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Quality evaluation of the Finasteride polymorphic forms I and II in capsules

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ABSTRACT

Finasteride (FNS) is a specific competitive inhibitor of steroid type-II 5α-reductase and is widely used for the treatment of benign prostatic hyperplasia, prostate cancer, and androgenetic alopecia. FNS has two polymorphic forms identified as Form I and Form II. It is known that polymorphism can cause significant differences in the physicochemical properties of a compound such as melting point, density, morphology, solubility, and color. Thus, proper qualitative and quantitative monitoring of the solid-state forms is crucial to ensure high-quality products. There are no published papers studying the influence of the FNS polymorphs on the physicochemical quality of capsules. Furthermore, the available analytical methods are time-consuming, expensive, use buffer or do not demonstrate stability-indicating capacity. The aim of this work was to validate a rapid high-performance liquid chromatography (HPLC) method to evaluate FNS in capsules and to study the physicochemical properties of polymorphic forms, evaluating their possible influence in the dissolution profile and stability of FNS in capsules. Capsules containing Forms I and II of FNS were prepared and subjected to quality control studies, dissolution profiles and a stability study at 50 °C. A significant effect of polymorphism on the FNS solubility and dissolution properties was observed. These results suggest that changes in the effects of FNS can occur if a suitable control study is not performed on the raw material used to produce the capsules.

1. Introduction

Finasteride, chemically named *N*-(1,1-dimethylethyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide (Fig. 1), is a specific competitive inhibitor of steroid type-II 5α-reductase, an intracellular enzyme that converts testosterone to dihydrotestosterone, and Finasteride is widely used for the treatment of benign prostatic hyperplasia (BPH), prostate cancer, and androgenetic alopecia [1–6]. A daily dose of 5 mg has been used for the treatment of BPH and prostate cancer, and a 1-mg dose has been used for the treatment of androgenetic alopecia. FNS has a molecular formula of $C_{23}H_{36}N_2O_2$, a molecular weight of 372.6 g/mol and a log *P*o/w value of 3.03. According to the Biopharmaceutics Classification System, FNS is a Class 2 drug with low solubility and high permeability [7].

Polymorphism is the ability of a solid material to exist in more than one form or crystal structure. It is estimated that approximately 80–90% of organic compounds can exist in multiple crystalline forms (polymorphs, hydrates, and solvates) [8]. Polymorphs are known to give rise to significant differences in the physicochemical properties of the compound, including melting point, density, morphology, solubility, and color. This in turn may have an impact on the physical and chemical stability, the bioavailability, and the processability during manufacturing and/or in the final product. In an extreme case, an undesired polymorph can even be toxic [9]. Thus, proper monitoring of the solid-state forms, both qualitative and quantitative, is crucial to ensure high-quality products [10].

The crystal structures of FNS polymorphs appear in the Cambridge Crystal Structure Database under the codes WOLXOK01 and WOLXOK02 for Form I and WOLXOK03 for Form II. Form I is orthorhombic, while Form II is monoclinic. The system is

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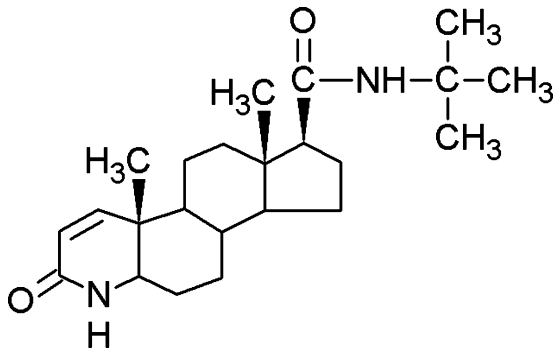

Fig. 1. Chemical structure of FNS.

enantiotropic, with transition from Form I to Form II observed to occur between 200 and 230 °C, depending on the heating rate [11].

Wawrzycka et al. [12] reported that FNS Form I is pharmaceutically preferred due to its better stability. In the literature, there are descriptions that characterize the FNS polymorphs by powder X-ray diffraction (PXRD), infrared spectroscopy (IR), nuclear magnetic resonance (NMR) spectroscopy, and differential scanning calorimetry (DSC) [11–14]. However, there are no published papers studying the effect of FNS polymorphs on the physicochemical quality of capsules.

The United States [15], European [16], and British [17] Pharmacopoeias describe different methods for the analysis of FNS in raw materials and tablets. However, some of these methods take longer than 40 min for the chromatographic run. Additionally, the published separation techniques are expensive as ultra-high performance liquid chromatography (UHPLC) methods, use buffer in the mobile phases or do not study the stability-indicating capacity for the determination of the FNS in the capsules.

In light of the considerations outlined above, the aims of this work were to validate a rapid reverse phase high performance liquid chromatography (RP-LC) method to evaluate the FNS in capsules and to study the physicochemical properties of the polymorphic forms, examining their possible effect on the dissolution profiles and the stability of the FNS in capsules.

2. Experimental

2.1. Chemicals and reagents

FNS United States Pharmacopeia Chemical Reference Substance, batch number G0K072 with a declared purity of 99.8%, was purchased from USP® (Maryland, EUA). The FNS raw material was acquired from Fagron do Brasil Farmacêutica Ltda (São Paulo, São Paulo, Brazil). The 5 mg FNS capsules and placebo were prepared in our laboratory. They were produced using the following pharmaceutical grade excipients: hydrous lactose (5.0%), starch (67.0%), magnesium stearate (0.5%), colloidal silicon dioxide (1.0%), sodium dodecyl sulfate (1.5%), and talc (25.0%). The average weight of the capsules was 142.8 mg.

2.2. Equipments

The HPLC equipment used was Shimadzu series LC-10A (Shimadzu, Kyoto, Japan). The liquid chromatography–mass spectrometry (LC–MS) experiments were carried out on a Micromass Platform LC system (Micromass, Manchester, UK). The dissolution tests were performed in an Electrolab TDT-08 L multi bath ($n = 8$) dissolution test system (Electrolab, Mumbai, India). Powder X-ray diffraction (PXRD) of samples was performed on a Rigaku ultima IV X-ray diffractometer (Rigaku Company Ltd., Tokyo, Japan). Infrared Spectra (ATR/FTIR) were obtained using an Affinity-1 Fourier Transform infrared spectrophotometer (Shimadzu, Tokyo, Japan). Differential scanning calorimetry (DSC) was performed using a Mettler Toledo instrument model DSC 1 STAR system (Mettler-Toledo, São Paulo, Brazil).

2.3. Solutions

The stock standard solution was prepared weighing 10 mg of FNS reference substance, transferring it to a 100 mL volumetric flask, and diluting to volume with mobile phase. For the dissolution analysis, the stock solution was diluted in the dissolution medium.

To prepare the sample solutions, 20 capsules were used. The weight equivalent to 2 capsules of each product was transferred to a 100 mL volumetric flask, 80 mL of mobile phase was added, and the flask was sonicated for 5 min. Mobile phase was used to bring the solution to volume and the solution was filtered. The filtrate was diluted immediately to appropriate concentration levels using mobile phase.

2.4. RP-LC method

The chromatographic separation was performed at 30 °C on a LiChroSpher RP-8 (Merck®, Darmstadt, Germany) packed column (150 × 4.6 mm i.d., 5 μm particle size) using acetonitrile:water (70:30, v/v) as mobile phase at flow rate of 1.0 mL/min. The mobile phases were prepared daily, filtered through a 0.45 μm membrane filter. The detector wavelength was set to 210 nm, and the injection volume was 20 μL.

2.5. LC–MS method

To evaluate selectivity of the method for degradation products, studies were also performed on a LC–MS system. The column used was a LiChroSpher RP-8 (Merck®, Darmstadt, Germany) packed column (150 × 4.6 mm i.d., 5 μm) at 30 °C. The mobile phase was acetonitrile:0.01% formic acid (70:30, v/v) at a flow rate of 1.0 mL/min, splitting 0.2 mL/min to the ESI-MS interface after the UV detector.

2.6. Dissolution method

The dissolution method used was previously developed and validated in our laboratory [18]. The experimental conditions were 500 mL of water at 37.0 ± 0.5 °C as dissolution medium, a basket as apparatus (USP Apparatus 1) and rotation speed of 100 rpm. Two milliliters of the dissolution medium were sampled after 3, 9, 15, 20, 30, 45, and 60 min, followed by immediate medium replacement.

2.7. Validation of the RP-LC method

The method was validated for the following parameters: specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), and robustness according to the International Conference on Harmonization (ICH) guidelines [19,20].

The stability-indicating capability of the method was determined by subjecting a reference sample solution (100 μg/mL) to accelerated degradation by acidic, alkaline, neutral, and oxidative conditions to evaluate the interference of the degradation products on the quantitation of FNS [19]. The samples were subjected to stress conditions in water, hydrochloric acid (0.1 M HCl), sodium hydroxide (0.1 M NaOH), and hydrogen peroxide (3% H_2O_2) at 60 °C for 24 h. Corresponding blanks were simultaneously carried out. A sample containing only placebo and a sample containing placebo spiked with FNS were also assessed. Then, the stability-indicating