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Research paper

Correlation between microstructure and bioequivalence in Anti-HIV Drug Efavirenz

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ABSTRACT

Polymorphism and particle size distribution can impact the dissolution behaviour and, as a consequence, bioavailability and bioequivalence of poorly soluble drugs, such as Efavirenz (EFV). Nevertheless, these characteristics do not explain some failures occurring in *in vitro* assays and in *in vivo* studies. EFV belongs to Class II and the High Activity Antiretroviral Therapy (HAART) is considered the best choice in the treatment of adults and children. EFV is a drug that needs bioequivalence studies for generic compounds. In this work, six raw materials were analyzed and two of them were utilized with human volunteers (*in vivo* assays or bioequivalence). All the routine pharmaceutical controls of raw materials were approved; however, the reasons for the failure of the bioequivalence assay could not be explained with current knowledge. The aim of this work was to study microstructure, a solid-state property of current interest in the pharmaceutical area, in order to find an explanation for the dissolution and bioequivalence behaviour. The microstructure of EFV raw materials was studied by Whole Powder Pattern Modelling (WPPM) of X-ray powder diffraction data. Results for different EFV batches showed the biorelevance of the crystalline domain size, and a clear correlation with *in vitro* (dissolution tests) and *in vivo* assays (bioequivalence).

1. Introduction

All solid-state characteristics of drugs can potentially impact their dissolution behaviour and, as a consequence, their bioavailability and bioequivalence. This is especially true in the case of poorly soluble drugs, for which crystalline structure or polymorphism, particle size distribution, apparent density, flowability and compressibility are all carefully controlled by solid-state pharmaceutical routine protocols. However, other solid-state properties should be studied to understand some failures occurring in *in vitro* assays (dissolution tests) and *in vivo* studies (bioequivalence), which cannot be explained by the currently used protocols.

According to the Joint United Nations Program on HIV/AIDS (UNAIDS), in 2012 the number of people living with AIDS worldwide was estimated at 35.3 million, and antiretroviral therapy averted 6.6 million AIDS-related deaths [1].

Efavirenz (EFV) was approved for the treatment of human immunodeficiency virus type 1 infection (HIV-1) in 1998 [2–4]. In the High Activity Antiretroviral Therapy (HAART), it is considered the best choice in the treatment of adults and children [5]. EFV is a Class II drug (low solubility, high permeability) according to the Biopharmaceutical Classification System (BCS) [6], showing

Abbreviations: EFV, Efavirenz; HAART, High Activity Antiretroviral Therapy; WPPM, Whole Powder Pattern Modelling; UNAIDS, United Nations Program on HIV/AIDS; HIV-1, human immunodeficiency virus type 1; BCS, Biopharmaceutical Classification System; API, active pharmaceutical ingredients; SLS, sodium lauryl sulphate; DE, Dissolution Efficiency; SD, standard deviation; SR, Synchrotron Radiation; XRPD, X-ray Powder Diffraction; SEM, Scanning Electron Microscopy.

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poor gastrointestinal absorption due to inadequate drug solubility in gastrointestinal fluids [2]. Besides that, the oral bioavailability of this drug is between 40% and 45% [5].

The generic version of EFV is a viable solution to offer quality medications to a much larger number of AIDS/HIV patients, provided that drug approval and registration are made according to existing national and international regulations covering the innovator's version. In this context, bioequivalence assessment is the most important quality control tool in the process of a generic product development and registration, in order to ensure its therapeutic efficacy [7,8]. Even when the known critical conditions of purity and solid state characteristics were routinely controlled for the raw materials, significant differences were detected when administrated to healthy volunteers in bioequivalence studies. That was the case for two raw material batches of EFV studied in this work, used in bioequivalence studies where one was approved while the other one failed. The bioequivalence studies were conducted on volunteers whose age ranged between 18 and 45 years. The analytical tests and details of these studies were reported by Bedor et al. [9] and Honorio et al. [10], who followed the Brazilian protocol for bioequivalence studies [11], in accordance also with international recommendations for this kind of evaluation. Apart from the *in vivo* studies, so far there are no clear explanations for the dissolution (*in vitro*) tests with the specific conditions used in this work. These results showed significantly different behaviour from batch to batch, after the micronization process, for reasons that were not understood. Therefore, it is important to understand this discrepancy not only *in vitro*, but also *in vivo* in order to guarantee the bioequivalence of generic drugs and the reproducible quality in batch to batch production. It is crucial to acquire a deeper physicochemical knowledge to control the properties and solid-state characteristics of the pharmaceutical raw materials.

Useful indications might be obtained by a detailed study of the micronization process, in particular of the mechanical effects on the microstructure of active pharmaceutical ingredients (APIs). This is a well-known subject in Materials Science, where the correlation between microstructure and performance of materials belonging to all major classes such as ceramics, metals and alloys has long been recognized [12,13]. So far, none of these concepts has been appropriately addressed in the pharmaceutical sciences.

The aim of this study was to demonstrate the effect of microstructure of EFV (AIDS drug) raw material, expressed in terms of correlation of crystalline domain size with both *in vitro* assays (dissolution profiles) and *in vivo* studies of human oral bioequivalence.

2. Materials and methods

2.1. Materials

Six EFV batches were provided by two Brazilian institutions, the governmental pharmaceutical laboratories Farmanguinhos-FIOCRUZ and LAFEPE. All batches were micronized.

During the bioequivalence studies, raw material bathes 1 and 5 were used, respectively, in the approved and not approved biobatches. The reference drug product (Stocrin®, 600 mg tablets) was commercially available at the time of the study. Details of clinical data from the approved biobatch can be found in Honorio et al. [10], and for the not approved biobatch in Bedor et al. [9]. Prior to volunteer recruitment, all protocols were approved by the research ethics committee, as detailed in each specific study.

The bioanalytical conditions used in the bioequivalence studies can be found in the same publications, also following ANVISA recommendations at the time of the studies [14], which closely adhere to the international guidelines.

2.2. Dissolution profiles

EFV raw materials (200 mg of pure drug) were added to 300 ml of sodium lauryl sulphate 0.25% (SLS, dissolution medium) at 37 ± 0.5 °C. The test was made with 75 rpm stirring in standard dissolution equipment (Nova Ética, Brazil). The apparatus 2 (USP) was used and samples of 5 ml were withdrawn at 5, 10, 15, 20, 30, 45, 60, 90 and 120 min. The medium was refilled with an equal amount of fresh solution to maintain a constant total volume. The specimens were analyzed by high-performance liquid chromatography. The mobile phase consisted of acetonitrile: ammonium acetate buffer pH 7.5 (50:50 v/v) and wavelength detection at 252 nm. The other chromatographic conditions were: a PerkinElmer® C18 (150 mm × 4.6 mm, 5 μm) column, flow rate of 1 ml/min and injection volume of 20 μl. The Dissolution Efficiency (DE) was calculated as the area under the dissolution curve (AUC_{0-120}) up to a certain time, t, expressed as a percentage of the area of the rectangle described (AUC_{TR}) by 100% dissolution over the same time interval [15]. The DE was calculated to compare the relative performance of the six batches. Results were expressed as mean values ± standard deviation (SD). Statistical comparisons were made by Student's t-test using the GRAPH PAD PRISM INSTAT Program (San Diego, CA, USA), considering $P < 0.05$ to be significant.

2.3. X-ray Powder Diffraction (XRPD)

XRPD pattern was recorded on a XPERT PANalytical diffractometer, equipped with X'Celerator detector, using Ni filtered kα radiation from a Cu tube operating with 40 kV and 45 mA, 2 Theta range from 5–30°, scan step size of 0.033° and scan step time of 45 s. The Soller, divergent and antiscattering slits used were 0.04 rad and 0.25E respectively.

2.4. Synchrotron Radiation (SR) X-ray Powder Diffraction (XRPD)

Structural and microstructural analysis was based on SR XRPD measurements made at the MCX beamline of the Italian synchrotron Elettra-Sincrotrone Trieste, using the Debye–Scherrer (capillary) geometry [16]. Specimens of the six EFV batches were loaded in Kapton® capillaries and measurements were carried out in duplicate. Structural information was obtained by modelling the data with the software TOPAS© [17,18]. All samples were identified as EFV polymorph 1, with space group $P2_12_12$ and cell parameters $a = 16.781$ Å, $b = 27.258$ (Å), $c = 9.698$ (Å) (data collected at 250 K [19]). This structural information was used as initial model in the refinement of data collected at 298 K, giving $a = 16.88(1)$ Å, $b = 27.335(5)$ (Å), $c = 9.765(2)$ (Å), where the number in parentheses is standard deviations of the distribution of values in the six batches. Microstructural information was provided by the analysis of the diffraction line profiles, using the software PM2K [20,21], implementing the Whole Powder Pattern Modelling (WPPM) approach [22–24]. WPPM is based on a physical model of the microstructure to generate theoretical expressions for the line profiles, and relies on the assumption that the observed diffraction line profile is a convolution of profile components produced by all contributing effects, such as the instrumental profile, coherent scattering domains size/shape, lattice distortions, etc. Microstructural parameters can then be obtained from a non-linear least squares fitting of the experimental powder pattern, using Fourier Transforms of the individual profile components to handle the usually complex convolution problem. Details can be found in the literature [22–24]: here the method was applied representing the EFV crystallites as equiaxed crystalline domains. The latter were modelled as spheres with a lognormal distribution of diameters D ($\langle D\rangle = d[1,0]$ = arithmetic mean, $\langle D\rangle s = d[3,2]$ = surface-weighted mean, $\langle D\rangle v = d[4,3]$ = volume-weighted mean). The modelling then