjected to repeated analysis (six occasions for each sample) in the GLC-system. Results were used in the calculation of the percent labelled amount as detailed in section 5.1.4 below.

For the percent recovery experiment, syrup No.2 only was employed. The six samples thus aliquotted were then paired off, and each pair which had a varying amount of standard added was then subjected to acetylation. After acetylating procedure, each of the final sample obtained was repeatedly analyzed (six times) in a GLC-system

5.1.1 Preparation of Standard Solution

20, 40, 60, 80 & 100 mg of paracetamol BP were put into each flask. Four ml each of pyridine and acetic anhydride was added. Acetylation reaction was allowed to take place for 3 hours at 220°c. After the reaction had completed, the reaction mixtures were transferred into 10-ml volumetric flasks and one ml of benzophenone solution-1 (section 2.1) was added to each flasks. The volume was adjusted with acetic anhydride then a

representative 0.2 µl of each of the five mixtures experimented was analyzed in GLC with conditions as detailed in section 3.2.6. A plot between peak area ratio and concentration was used as standard curve.

5.1.2 Preparation of Chromatographic Column

The column was set up and employed as in the assay of paracetamol syrup as detailed in the USP XXII recommended methods below in section 5.2.2.

5.1.3 Preparation of Sample

A 10 ml pycnometer was accurately weighed when emptied and when filled with paracetamol syrup. The weight different represents paracetamol syrup, and can be used in the density calculation using the following formula :-

$$D = M/V$$

Where D = density of syrup, in g/ml

M = mass or weight of syrup in pycnometer, in g

V = volume of pycnometer used, in ml

A portion of syrup equivalent to about 48 mg of paracetamol was accurately weighed in a 100-ml beaker, and 0.2 ml of 2N NaOH was added with thorough mixing. A drop of HCl was further added, and beaker was swirled to mix. Three grams of purified siliceous earth was added in and the mixture was transferred to column. Traces of the compound left on the beaker was removed with additional gram of purified siliceous earth mixed with 2 drops of water, and the washing was transferred to column with gentle tapping to

ensure uniform packing. A small pledget of cotton wool was placed on top of the packed column, and the column was repeatedly washed with 100 ml water-saturated chloroform. Eluent was discarded. After washing, paracetamol was eluted with 200 ml of water-saturated ether. The ether was removed by evaporation under a gentle stream of air in a steam-bath. Residue was redissolved in 4 ml each of pyridine and of acetic anhydride, and the solution was then subjected to acetylating reaction at 220°c for 3 hours as detailed in section 3.1.2. The reaction mixtures were transferred to 10-ml volumetric flasks then 1 ml of benzophenone solution-1(section 2.1) was added into each flasks. The volume was adjusted with acetic anhydride and mixed throughly.

5.1.4 Procedure

0.2 µl of acetylated products obtained above were subjected to analysis in GLC system described in section 3.2.6. The quantity of paracetamol presented in the syrupy solution can be obtained with the use of formula :-

(Y-C)/M

$$\text{PERCENT LABELLED AMOUNT} = \frac{W(Y-C) \times 100}{MLN}$$

where W = weight of 5 ml syrup, in mg

Y = peak area ratio of DAAP/benzophenone

C = standard curve's intercept at y-axis

M = slope of the standard curve

L = weight of syrup containing 48 mg of paracetamol, in mg

N = amount of paracetamol in 5 ml, in mg

5.1.5 Percent Recovery

Three sets of syrup preparation No.2, each containing an equivalent of 48 mg of paracetamol were weighed in 100-ml beakers. After which, either 12, 22 or 32 mg of paracetamol BP was added Followed by an addition of 0.2 ml of 2N NaOH. Swirled to dissolve. Proceed as directed for preparation of sample in paracetamol syrups as detailed in section 5.1.3, beginning with ....."3.0 g of purified siliceous earth was added.....". The whole procedure such described was repeated once.

The above products obtained was analyzed as detailed in section 3.2.6. Calculation for the percent recovery is carried out with the following formula :-

$$\textbf{Percent Recovery} = \frac{(Wm - Ws)}{Wa} \times 100$$

where Wm = weight of paracetamol measured in mixture, in mg

Ws = weight of paracetamol in sample, in mg

Wa = weight of paracetamol BP added, in mg

5.2 USP XXII METHOD (55)

5.2.1 Preparation of Standard Solution

Eighty mg of accurately weighed paracetamol BP (equivalent to USP acetaminophen RS) was transferred to a 100-ml volumetric flask. Methanol was added to volume, and mixed. Ten millilitres of this solution was transferred to the second 100-ml flask and further diluted to volume with more methanol. Again mixed well, and 10 ml of this was re-transferred to the

third 100-ml volumetric flask. One ml of 0.1N HCl was added followed by methanol to volume. Mixed to obtain a final solution which now contained 8 µg of paracetamol per ml.

5.2.2 Preparation of Chromatographic Column

A glass chromatographic column (25mm x 250mm) had at its lower end packed with a small pledget of cotton wool. A small glass tube (5cmL x 7mmW) was fused to tip of this end of the column. Two grams of purified siliceous earth in 2.0 ml of carbonate/bicarbonate solution (1g $NaHCO_3$ + 4.5g $NaCO_3$ in 100 ml) was prepared, mixed well until solution became fluffy before being transferred to the column. A good and uniform packing of the column was achieved with aid of a tapering solid glass rod (45cmL) with a small round disk (approx.6mmD) attached to its packing end.

5.2.3 Preparation of Sample

An accurately measured volume of syrup containing 240 mg of paracetamol was transferred to a 250-ml volumetric flask. To which 0.2 ml of 1N NaOH had been added. This was diluted with water to volume, and mixed. Transferred 2.0 ml of this mixture to a 100-ml beaker, one drop of conc. HCl and 3.0 g of purified siliceous earth were added. Mixed and transferred to the chromatographic column. Beaker was scrubbed with additional gram of purified siliceous earth mixed in 2 drops of water, and washing was transferred to the column. The column washed with 100 ml of water-saturated chloroform and the eluent was discarded. Paracetamol was eluted with 150 ml of water-saturated ether, and eluent was collected in 400-ml beaker. Ether was evaporated to dryness on a steam bath with aid of air current (prolonged drying

was avoided to prevent possible loss of the paracetamol). As soon as the eluent had dried, the residue was redissolved in a solvent mixture containing 1 ml of 0.1N HCl in 100 ml of methanol, and was transferred to a 50-ml volumetric flask. Beaker was thorough rinsed with solvent mixture and added to volume and mixed. Transferred 10.0 ml of this mixture to a second 50-ml flask, diluted again with the same solvent mixture to volume.

5.2.4 Procedure

Absorbances of both standard and sample were determined in 1-cm cell at 249 nm, employing solvent mixture as blank. The quantity of $C_8H_9NO_2$, in mg, in each ml of the syrup was calculated with the following formula :-

$$31.25\,(C/V)(A_u/A_s)$$

$$\textbf{Percent Labelled Amount} = \frac{156.25CAu}{VLAs} \times 100$$

where C = concentration of paracetamol BP., in µg/ml, in the standard preparation

V = volume, in ml, of syrup employed in the assay

Au = absorbance of the sample assay preparation

As = absorbance of standard preparation

L = labelled amount of syrup, in mg/5 ml

5.2.5 Percent Recovery

Transferred an accurate measured volume of paracetamol syrup No.2 containing 240 mg of paracetamol to a 250-ml volumetric flask. Two ml of 1N NaOH was added, followed by water to volume, and mixed. Three sets

each of 2.0 ml of this mixture was transferred to 100-ml beakers. Each was added with either 12, 22 or 32 mg of paracetamol BP. The rest of the procedure was the same as that reported in section 5.2.3.

Proceed as directed for procedure in the preparation of sample for paracetamol syrup in section 5.2.4. The results can then be calculated as detailed in section 5.1.5.

6. QUANTITATIVE DETERMINATION OF PARACETAMOL IN TABLET PREPARATIONS

6.1 ACETYLATION - GLC METHOD

Five preparations of tablets, i.e. tablet number 1 to 5, utilized in this experiment. Each preparation was weighed (in powder form) six times, and each was subjected to acetylating procedure. Each acetylating sample was then subjected to repeated analysis (six occasions for each sample) in the GLC-system. Results were used in the calculation of the percent labelled amount as detailed in section 6.1.3. below.

For the percent recovery experiment, tablet No.1 only was employed. The six samples thus weighed were then paired off. And each pair which had a varying amount of standard added was then subjected to acetylation. After acetylating procedure, each of the final sample obtained was repeatedly analyzed (six times) in a GLC-system

6.1.1 Preparation of Standard Solution

The standard curve was constructed by plotting the varying amounts of paracetamol BP , i.e. 100, 200, 300, 400 and 500 mg; against their corresponding values of the peak area ratio.

6.1.2 Preparation of Sample

Twenty paracetamol tablets obtained commercially were carefully weighed and grounded into fine powder. Powder equivalent to about 300 mg of paracetamol was used in the acetylation reaction as detailed in section 3.1.2 followed by transferring the reaction mixture into 10-ml volumetric flasks with an addition of 1 ml of benzophenone solution-1 (section 2.1). The volume was adjusted with acetic anhydride.

6.1.3 Procedure

The quantity of paracetamol injected (as directed in section 3.2.6) relates directly to the peak area ratio of DAAP/benzophenone. Therefore, percent of the labelled amount of this compound can be calculated using the following formula :-

$$\textbf{PERCENT LABELLED AMOUNT} = \frac{A(Y-C) \times 100}{MLN}$$

where Y = peak area ratio of DAAP/benzophenone

C = intercept at y-axis of the standard curve

M = slope of the standard curve

L = weight of paracetamol in tablet taken, in mg

A = weight of one tablet, in mg

N = amount of paracetamol per tablet, in mg

6.1.4 Percent Recovery

Twenty paracetamol tablets No.1 obtained commercially were weighed and grounded into fine powder. Three sets each of about 300 mg of this fine powder were weighed in pear-shape flasks. 30, 60, and 90 mg of paracetamol BP was added into each flask, respectively. The acetylation reaction was then allowed to take place as detailed in section 3.2.6. The reaction mixtures were transferred into 10-ml volumetric flasks and one ml of benzophenone solution-1 (section 2.1) was added.to each flasks. The volume was adjusted with acetic anhydride and mix throughly.

The product obtained was then subjected to the GLC system with the condition as detailed in 3.2.6. Calculation for the percent recovery is carried out with the formula as in section 5.1.5.

Again the results can then be calculated as detailed in section 5.1.5.

6.2 USP XXII METHOD

6.2.1 Preparation of Standard Solution

Standard sample was preparation as detailed in the syrup section (section 5.2.1).

6.2.2 Preparation of Chromatographic Column

Column was prepared as in same manner as in the syrup section (section 5.2.2).

6.2.3 Preparation of Sample

Twenty paracetamol tablets were accurately weighed, and grounded into fine powder. About 250 g of accurately weighed portion of this powder was transferred to a 250-ml volumetric flask. 2.0 ml of 1N NaOH was added, and this was further diluted with water to volume, mixed and filtered. The first 20 ml of the filtrate was discarded. Thereafter, proceed as directed in the preparation of sample for paracetamol syrup, beginning with ...."Transferred 2.0 ml of this solution to a 100-ml beaker....." (section 5.2.3).

6.2.4 Procedure

The procedure employed was similar to that described for assay for syrup (section 5.2.4). The quantity of $C_8H_9NO_2$ in the portion of tablets employed in assay preparation by the following formula :-

$$31.25\ C\ (\ Au/As)$$

$$\textbf{Percent Labelled Amount} = \frac{31.25\ CW\ Au}{LVAs} \times 100$$

where C = concentration, in mg, of paracetamol BP

Au = the absorbance of assay preparation

As = the absorbance of standard preparation

L = weight of paracetamol taken, in mg

W = weight of one paracetamol tablet, in mg

V = amount of paracetamol in a tablet, in mg

6.2.5 Percent Recovery

Twenty paracetamol tablets No.1 were accurately weighed and made into fine powder. About 250 mg portion of this powder was transferred to a 250-ml volumetric flask. 2 ml of 1N NaOH was added and solution was adjusted to volume with water. Mixed and filtered. The first 20 ml of filtrate was discarded. Three sets each of 2 ml of this filtrate was transferred to separate 100-ml beaker. Either 30, 60 or 90 mg of paracetamol BP was added into each beaker. Swirled until filtrate had completely dissolved. Thereafter, proceed on as for preparation of sample in the tablet section 6.2.3, beginning with ...."added a drop of HCl, and 3.0 g of siliceous earth. Mixed and transferred.....".

Proceed as directed for procedure in the assay under paracetamol tablet in section 6.2.4. Results can be calculated as detailed previously in section 5.1.5.

7. QUANTITATIVE DETERMINATION OF PARACETAMOL IN INJECTION PREPARATIONS USING ACETYLATION-GLC METHOD

Two preparations of injections, i.e. injection No.1 and 2 , utilized in this experiment. Each preparation was weighed six times, and each was subjected to acetylating procedure. Each acetylating sample was then subjected to repeated analysis (six occasions for each sample) in the GLC -system. Results were used in the calculation of the percent labelled amount as detailed in section 7.3. below.

For the percent recovery experiment, injection No.1 only was employed. The six samples thus weighed were then paired off. And each pair which had a varying amount of standard added was then subjected to

acetylation. After acetylating procedure, each of the final sample obtained was repeatedly analyzed (six times) in a GLC-system

7.1 PREPARATION OF STANDARD SOLUTION

Standard curve previously constructed in section 6.1.1 for use in tablet preparation was employed here.

7.2 PREPARATION OF SAMPLE

The density was determined with pycnometer as described previously in section 5.1.3. Thereafter, the injectable solution containing an equivalent of about 150 mg of paracetamol was subjected to acetylation (as detailed in section 3.1.2). The reaction mixtures were transferred into 10-ml volumetric flasks followed by an addition of 1 ml of benzophenone solution-1 (section 2.1). The volume was adjusted with acetic anhydride and mix throughly.

7.3 PROCEDURE

The procedure employed for this section of the experiment was the same as that previously described in section 3.2.6. The percent labelled amount was calculated with the following formula :-

$$\text{PERCENT LABELLED AMOUNT} = \frac{W(Y-C) \times 100}{MLN}$$

where Y = peak area ratio of DAAP/benzophenone

C = intercept at y-axis of the standard curve

M = slope of the standard curve

W = weight of solution in an ampule, in mg

L = weight of solution taken, in mg

N = amount of paracetamol in an ampule, in mg

7.4 PERCENT RECOVERY

An amount equivalent to about 150 mg each of paracetamol injection No.1 were weighed in pear-shape flasks. Either 30, 60 or 90 mg of paracetamol BP was further added to each flask. These were then subjected to acetylation reaction.

Proceed as directed for procedure for the assay for paracetamol injection in section 7.2. Results, again, can be calculated as detailed in section 5.1.5.

8. QUANTITATIVE DETERMINATION OF PARACETAMOL IN HUMAN SERUM USING ACETYLATION-GLC METHOD

8.1 STOCK SOLUTION OF PARACETAMOL FOR THE PREPARATION OF STANDARD SOLUTION

One hundred mg of paracetamol BP was weighed in a pear-shape flask. 5.0 ml each of pyridine and acetic anhydride was added to the flask, and acetylation reaction was allowed to take place at 220°c for 3 hours as described previously. After reaction had completed, The reaction mixtures were transferred into a 10-ml volumetric flask and adjusted to volume with acetic anhydride. 0.2 ml of this solution was transferred to another 10-ml volumetric flask, and acetic anhydride was added to volume so that a final concentration of 200 µg per ml was obtained.

8.2 PREPARATION OF STANDARD SOLUTION

0.2, 0.4, 0.6, 0.8 and 1.0 ml of the above stock solution in section 8.1 were transferred each to individual 10-ml volumetric flask previously prepared containing 1.0 ml of benzophenone solution-2 (section 2.2).

8.3 PREPARATION OF CHROMATOGRAPHIC COLUMN

A chromatographic column was carefully packed with siliceous earth and employed in separation of paracetamol in human serum. The procedure adopted here was the same as that detailed in section 5.2.2.

8.4 STOCK SOLUTION OF PARACETAMOL FOR THE PREPARATION OF SAMPLE

One hundred milligrams of paracetamol BP. was weighed in a 10-ml volumetric flask and methanol was used to adjusted the volume. 0.2 ml of the solution was pipetted into another 10-ml volumetric flask and again the solution was adjusted to volume with methanol so that the final concentration of 200 µg/ml was obtained.

8.5 PREPARATION OF SAMPLE

1.6 ml of serum and 0.4 ml of the stock solution in section 8.4 were employed in part of the study. They were carefully pipetted into a 100-ml beaker and treated with NaOH, HCl and purified siliceous earth before being transferred to the column as detailed in section 5.1.3. The residue was acetylated with acetic anhydride in the presence of pyridine. After acetylation reaction had completed, the reaction mixtures were transferred into a 10-ml volumetric

flask and 1.0 ml of benzophenone solution-2 (section 2.2) was added. The solution was adjusted to volume with acetic anhydride.

8.6 PROCEDURE

0.2 µl of this solution mixture was injected into GLC system as detailed previously in section 3.2.6 and the amount of paracetamol in serum was calculated as followed :-

$$\frac{5(Y-C)}{M}$$

where Y = peak area ratio of DAAP/benzophenone

C = intercept at y-axis of the standard curve

M = slope of the standard curve