CHAPTER I

INTRODUCTION

Methyltestosterone is an androgenic hormone used in therapy primarily in the event of impaired function of the testicle: castration symptoms, infantidism, impotence, and male climax symptoms. It used for the palliative treatment of certain cases of metastatic breast carcinoma in women. Methyltestosterone is often applied as a component of multivitamin preparations for its anabolic properties. It increases protein synthesis, causes retention of nitrogen and a gain in weight, in both normal and pathological body. It is useful to promote healing of fractures in old men, and in combination with an estrogen, is used in the treatment of postmenopausal osteoporasis(1,2). Methyltestosterone in single drug preparations and preparations of various mixtures with estrogen, vitamins and other agents are distributed in the forms of tablet and capsule.

There were many analytical methods of methyltestosterone in pharmaceutical preparations. For the quantitative determination, of bulk materials and single drug dosage forms, spectrophotometric methods are most frequently used(3-5). One official analytical method of methyltestosterone prepparations is a direct measurement with a maximum absorption at about 241 nm after extraction with chloroform(3). Other method based on a chemical reaction, for example the reaction

of Δ^4-3-keto group of methyltestosterone with 2,4-dinitrophenylhydrazine(4). Hosangadi and Farias(5) had developed two methods for colorimetric determination of its 2,4-dinitrophenylhydrazone. The excess reagent was separated, by using a strong acidic cation exchange resin in the first method, and using solvent extraction with hexane in the second one. Determination of methyltestosterone by infrared spectrophotometric method have been reported by Carol(6) and Ito and Amakasu(7).

Powelezyk et al.(8) developed a gas chromatographic method for methyltestosterone preparations. Bruschi(9) also described a gas chromatographic procedure which was used to determine both methyltestosterone and vitamin E (tocopheryl acetate) in combined preparations.

Jarzebinski et al.(10) recommended a densitometric method for the determination of methyltestosterone and dieneestrol tablets. They used thin-layer chromatographic separation with a suitable mobile phase and detected the colored spots after spraying with methanolic solution of isonicotinohydrazide. Ivanova and Sokolov(11) developed a chromatospectrophotometry for methyltestosterone determination, using the absorbance measurement at 242 nm.

Densitometry is a quick and reliable method for the direct determination of methyltestosterone without preliminary purification. Because of the very low concentration of methyltestosterone in vitamin-hormone preparations, fluorescence-inducing procedure with thin-layer chromatography is

now used.

The purpose of this thesis is to find the best method which can be used in quality control of methyltestosterone in vitamin-hormone preparations. The method was based on the specific reaction of Δ^4-3-keto group of methyltestosterone with atmospheric oxygen leading to 4-hydroxy-3-oxo-$\Delta^{4,6}$-steroid. Methyltestosterone should be separated from vitamins and other substances with TLC plate aluminum oxide 150 F_{254} (type T) in optimum solvent system. The blue fluorescent compound, 4-hydroxy-3-oxo-$\Delta^{4,6}$-steroid, could be formed by heating at high temperature, in which aluminum oxide reacted as a catalyst. The intensity of fluorescence was determined by using spectrofluorodensitometer. This method is very sensitive and selective for the preparations of methyltestosterone containing vitamins, minerals and other hormone such as ethinyl estradiol.