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Solution and Solid State Nuclear Magnetic Resonance Spectroscopic Characterization of Efavirenz

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ABSTRACT

Samples of efavirenz (EFZ) were evaluated to investigate the influence of the micronization process on EFZ stability. A combination of X-ray diffraction, thermal analysis, FTIR, observations of isotropic chemical shifts of ^{1}H in distinct solvents, their temperature dependence and spin-lattice relaxation time constants (T_1), solution (1D and 2D) ^{13}C nuclear magnetic resonance (NMR), and solid-state ^{13}C NMR (CPMAS NMR) provides valuable structural information and structural elucidation of micronized EFZ and heptane-recrystallized polymorphs (EFZ/HEPT). This study revealed that the micronization process did not affect the EFZ crystalline structure. It was observed that the structure of EFZ/HEPT is in the same form as that obtained from ethyl acetate/hexane, as shown in the literature. A comparison of the solid-state NMR spectra revealed discrepancies regarding the assignments of some carbons published in the literature that have been resolved.

Introduction

In recent years, much attention has been focused on the predisposition of pharmaceutical solids to crystallize in several crystalline forms.[1-3] Distinct crystalline forms of a drug can exhibit different chemical and physical properties, the most important being stability, dissolution, and bioavailability. The thermodynamically stable form is generally chosen for pharmaceutical development into the final dosage product.

Efavirenz ([4S]-6-chloro-4-[2-cyclopropylethynyl]-4-[trifluoromethyl]-2,4-dihydro-1H-3,1-benzoxazin-2-one [EFZ]) is one of the drugs used in an anti-HIV cocktail distributed at no charge by the Unified Health System of Brazil, and its structure presents different hydrogen bonding sites that increase the possibility of exhibiting distinct crystal packing behaviors on recrystallization. Until now, the patents of EFZ reported 23 different polymorphic structures, as well as solvated hydrates and amorphous forms.[4-9] However, there remains ambiguity about the actual number of solid forms (polymorphs and solvates) of EFZ. Patent data show that form I is the most stable polymorph, and all other forms revert to this one under specific conditions. Therefore, several authors have investigated the solid-state structures of recrystallized products of EFZ.[10-13] In addition, these forms have not been adequately characterized and distinguished. The different recrystallization conditions, such as refluxing and/or heating, cooling and stirring, the use of solvent mixtures with varying polarity, and antisolvent addition or drying under vacuum, yielded different forms of EFZ. Cuffini et al.,[10] Ravikumar and Sridhar,[11] and Mahapatra et al.[12] reported distinct packing behaviors via hydrogen bonds from the N-H and oxygen atom of the carbonyl group to polymorph I, but the 3 structures were distinct and did not match well with those reported in several patents.

Cuffini et al.[10,13] reported the crystallography of the EFZ structure obtained in MeOH/H_2O solvent. X-ray diffraction data showed the presence of 2 symmetric independent molecules in the

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crystallographic asymmetric unit, with remarkable differences in the orientation of the 2-cyclopropylethynyl residue. The major distinction occurs in the O_{ring}—C2—C9—C10 (Fig. 1) torsion angles, which guide different orientations of the cyclopropyl moiety, with the formation of a cyclic dimer through the N-H···O linkage. The authors also published a characterization of the anhydrous polymorph I (raw material) and II (obtained by MeOH crystallization) using solid-state techniques to address the thermodynamic stability and strategies to improve the dissolution properties. Those studies showed that polymorph II was more stable and was 10-fold more soluble than polymorph I due to morphology modifications. However, Mahapatra et al.[12] reported that form I could be obtained at the interface of an acetonitrile–water solvent system and from a methyl cyanide–water mixture. The products were characterized by differential scanning calorimetry (DSC) and X-ray powder diffraction (XRPD), and it was observed that the EFZ structure shows a significant degree of conformational disorder in the cyclopropyl group. According to the authors, the packing involves the formation of helical hydrogen bonds between the amide NH and the neighboring amide carbonyl (N-H···O).

Currently, 90% of drugs are administered in the solid form, which implies formulation and drug processing into a powder. Reduction of the particle size of the drug is generally achieved using micronization and milling processes. These processes increase the solubility and dissolution rate of the drug and are fundamental to the therapeutic effectiveness. However, it is known that the size reduction process of the drug particles can modify the structural properties of the raw material, leading to the formation of polymorphs,[14] which often present significant differences in solubility, bioavailability, processability, and physical–chemical stability.

In this work, the EFZ structure of a nonprocessed sample (EFZ/NPR) was investigated by solution and solid-state nuclear magnetic resonance (NMR) spectroscopy. The micronization process and recrystallization in heptane were also evaluated using different analytical techniques, such as XRPD, thermogravimetric analysis (TGA), DSC, FTIR, scanning electron microscopy (SEM), and ^{13}C solid state NMR. EFZ recrystallized in heptane (EFZ/HEPT) sample was also investigated by single crystal X-ray diffraction technique to control its crystal structure corresponding to the form I reported in the literature by Ravikumar and Sridhar.[11]

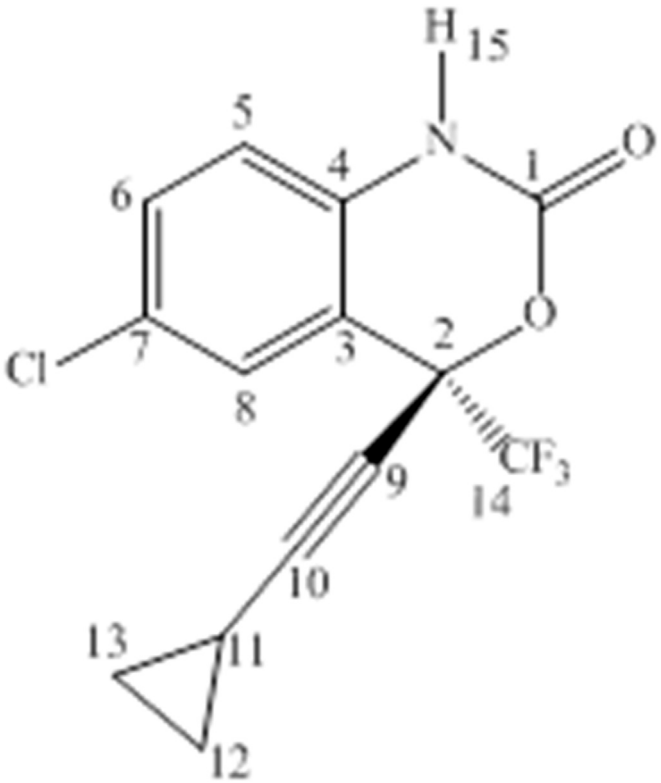

Figure 1. EFZ chemical structure.

Materials and Methods

Materials

EFZ/NPR and micronized EFZ (EFZ/MIC) samples were provided by Farmanguinhos (FIOCRUZ, Rio de Janeiro, Brazil). All reagents and solvents used were of analytical grade.

Procedures

A sample of EFZ/HEPT was prepared by dissolving approximately 100 mg of EFZ/MIC in 50 mL of boiling heptane (98°C) for 5 min. The solution was cooled, and the material began to precipitate at approximately 64°C. The crystals obtained were filtered and stored in a desiccator.

Melting Points (MPs)

MPs were determined using a BÜCHI Melting Point B-545 apparatus with a heating rate of 5.0°C/min.

TGA and DSC

TGA and DSC analyses were performed on a NETZSCH STA 449 F3 Jupiter® thermal balance using 4.0-8.0 mg of samples with a heating rate of 10°C/min. The parameters for TGA analysis were dry nitrogen flow rate of 50 mL/min and heating from 25 to 400°C, whereas for the DSC tests, a flow rate of 80 mL/min of dry nitrogen and heating from 25 to 210°C was used. The instrument was calibrated using indium as a reference standard.

X-Ray Powder Diffraction

The XRPD patterns of the samples were recorded on an X-ray diffractometer (ULTIMA IV, Rigaku) equipped with standard sample holders, with Cu as the tube anode. The diffractograms were recorded under the following conditions: voltage 40 kV, 20 mA, and fixed divergence slit using the configuration of 2θ range from 5° to 80°, with a step size of 0.02 and a 10-s dwell time. Phase transformations were avoided during sample preparation. Approximately 200 mg of samples were loaded into the sample holder. The preferred orientation effects were avoided by side loading the sample holder rather than vertically loading.

Single Crystal X-Ray Structure Determination

X-ray diffraction data collection (φ scans and ω scans with κ offsets) were performed on an Enraf–Nonius Kappa–CCD diffractometer (95-mm CCD camera on κ–goniostat) using graphite-monochromatedMoKα radiation (0.71073 Å). The software COLLECT[15] and Denzo–Scalepack package of softwares[16] were applied for acquisition, indexing, integration, and scaling of Bragg reflections. The final cell parameters were obtained using all reflections.