CHAPTER V

SUMMARY AND CONCLUSION

The work done indicated that methyltestosterone in vitamin-hormone preparations could be determined by spectrofluorodensitometric method. Methyltestosterone was separated from other substances by alumina TLC plate in optimum developing solvent. The fluorescent compound was formed by heating at 150° for 20 minutes and detected by using a fluorescence spectrophotometer in connection with a thin-layer chromatography accessories with the wavelength of maximum excitation at 366 nm and the wavelength of maximum emission at 454 nm.

The completely separation of methyltestosterone will be accomplished by using the optimum mobile phase of the 60:40 mixture of benzene and diethyl ether. The TLC plate should be activated before application of the test material in order to obtain reproducible R_f values. The application volume of the assay solution must be accurate and constant in order to obtain reproducible spot areas and R_f values.

The fluorescence intensity between spots within the same plate was reproducible. The data-pair technique was carried out in order to minimize the systematic errors due to the chromatographic parameters. The fluorescent compound, 4-hydroxy-3-oxo-$\Delta^{4,6}$-steroid, was also stable to time. The relationship between concentration of methyltestosterone and peak heights of the fluorescence was linear in the

range 0.025-0.200 mg per ml for a 1 µl application volume.

The proposed method and USP method were used to determine methyltestosterone in commercially single drug dosage form. The amounts of methyltestosterone obtained by both methods showed close relationship. However, this proposed method has more advantage in combined drug dosage forms. Since the other substances such as hormone, vitamins, minerals and other excipients in vitamin-hormone preparations were separated by thin-layer chromatography before determining the amount of methyltestosterone by densitometer, there were no interference from those substances. Although the method required a special technique and availability of certain equipments. The pretreatment and procedure were simple, and the method was sensitive, specific and accurate. This method was suitable for routine analysis of methyltestosterone in vitamin-hormone preparations.

The results obtained from the study of the proposed method could be summerized as followed:

1. The separation technique could be employed successfully for thin-layer chromatography.

2. The fluorescence-inducing procedure for methyltestosterone was carried out by heating in which aluminum oxide acted as a catalyst and the fluorescence intensity remained constant for a few days.

3. The procedure was very sensitive to micro-amount of methyltestosterone in formulations.

4. The method was specific, accurate with high reproducibility, and could be used to determine methyltestosterone in combined drug preparations e.g. vitamin-hormone preparations.