CHAPTER III

RESULTS AND DISCUSSION

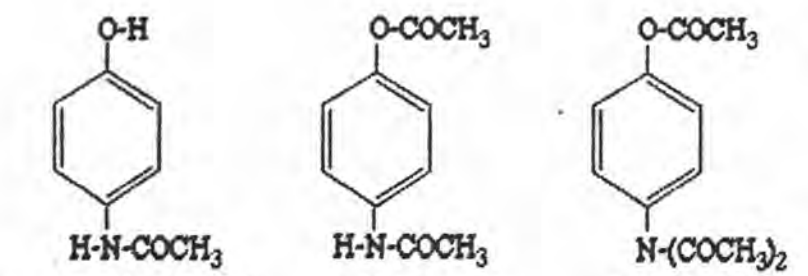
Paracetamol, an analgesic and anti-pyretic, produces two distinct derivatives when reacts with acetic anhydride and pyridine, namely monoacetyl-p-acetaminophenol (MAAP) and diacetyl-p-acetaminophenol (DAAP). These derivatives can be identified by their specific physical properties such as melting points, chromatographic and spectrometric characteristics as detailed below :-

1. IDENTIFICATION OF PARACETAMOL AND ITS DERIVATIVES

1.1 MELTING POINT CHARACTERISTICS

Paracetamol and its derivatives were identified by determining their melting points. The results obtained appeared in table-1.

The melting point of substances depends on a number of hydrogen atom attached to an electronegative atom (56). Such hydrogen atom could form an intermolecular hydrogen bond, so that more energy is needed to separate the molecule such as when it changes from solid to that of the liquid state.



p-acetaminophenol MAAP DAAP

From the structures shown above, paracetamol appears to have two such hydrogen atoms, and MAAP has only one whereas DAAP has none. It is evident, therefore, that paracetamol will have highest melting point, while DAAP has the lowest, and that of MAAP falls in between.

Due to the lowest melting point of DAAP, we chose to measure it for determined the amonut of paracetamol in this study.

1.2 THIN LAYER CHROMATOGRAPHY (TLC)

Paracetamol and its two derivatives were also identified by comparing their R_f-values with those of the standards in a silica gel chromatographic system operated in solvent mixture of hexane and ethyl acetate (1:1). The R_f-values were calculated after the solvent had travelled to a distance of approximately 10 cms, the distance known to be adequate for satisfactory separation of these compounds (see figure-1). The values of R_f obtained from such an experiment appeared in table-2.

The R_f-values of derivatives were different from its parent compound due to changes of polarities. The degree of this change was related to the number of acetyl groups introduced into the compound. If, in a silica gel chromatographic set-up, silica has a relatively high polarity, DAAP which is the most non-polar of the three, should ,therefore,have the highest R_f-value in this system; while paracetamol which is more polar therefore have the lowest R_f-value. This was indeed the case and the results that we have obtained are shown in figure-1.

1.3 ULTRAVIOLET SPECTROPHOTOMETRY (UV-SPECTROPHOTOMETRY)

The absorption spectra of paracetamol and its derivatives in chloroform were shown in figure-2. Paracetamol has a maximum absorption peak at the wavelength of about 251 nm, while MAAP and DAAP were maximal at 249 and 248 nm, respectively. The number of acetyl group substituted appears to have shorten the wavelength of absorption spectrum, phenomenon commonly known as hypsochromic shift (57). This is because the carbonyl group withdraws lone-pair electrons from auxochrome, thus making electrons in the benzene ring difficult to delocalize. There is a shoulder on the long wavelength side which is the n-->π^* transition of the chromophore (C=O). This shoulder is, however, small for MAAP, and virtually non-existence for DAAP. This finding strongly suggests that the substituted group on nitrogen atom in the DAAP structure (which is an auxochrome to the carbonyl chromophore) had bigger withdrawing force than those present in paracetamol, therefore the mobility of lone-pair electrons in the carbonyl chromophore becomes somewhat more restricted. With the final result that more energy is needed for changing transition state into π^* and consequential shifting into a shorter wavelength region. In DAAP, besides group substitution similar to MAAP, it also has an additional acetyl group added on to the nitrogen atom of its molecule thus rendering its absorption spectrum even shorter.

1.4 INFRARED SPECTROPHOTOMETRY (IR-SPECTROPHOTOMETRY)

The infrared spectra of paracetamol and its derivatives in potassium bromide disc were shown in figure-3, 4 and 5. The elucidation of the absorption peaks (58) for each of these three compounds are as followed :-

PARACETAMOL

3350 CM^{-1}	single N-H stretch of secondary amide
3180 CM^{-1}	phenolic O-H stretch
1660 CM^{-1}	amide C=O stretch
1615, 1510, 1440 CM^{-1}	ring C=C stretch
1565 CM^{-1}	overtone of N-H
1330 CM^{-1}	aryl C-N stretch
1260 - 1225 CM^{-1}	aromatic C-O stretch
1110 CM^{-1}	alkyl C-N stretch
840 CM^{-1}	para-disubstituted aromatic ring

MAAP

3380 CM^{-1}	single N-H stretch of secondary amide
1750 CM^{-1}	C=O stretch of phenolic ester
1690 CM^{-1}	amide C=O stretch
1610, 1505, 1430 CM^{-1}	ring C=C stretch
1540 CM^{-1}	overtone of N-H
1310 CM^{-1}	aryl C-N stretch
1230 - 1190 CM^{-1}	acetate carbonyl-oxygen stretch
1105 CM^{-1}	alkyl C-N stretch
850 CM^{-1}	para-disubstituted aromatic ring

DAAP

1750 CM^{-1}	C=O stretch of phenolic ester
1710 CM^{-1}	tertiary amide C=O stretch
1610, 1515, 1430 CM^{-1}	ring C=C stretch

1295 CM^{-1}	aryl C-N stretch
1170 -1240 CM^{-1}	acetate carbonyl-oxygen stretch
1100 CM^{-1}	alkyl C-N stretch
855 CM^{-1}	para-disubstituted aromatic ring

The spectrum obtained showed difference very clearly between the three compounds studied. That is in paracetamol, the single NH stretch absorption of secondary amide appears at 3350 CM^{-1} with an overtone at 1565 CM^{-1}. The phenolic OH stretch absorbed at 3180 CM^{-1} with a broad band due to intermolecular hydrogen bond. There was also the C=O stretch of amide at 1660 CM^{-1} and the aromatic C-O stretch at 1260-1225 CM^{-1} usually split into the number of maxima.. Comparing MAAP to paracetamol, the phenolic OH stretch at 3180 CM^{-1} disappeared when the phenolic ester C=O stretch shooted. And in comparing DAAP to paracetamol and to MAAP, the single NH stretch of secondary amide disappeared but the C=O stretch of amide becomes broader than MAAP. The suggestion was that there were 2 amide carbonyl groups.

1.5 PROTON NUCLEAR MAGNETIC RESONANCE (^{1}H-NMR)

^{1}H-NMR spectra of paracetamol and its derivatives were obtained by using DMSO-d_6 as solvent for paracetamol, and $CHCl_3$-d as solvent for derivatives. Tetramethylsilane was used as reference standard. The spectra were shown in figure-6, 7, and 8, and were elucidated (57,59) as detailed below:-

PARACETAMOL

δ	2.086	singlet	3H of -$NCOCH_3$
δ	7.036	quartet	protons of aromatic ring
δ	8.790	singlet	1H of -NH

δ	9.020	singlet	1H of -OH

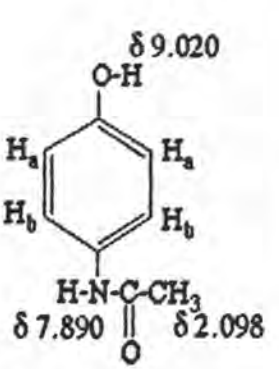
MAAP

δ	2.103	singlet	3H of $-NCOCH_3$
δ	2.288	singlet	3H of $-OCOCH_3$
δ	7.220	quartet	protons of aromatic ring
δ	7.890	singlet	1H of -NH

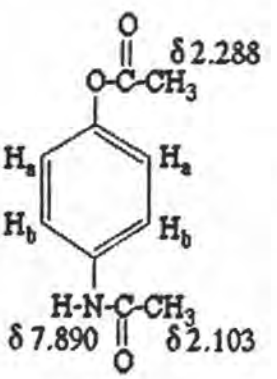
DAAP

δ	2.253	singlet	6H of $-N(COCH_3)_2$
δ	2.266	singlet	3H of $-OCOCH_3$
δ	7.160	singlet	protons of aromatic ring

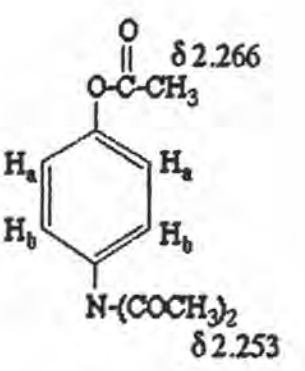
In the spectrum of paracetamol, we could see the signal of hydroxyl proton and proton attached to nitrogen atom at δ 9.020 and δ 7.890 respectively. A group of protons at δ 7.036 were of aromatic ring (it was integrated as having 4 protons) which would suggest that the pair of signal at δ 7.376 and δ 7.278 were of H_a (as shown below on the left) and the pair of signal at δ 6.794 and δ 6.699 were of H_b.



p-acetaminophenol



MAAP



DAAP

Electrons surrounded H_a were withdrawn by oxygen atom whereas of H_b were withdrawn by nitrogen atom. The withdrawing property of oxygen is higher than that of nitrogen so that H_a were deshielded more than H_b causing signal more down field when compared to H_b's The signal generated at δ 2.098 (which was integrated as having 3 protons) by the methyl protons of amide acetyl.

In the spectrum of MAAP, protons attached to nitrogen atom could be seen easily at δ 7.890. The methyl protons of phenolic ester which just been added generate signal at more down field (δ 2.288) when compared to the signal of methyl protons of amide acetyl (δ 2.103). This is due to the fact that these new protons were deshielded by the carboxyl group attached to them. The H_a and H_b (as shown above on the right) signal were also shift to down field (δ 7.220) because of the ester carbonyl group which also withdrawn the electrons around these protons too.

In the spectrum of DAAP, we noted that the signal of protons of aromatic ring changed from quartet to singlet due to an AB-coupling (60). This means that the differences in chemical shift and the coupling constant (i.e. $\delta\nu/J$) of H_a and H_b were almost the same. The two inner peaks of MAAP's quartet fused and became one large signal, while the two outer peaks disappeared. The added acetyl group affects the shielding of electrons around the aromatic protons, so that the signal shifted further down field (δ 7.160).

Since the surrounding effects on methyl protons of phenolic ester, and those on amide acetyl's are similar. This results in the movement of chemical shift of amide acetyl protons to more down field (δ 2.253). With a

consequence that the amide acetyl protons move closer to those of phenolic ester (δ 2.266).

It was shown in figure-8 that the ratio of protons in aromatic protons region and the methyl protons region (both of amide acetyl and phenolic ester) was integrated as 4:9

1.6 GAS-LIQUID CHROMATOGRAPHY (GLC)

Each peak of paracetamol derivatives was identified by comparing the retention time with that obtained with pure derivative products. The retention time of MAAP was about 6 minutes, whereas that of DAAP was about 4 minutes. Paracetamol, on the other hand, is a more polar compound and therefore tended to bond to the polar stationary phase (Carbowax 20M) coated onto the solid support of the GLC column. Since the compound was trapped to the stationary phase inside the column, so there was no free paracetamol available to give rise to a peak. The chromatograms of paracetamol derivatives are shown in figure-9.

2. STUDIES OF FACTORS AFFECTING DERIVERTIZATION REACTION

2.1 EFFECT OF TEMPERATURE

Nine flasks, each containing mixture of paracetamol, pyridine and acetic anhydride were incubated at nine different temperature settings. Afterwhich, benzophenone was added, and these mixtures were then injected into GLC system. Peak area ratios between DAAP/benzophenone and MAAP/benzophenone obtained, and were detailed in table 3 and figure 10.

At a lower temperature range, relatively more MAAP was produced than DAAP. On the other hand, this ratio is reversed at higher temperature. Maximal production of DAAP was achieved with the incubating temperature of about 200°C. After this, further rise does not appear to cause further production increased.

We have chosen to measure DAAP rather than MAAP in our study as the formation of the later compound is so temperature sensitive. A minor variation in the incubating temperature, which is extremely difficult to control accurately, will effect big changes in its production. On the other hand, DAAP production which was relatively temperature insensitive and reaches its maximal production fairly quickly at the temperature of about 200°c and over, and therefore has a very much better reproducibility. Due to temperature sensitive of MAAP, we chose to measure DAAP for determined the amount of paracetamol in this study.

2.2 EFFECT OF INCUBATION TIME

Nine flasks, each containing a mixture of paracetamol, pyridine and acetic anhydride were incubated at 220°C for a varying period of times as detailed in table-4. After acetylation procedure, each product mixture had benzophenone added before being subjected to analysis in the GLC system. Peak area ratio of DAAP/benzophenone and MAAP/benzophenone were tabulated in table-4 and plotted in figure-11.

At the beginning of reaction, MAAP formed quickly in a fairly large amount while DAAP did hardly at all. Both MAAP and DAAP were time sensitive until about 3 hours, the amount of MAAP formed tended to be small while that of DAAP was large and constant while that of MAAP was the reverse.

Because of this findings, we have therefore chosen to incubate at 220°c for 3 hours.

2.3 EFFECT OF AMOUNT AND COMPOSITION OF ACETYLATING REAGENTS

Eleven flasks, each containing a mixture of paracetamol with varying amount and composition of pyridine and acetic anhydride were incubated at 220°c for 3 hours. After the reaction had been completed benzophenone was added, and the mixtures were then injected into a GLC system. Peak area ratios of DAAP/benzophenone obtained from employing different amount and composition of acetylating agents are recorded in table-5 and plotted in figure-12.

It was shown that when the amount of pyridine was fixed and acetic anhydride was varied, or when the amounts of both pyridine and acetic anhydride were varied in the same direction and with the same amount, the peak area ratio of DAAP/benzophenone remained constant. But if the mount of pyridine was increased and acetic anhydride was fixed, the peak area ratio of DAAP/benzophenone decreases as pyridine was increased. This later finding is believed to be the effect of pyridine which reacted with acetic anhydride to yield acetyl pyridinium. Consequently, reduced the amount of acetic anhydride and of DAAP in manner. It has been shown that if the volume of acetic anhydride employed is less than that of pyridine, this may favour MAAP rather than DAAP formation, and since small amount of pyridine may be inadequate to completely dissolve paracetamol, a volume smaller than 4 ml has not therefore, been used in this experiment. Because of the fore-going reasons, four

millilitres each of pyridine and acetic anhydride were selected for acetylation reaction in this study.

2.4 EFFECT OF VARYING CONCENTRATIONS OF PARACETAMOL

Thirteen flasks, each containing a varied amount of paracetamol with 4 ml each of pyridine and acetic anhydride were incubated at 220°c for 3 hours. At the end of the incubation, benzophenone was added, and each mixture was injected into GLC system. The peak area ratios of DAAP/benzophenone obtained with varying amount of paracetamol were recorded in table-6 and plotted in figure-13.

A linear relationship between paracetamol concentration (20 to 1000 mg) and peak area ratio of DAAP/benzophenone was obtained ($r = 0.9954$)

More than 1000 mg of paracetamol would tend to give a line that the upper end started to get flatten as the acetylating reaction has reached its plateau level. Less than 20 mg, however, the linear relationship between these two parameters still exist. A more detailed discussion of this would be made later in section.6.1.

3. QUANTITATIVE DETERMINATION OF PARACETAMOL IN SYRUP PREPARATIONS

3.1 ACETYLATION-GLCMETHOD

All syrup preparations were allowed to passed through the column similar to those in the USPXXII prior to acetylation reaction due to some interferences in the preparations such as

a.) Water in the preparations caused acetic anhydride to be hydrolyzed and turn into acetic acid. Hence, the amount of acetic anhydride was depleted.

b) By passing through the column, colour and preservative in the preparations would be trapped inside the column.

c) Sugar in the preparations could reacted with acetic anhydride so there is no acetic anhydride left for acetylating paracetamol.

3.1.1 Preparation of Standard Solution

Five concentrations of paracetamol were reacted with pyridine and acetic anhydride. After acetylation had completed, the mixture was transferred to 10-ml volumetric flask and equal volume of benzophenone was added. Each mixture was then adjusted to volume with acetic anhydride and injected into the GLC-system.and the peak area ratio of DAAP/benzophenone obtained were included in table-7 and data was used in figure-14.

It is evidence from the graph constructed in figure-14 that a good linear relationship between the varying concentrations (20-100 mg) of paracetamol and peak area ratio of DAAP/benzophenone was obtained, with a correlation coefficient of 0.9998 ($y = 0.00548x - 0.0132$).

3.1.2 Preparation of Chromatographic Column

Sodium carbonate-sodium bicarbonate solution was mixed with siliceous earth. The resultant fluffy mixture was transferred to the column. Since this fluffy material is so alkaline that any strong acidic

compound would form salt with it and be retained in the column, allowing non-acidic or weakly acidic compound such as paracetamol to pass through

3.1.3 Preparation of Sample

All syrup samples were weighed and treated with base. Aliquots were then treated with acid and mixed with siliceous earth and finally transferred to the chromatographic column. The packed column was flushed with water-saturated chloroform followed by water-saturated ether to elute paracetamol and other solvent materials including water that were originally mixed in syrup solution from siliceous earth. Eluent containing paracetamol was subjected to evaporation under stream of room air to remove ether and thereafter subjected to acetylation reaction. Details of the results obtained are presented in table-8.

Since the syrupy solutions were so viscous and was virtually impossible to pipet accurately. We have, therefore, to resorted to accurate weighing instead. Sodium hydroxide was added to help in dissolving paracetamol. The sample was thereafter changed back to its original neutral form with acid.

Siliceous earth -$NaHCO_3$/$NaCO_3$-paracetamol chromatographic column was repeatedly washed with water-saturated chloroform to get rid of the contaminated non-polar compounds in the syrup, but the neutral paracetamol itself was eluted with water-saturated ether.

Both chloroform and ether used in this study needed to be saturated with water to mke the solvent miscible with water presence in syrup preparations.

3.1.4 Procedure

After benzophenone has been added, a portion of the mixture was injected into GLC-system to obtain peak area ratios of DAAP/benzophenone, and these values were presented in table-8. The calculation of percent amount and percent labelled amount were also included in the same table. Chromatogram of samples tested can be seen in figure-15 which suggested that the method employed in the analysis of DAAP/benzophenone performed satisfactory, and that no interfering substances detected under the conditions of the assay described.

Table-21 demonstrates the calculation for the percent labelled amount of paracetamol presented in the syrup samples compared between the acetylation-GLC method with those of the USP XXII. It was found that values obtained from both methods were not statistical significant .in different from each other

Compound analyzed	Percent labelled amount*			
	Acetyl.-GLC	%CV	USP XXII	%CV
Syrup No. 1*	99.54	1.0433	99.06	0.9039
Syrup No. 2**	100.94	0.9065	99.84	0.8320
Syrup No. 3	101.19	0.5001	-	-
Syrup No. 4	104.47	0.6360	-	-
Syrup No. 5	103.57	0.9160	-	-

* = mean of six separate experiments, * t-value = 0.864 with 10 degree of freedom
** t-value = -2.184 with 10 degree of freedom

Table 21 Comparing the Percent Labelled Amonut of Paracetamol in Syrup Preparations Obtained from Acetylation-GLC and USP XXII Method

3.1.5 Percent Recovery

Three aliquots of syrup No. 2 were transferred to separate flask to which either 12, 22 or 32 mg of paracetamol BP had been added. Neutral paracetamol was obtained when each of these samples were processed with siliceous earth chromatographic column and eluted with ether as detailed previously in section on the preparation of sample 3.1.3 above.

Acetylation was carried out, benzophenone was added and the mixture was injected into GLC system. Weights of both paracetamol syrup and the standard added together with the peak area ratio obtained were used in the calculation. The results of this appears in table-9.

Table-22 constructed from data obtained in table 9 and 11, shows quite clearly that the percent recovery of paracetamol in syrup No.2 carried out with either Acetylation-GC method or the USP XXII method gave identical statistical non-significant values

Paracetamol BP added (in mg)	Percent recovery*	
	Acetylation-GLC Method	USP XXII Method
12*	98.99	98.97
22**	100.06	101.58
32***	99.92	100.77

*=mean of two separate experiments, * t-value = -0.013 with 2 d.f, p > 0.05
** t-value = 0.483 with 2 d.f., p > 0.05, *** t-value = 0.465 with 2 d.f., p > 0.05

TABLE 22 Comparing the Percent Recovery of Paracetamol in Syrup No.2 Obtained from Acetylation-GLC and USP XXII Method

3.2 USP XXII METHOD

3.2.1 Preparation of Standard Solution

Paracetamol was dissolved and diluted with methanol. Two different dilutions were prepared, and the second solution had small volume of acid added before final volume had been adjusted. This was to ensure that paracetamol remained in the neutral form. The solution was then measured for absorbance at 249 nm, and the data obtained were used in the construction of table-10.

3.2.2 Preparation of Chromatographic Column

The preparation and setting-up of Chromatographic column were the same as detailed previously in section 3.1.2 above.

3.2.3 Preparation of Sample Solution

All syrup samples were pipetted into flasks containing base, and further diluted to volume with water (as instructed in the manual). These aliquots were then treated with acid, mixed with siliceous earth and transferred to column for packing. Chloroform was added to flush column. This was followed by eluting paracetamol with ether. The eluent was evaporated to dryness with room air precipitate was re-dissolved in acidic methanol to ensure that paracetamol remained in neutral form.

In the USP XXII method, pipetting was suggested for sample transferred. We had followed their suggestion faithfully, despite the fact that the syrup sample was rather viscous and has great tendency to stick to sides of glass pipet. We minimized errors by

i) allowing pipet to stand up right for some time to ensure that excess portion which sticked to the sides had completely trickled down to join the rest of solution giving a proper miniscus before volume was transferred.

ii) allowing the syrup to drain for quite a long time to ensure that the volume of syrup was as close to 10.0 ml as possible.

3.2.4 Percent Recovery

Three aliquots of syrup solution No.2 in section 3.2.3 were transferred to reacting beakers either 12, 22 or 32 mg of paracetamol BP standard was added. Thereafter, they were subjected to treatment with acid, mixed with siliceous earth and packed into the column. The rest of the procedures were the same as those reported in sections 3.2.3 and 3.2.4 above. Weights of both paracetamol syrup and the standard added together with the peak area ratio obtained were used in the calculation. The results of this appears in table-11.

4. QUANTITATIVE DETERMINATION OF PARACETAMOL IN THE TABLET PREPARATIONS

4.1 ACETYLATION-GLCMETHOD

4.1.1 Preparation of Standard Solution

Five concentrations of paracetamol were reacted with pyridine and acetic anhydride, afterwhich benzophenone was further added and the final mixture were injected into the GLC system. The peak area ratio of DAAP/benzophenone for each original concentration obtained displayed in table-12 and in figure-16.

Figure-16 shows plot exhibiting relationship between peak area ratios of DAAP/benzophenone and paracetamol concentrations employed (100-500 mg) with the values of 1.0000 and $y = 0.005712x + 0.00688$ for correlation coefficient and slope, respectively.

4.1.2 Preparation of Sample

Tablet samples were weighed accurately and subjected to acetylation reaction with pyridine and acetic anhydride. After incubation, benzophenone was added to each sample, and results obtained appears in table-13.

4.1.3 Procedure

A portion of mixture prepared were injected into the GLC system, and the results of the peak area ratio of DAAP/benzophenone obtained are included in table-13 together with calculation of the percent amount, percent labelled amount. Chromatograms obtained for each of the sample are shown in figure-17. This figure also indicate further that there were no interference detected in this analytical system.

Table-23 shows that the percent labelled amount of paracetamol in the tablet preparations estimated by acetylation-GLC method was similar if not identical with statistical non-significant in difference to those given by the USP XXII method.

Compound	% Labelled Amount*			
	Acetyl-GLC	% CV	USP XXII	% CV
Tablet No. 1*	101.99	0.1221	102.65	1.6210
Tablet No. 2**	100.24	0.8435	100.73	1.3440
Tablet No. 3	101.19	0.6975	-	-
Tablet No. 4	104.94	0.4983	-	-
Tablet No. 5	103.08	0.6433	-	-

*= mean of six separate experiments, **t-value = 0.968 with 10 d.f, p > 0.05
***t-value = 0.755 with 10 d.f., p > 0.05

TABLE 23 Comparing the Percent Labelled Amonut of Paracetamol in Tablet Preparations Obtained from Acetylation-GLC and USP XXII Method

4.1.4 Percent Recovery

Three sets of powder obtained from tablets No.1 were separately weighed and added to flask containing either 30, 60 or 90 mg of paracetamol BP. They were then subjected to acetylation reaction benzophenone was further added after reaction had completed, and the mixtures obtained were injected into the GLC system. Results of the peak area ratio appears in table-14 calculation for percent amount and percent recovery also appear in this same table.

Table-24 compared results of percent recovery of paracetamol in tablet No.1 obtained with acetylation-GLC versus those with the

USP XXII method. As can be readily seen, the results are almost identical and were statistically non-significant.

Paracetamol BP added (in mg)	Percent Recovery*	
	Acetylation-GLC method	USP XXII Method
30*	100.17	97.93
60**	98.21	98.81
90***	100.60	99.24

* = mean of two separate experiments, * t-value = -3.025 with 2 d.f., $p > 0.05$
**t-value = 0.370 with 2 d.f., $p > 0.05$, ***t-value = -1.143 with 2 d.f., $p > 0.05$

TABLE 24 Comparing the Percent Labelled Amount of Paracetamol in Tablet No.1 Obtained from Acetylation-GLC and USP XXII Method

4.2 USP XXII METHOD

4.2.1 Preparation of Standard Solution

The preparation of the standard solutions as recommended in USP XXII and similar to that detailed in section 3.2.1 above. Results of the absorbance measurements of these solutions appear in table-15.

4.2.2 Preparation of Chromatographic Column

The preparation is identical to that detailed in section 3.1.2 above.

4.2.3 Preparation of Sample

Tablet sample was carefully weighed and transferred to reacting flask containing base this was diluted to volume with water the remaining of the procedure was the same as those reported in section 3.2.3, and as discussed in section 3.1.3. Data obtained appear in table-15.

4.2.4 Procedure

Absorbance for both standard and sample solutions were made at 249 nm, and results appear in table-15, together with results of calculation for % amount and % labelled amount.

4.2.5 Percent Recovery

Again three aliquots of the solution made from tablet No.1 were prepared. Either 30, 60 or 90 mg of paracetamol BP was added, and they were subjected to treatments as detailed previously in section 3.2.3 and 3.2.4 above. Results of weights of paracetamol samples and standards, their absorbances after acetylation and calculations of % recovery are reported in table-16.

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

5.1 PREPARATION OF STANDARD SOLUTION

Preparation of standard solutions as wells as standard curve were the same as detailed in section 4.1.1.

5.2 PREPARATION OF SAMPLE

Both samples of injectable preparations were accurately weighed (as solution was quite viscous), and were subjected to acetylation reaction in the presence of pyridine and acetic anhydride. Afterthat, benzophenone was added, and mixtures were analyzed in a GLC system.

5.3 PROCEDURE

All of the reaction flasks (2 x 6 = 12) used in this experiment were analyzed in the GLC system and the peak area ratio of DAAP/benzophenone obtained are included in report in table-17. Calculation for the % amount as well as for % labelled amount were also included in this same table. The chromatographic results of the samples analyzed appear in figure-18. No interfering substance seen in the tracing.

Table-25 contains calculation results of % labelled amount under studied here. It is of great interest to note that injectable preparation No.2 employed in this study contains only 81% or 4/5 of what was labelled on the ampules. Different batch of the same make was re-analyzed, and again similarly low value was obtained. It was shown that

Compound	% Labelled Amount*	% C V
Injection No. 1	100.20	0.6388
Injection No. 2	81.01	0.5804

* mean of six separate experiments

TABLE 25 The Percent Labelled Amount of Paracetamol Obtained from Acetylation-GLC Method

5.4 PERCENT RECOVERY

Three sets of aliquots of injectable preparation No. 1 were accurately weighed and transferred to each flask. Either 30, 60 or 90 mg of paracetamol BP was added. These were subjected to acetylation reaction and benzophenone was added. Mixture was injected into GLC system and the peak area ratio of DAAP/benzophenone were obtained. Data on weights of both sample and standard, peak area ratio & the percent recovery appear in table-18.

Table-26 shows the percent recovery of paracetamol in injection No.1 and the statistical analysis shows that the percent recovery obtained from different concentration of paracetamol added are not dufferent.

Paracetamol added (in mg)*	Percent recovery*
30	97.47
60	99.27
90	99.44

* = mean of two separate experiments, * F-value = 3.934 with v_1=2 and v_2=3, $p > 0.05$

TABLE 26 The Percent Recovery of Paracetamol in Injection No.1 Obtained from Acetylation-GLC Method

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

6.1 PREPARATION OF STANDARD SOLUTION

Five different concentrations of paracetamol were prepared and were allowed to undergone acetylation reaction with pyridine and acetic anhydride. Afterwhich benzophenone was added and mixtures were then injected into a GLC system. Peak area ratio of DAAP/benzophenone obtained appear in table-19 and figure-19, respectively.

It was evidence that plot between paracetamol concentrations and peak area ratios was linear, and that the correlation coefficient and slope were 0.9994 and $y = 0.03745x + 0.02468$, respectively.

6.2 PREPARATION OF SAMPLE SOLUTION

The serum containing known amount of paracetamol was treated with a base followed by acid before being packed on the column. The column was repeatedly washed with chloroform, and paracetamol eluted with ether. The dried residue obtained (containing paracetamol and the serum) was subjected to acetylation reaction, followed by addition of benzophenone and adjusted to volume with acetic anhydride.

6.3 PROCEDURE

The reaction flask containing mixture above (6.2) was injected into a GLC system, and the peak area ratio of DAAP/benzophenone obtained appear in table-20, while the chromatogram of unknown sample and the serum blank display in figure 20.