CHAPTER IV

SUMMARY AND CONCLUSION

Paracetamol was subjected to derivatization reaction (acetylation) with acetic anhydride with aid of pyridine as solvent and as catalyst. Paracetamol derivatives obtained composed mainly of MAAP and DAAP which were different from their parental compound, such as melting point, R_f-value on TLC plate, absorption in the UV-region, the IR-spectrophotometric pattern, ^{1}H-NMR signals as well as retention time in the GLC system. The GLC chromatogram also indicates that DAAP has a shorter retention time than MAAP, and that it has a symmetrical but narrow peak shape

Various conditions affect derivatization reaction, for example, temperature, incubation time, amount of acetylating agents (pyridine and acetic anhydride) and varying concentrations of paracetamol. All of these factors were included in the study. Preliminary data together with those obtained on temperature and time study have encouraged us to look further into DAAP rather than MAAP. Because of this, the main trust of efforts was therefore directed toward finding conditions that would give the highest DAAP yields. In full-filling this objective, it was found that, i) the refluxing time, i.e. time allowed for complete acetylation, should not be less than 3 hours. Longer incubation, however, did not give a higher yield, ii) the optimum incubation temperature was found to be about 220°c, but a higher temperature did not give a significantly higher yield, and iii) pyridine works well as catalyst in this system when it was presented in the same amount and ratio as that of acetic anhydride, and that their optimum volume was found to be about 4 ml.

Under strict conditions as detailed above, as little as 20 µg of paracetamol in one ml of plasma or serum could be easily measured with this method. On the other hand, a concentration of more than 1000 mg falls on the less sensitive part of the standard curve so it should be first diluted before assayed. But this is neither difficult or complicated, and does not alter other assay's attributes.

Only 0.2 µl of sample was injected into the GLC system with benzophenone as internal standard. The column employed in this study has a Carbowax 20M coated on the solid support (10m x 0.53mm x 1.33µm film thickness) and N_2 was used as carrier gas. The oven temperature was programmed to start at 140°c for one minute followed by an increase rate of 20°c per minute until the oven temperature reaches 200°c. The injection port and detector temperature were setted at 250°c.

This method was employed in the measurement of paracetamol in tablet, syrup and injectable formulations. The result compared favourably with those recommended in the USP XXII, with no statistical significant in difference (α =0.05). It was also found that other active ingredients and excipients did not interfere with this method. Furthermore, if this method was to applied to human plasma and serum, we would need to document whether or not the plasma constituents extracted together with the drug interfered in any way with the assay system. Since paracetamol, (which can not, in any case, be assayed directly without prior derivatization), will have to be first extracted from this physiological fluid before it can be derivatized and measured in this assay system. This information is, therefore, of utmost important if the method is to be used in clinical or pharmacological settings, such as in the pharmacokinetic and pharmacodynamic or toxicological studies.

It should be mentioned here that drugs or compounds that are difficult to volatile and therefore can not readily be measured or identified in the GLC system that have hydrogen attached to high electronegative atom can now be done so with derivatization reaction technique described in this study.