Polymorphic stability of darunavir and its formulation

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Abstract Darunavir (DAR) is a synthetic antiretroviral drug widely used around the world as an important component of HIV therapy. Usually, DAR is commercialized at ethanolate crystalline form. Under different environmental conditions, different pseudo-polymorphic forms arise and convert each other. The importance of the drug stability control is to ensure their quality, efficacy and safety. Therefore, the aim with this work was to evaluate the solid-state stability of DAR under stressed conditions and assess the role played by the formulation and package in providing stability to the drug. In this work, DAR ethanolate raw material and its tablet formulation were stocked under stress condition (55 °C) and monitored for 94 days. Thermal analysis (DSC and TG) and powder X-ray diffraction were used to evaluate possible polymorphic changes. There has been alteration of the crystalline structure of the raw material and the tablets stored outside of the primary packaging. However, the tablets kept within the primary package during the entire time remained with intact crystal structure, suggesting that as important as the formulation or coating is the protection provided by the packaging to the product used today. Thus, the packaging material to be used in the product containing DAR should be chosen carefully.

Keywords Darunavir ethanolate · Stability · Polymorphism · Drug formulation

Introduction

The study of the physical stability of drugs should be properly conducted in order to know the aspects of the proposed formulation and predict possible changes in the solid state of the drug [1]. The pharmaceutical processes (granulation, drying, compression) can lead to polymorphic transformations, and therefore, the critical steps in this process must be identified and controlled to ensure its stability until the end of its shelf life [1, 2].

As highlighted by Bonfilio et al. [3], the polymorphism in pharmaceutical solids can be a critical manufacturing variable and therefore has to be investigated.

Darunavir (DAR) is a synthetic non-peptidic protease inhibitor (Fig. 1). Since its emergence, many studies have demonstrated the efficacy of DAR against HIV [4, 5], and this antiretroviral drug has become an important component of the high active antiretroviral therapy. DAR inhibits dimerization and catalytic activity of the protease of HIV-1, selectively inhibiting the cleavage of Gag/Pol polyproteins encoded in the HIV virus-infected cells, preventing the formation of mature infectious viral particles [5].

DAR is marketed in ethanolate form under the brand name of PrezistaTM, and it is available in different dose strengths such as 75, 150, 300, 400 and 600 mg in tablet formulation and as an oral suspension 100 mg mL⁻¹. The dosage form used in this study was 300 mg DAR tablets, and it is the unique dose strength available in Brazil. This dosage has the following compositions: 325.24 mg of DAR ethanolate and the inactive ingredients (crospovidone, magnesium stearate), Opadry[®] II White (partially

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Fig. 1 Chemical structure of DAR

hydrolyzed polyvinyl alcohol, titanium dioxide, macrogol and talc) and Prosolv[®] (microcrystalline cellulose and colloidal silicon dioxide) [6].

As HIV is a global disease, DAR is used throughout the world, and consequently, it is stored under different environmental conditions. It is extremely important to study the stability of DAR keeping in mind that pharmaceuticals are especially sensitive to environmental factors.

Stability can be defined as the ability of a drug substance or product to maintain its established specifications as identity, strength, quality and purity throughout expiration dating period. The understanding of factors that contribute to change the stability of pharmaceuticals and identifying ways to guarantee their stability are very important [7].

The polymorphic behavior of DAR was first explained by Gyseghem et al. [8], who show that pseudo-polymorphic forms are environmental conditions dependent. DAR ethanolate's crystal structure changes to the hydrate form under moderate or high relative humidity (RH); moreover, it can change to amorphous form at high temperature. DAR hydrate can convert into the ethanolate form under ethanol atmosphere and into the amorphous form at high temperature or at low RH (<5 %). The amorphous form can convert into the hydrate at moderate or high RH and into the ethanolate form in an ethanol atmosphere [8].

This behavior of DAR is quite disturbing when one takes into account the fact that the HIV presents global prevalence and DAR be considered by WHO as essential medicament for the treatment of this disease [9]. This behavior is worrying because the Prezista[™] drug can be used around the world and is subject to different climatic characteristics of each part of the globe.

Brazilian government has a policy of free and universal access to antiretroviral medicines for people with AIDS, which has encouraged the development of generic formulations. To develop a better formulation of DAR, it is necessary to understand the chemical and physical stability of the drug as well as the role played by the formulation and package in providing its stability.

In agreement with American Pharmacopeia, the dissolution and biodisponibility of drugs may suffer influence from its solid-state properties such as crystallinity,

polymorphism, particle size and surface [10]. Our study group has used the thermal analysis to evaluate the stability of several drugs [11, 12]; following this practice, the aim with this work was to evaluate the solid-state stability of DAR under stressed conditions and assess the role played by the formulation and package in providing stability to the drug.

Methods and materials

DAR chemical reference (assigned purity of 98 %) was obtained from Sequoia Research (Pangbourne, UK). DAR ethanolate tablets (300 mg (Prezista[™])) were purchased from local markers.

DAR raw material and tablets were stocked under stress stability condition of 55 °C (oven) for 20, 40, 60 and 90 days using a dry oven. The raw material was kept in Petri plate; tablets were kept in Petri plates or the commercial package. Differential scanning calorimetry and thermogravimetry (SII Nanotechnology, Japan) and X-ray powder diffraction (Rigaku, Japan) were used to evaluate polymorphic changes in the samples.

Differential scanning calorimetry

The DSC curves were obtained using a DSC calorimeter (model DSC7020, SII Nano Technology, Japan) under a dynamic atmosphere of nitrogen (50 mL min⁻¹) with a heating rate of 10 °C min⁻¹ from 30 to 300 °C using open aluminum crucibles. The instrument was calibrated with an indium standard. The calibration parameters were obtained by using the temperature of the extrapolated peak to determine the fusion temperature ($T_{\text{onset}} = 156.6$ °C) and fusion enthalpy ($\Delta_{\text{fus}}H = 28.5$ J g⁻¹) of indium standard.

Thermogravimetric analysis

TG measurements were performed using a thermobalance (model TG-DTA7300, SII Nano Technology, Japan) under a dynamic atmosphere of nitrogen (50 mL min⁻¹) with a heating rate of 10 °C min⁻¹ from 30 to 300 °C using open aluminum crucibles. The equipment was calibrated using an indium standard for temperature and an alumina calibration weight for mass.

Powder X-ray diffraction

PXRD was performed using a diffractometer (model Ultima IV, Rigaku, Japan) with measurements of 2θ ranging from 5° to 55° at 40 kV and 30 mA. The sample was prepared according to USP 35 instructions (procedure 941) [10].

Results and discussion

The overlapping diffraction patterns of the raw material at the initial time (without having been subjected to stress) and after being subjected to dry heat for 20, 40, 60 and 90 days are shown in Fig. 2. It is possible to observe that the crystalline structure suffered little changes during the study. Specially, a band formation of the amorphous material near $20^\circ 2\theta$ can be observed by comparing the XRD diffractograms of the samples with the raw material for the initial time (not subjected to stress). These small changes may mean the loss of ethanol, DAR leaving to be the ethanolate form and a possible initial amorphization of drug; they may also mean that 55°C was not sufficient to lead to complete amorphization of raw materials when kept in a Petri dish.

Figure 3 shows the overlapping XRD patterns of PrezistaTM tablets kept in their original packaging, at the initial time and after being subjected to dry heat for 20, 40, 60 and 90 days. It is possible to verify that all the diffraction peaks are perfectly superimposed. These results show the important role played by the primary packaging of the product keeping the drug crystal characteristics. The analysis of X-ray diffraction showed that the coated tablets in the primary packaging remained its crystalline structure throughout the time they were subjected to dry heat.

Coated tablets stored outside the primary packaging, but in Petri plates suffered the major changes in DAR ethanolate crystals. This is shown in Fig. 4, where it is possible to observe that the peaks were continuously reduced from the 20 days sample until the 90 days sample, when they have completely disappeared. Figures 2 and 4 show the XRD of DAR raw material and XRD of DAR tablets,

Fig. 2 XRD of DAR raw material stored in Petri plates of non-stressed samples (*initial time*) and subjected to dry heat (55°C) for 20, 47 and 90 days, samples A, B, C and D, respectively

Fig. 3 XRD of DAR tablets stored in the primary packaging of non-stressed samples (*initial time*) and subjected to dry heat (55°C) for 20, 40, 60 and 93 days, samples A, B, C, D and E, respectively

Fig. 4 XRD of DAR tablets stored in Petri dishes of non-stressed samples (*initial time*) and subjected to dry heat (55°C) for 20, 40, 60 and 90 days, samples A, B, C, D and E, respectively

respectively, stored in Petri dishes of non-stressed samples (*initial time*) and subjected to dry heat at 55°C . It is possible to realize that DAR in tablets (Fig. 4) suffered higher amorphization than the raw material, which leads to the suspicion that the drug after being manipulated and compressed may have undergone changes and possibly interacted with adjuvants of formulation; all these possible events do not contribute to DAR solid stability and seem to speed up the amorphization process when kept out of primary packaging and under dry heat.

By applying DSC, the non-stressed DAR raw material (*initial*) showed only a thermal event, showing that ethanol molecule is trapped in a crystal grid (Fig. 5). Along the

Fig. 5 DSC curves from DAR ethanolate raw material of non-stressed samples (*initial time*) and subjected to dry heat (55 °C) at 20, 47 and 94 days, samples A, B, C and D, respectively

time, samples were subjected to dry heat, ethanol leaves the crystal grid and a second thermal event near to 60 °C is detected (Fig. 5). Probably, the loss of ethanol out the crystal lattice occurs near to 60 °C and the loss of ethanol in the crystal grid occurs near to 100 °C.

Thermogravimetric analysis of raw materials stored at 55 °C in Petri dishes (Fig. 6) showed loss of mass that decreased over time. For the DAR raw material in initial time, the calculated mass loss was 7.5 %, which is close to the percentage of ethanol in the mass of DAR ethanolate molecule (325.24 mg of DAR ethanolate is equivalent to 300 mg of DAR). For the samples stored for 20 and 40 days, the mass losses were 4.1 and 3.4 %, respectively. The decrease in mass loss detected in the assay

Fig. 6 Thermogravimetric curves for DAR ethanolate raw material of non-stressed samples (*initial time*) and subjected to dry heat (55 °C) for 20, 40 and 93 days, samples A, B, C and D, respectively

demonstrates that DAR ethanolate maintained at 55 °C without humidity control lost ethanol, which was fastened in its crystal grid.

The coated tablets, stored outside of the primary packaging (Petri dish), showed changes in their calorimetric profile (Fig. 7), similarly to what can be seen for the raw material (Fig. 5). Along the time, tablets were stored outside of the primary package, and it was observed that the enthalpy of the second event was decreasing and the enthalpy of the first event was increased, confirming the loss of DAR ethanolate crystal and formation of free ethanol (Figs. 5, 7).

DAR tablets, even with its coating, stored outside the primary packaging (Petri dish), showed huge changes in their calorimetric profile, similarly to what can be seen to raw material (Fig. 7; Table 1). Throughout the time, they were stored outside of the primary package, and it was found that the enthalpy of the event near to 100 °C decreased (Fig. 7) and the enthalpy of the first event was increased, confirming the loss of ethanol from crystal lattice and the free ethanol formation (Fig. 7).

Little or no change could be observed in the calorimetric profile of the samples subjected to dry heat and stored in the primary packaging (Fig. 8; Table 2). This suggests that packaging was essential to avoid ethanol loss and ensure the polymorphic stability of DAR.

Analyzing TG curve of DAR ethanolate tablets in their sealed primary packaging is possible to state that there was no change in the profile of mass loss during the days of storage. The first loss is observed in the range 40–100 °C and the second loss in the range 230–430 °C (Fig. 9).

The raw material and tablets stored outside the primary container, in Petri plates, showed a decrease in the ethanolate

Fig. 7 DSC curves for DAR ethanolate tablets stored in Petri dish of non-stressed samples (*initial time*) and subjected to dry heat (55 °C) for 20, 40, 60 and 93 days, samples A, B, C, D and E, respectively

Table 1 Enthalpy values calculated by integrating the DSC curves of the samples of tablets of DAR stored in Petri dish and subjected to 55 °C

Time exposure to dry heat/days	Enthalpy/mJ mg ⁻¹
0	106.0
20	44.4
40	41.9
93	35.3

Fig. 8 DSC curves for DAR ethanolate tablets stored in primary packing of non-stressed samples (*initial time*) and subjected to dry heat (55 °C) for 20, 40, 60 and 93 days samples A, B, C, D and E, respectively**Table 2** Enthalpy values calculated by integrating the DSC curves of the samples of tablets of DAR stored in the primary packaging and subjected to 55 °C

Time exposure to dry heat/days	Enthalpy/mJ mg ⁻¹
0	106.0
20	106.0
40	105.0
61	94.3
93	86.7

form. As the loss of ethanol accompanying changes in the crystalline form of the drug, the amorphization for the DAR ethanolate tablets was faster than for the raw material (Figs. 2, 4). That leads to the suspicion that the drug after having been manipulated and compressed can have undergone changes and, may be, interactions with the adjuvant formula that does not contribute to the solid stability and appears accelerate the amorphization when kept outside the primary packaging under dry heat.

Fig. 9 Thermogravimetric curves for DAR tablets stored in the primary packaging of non-stressed samples (*initial time*) and stored at 55 °C for 20, 40, 60 and 93 days, samples A, B, C, D and E, respectively

All these changes may affect other so important properties as solubility and hence the bioavailability of DAR. There are no published studies on the impact of this transformation undergone by DAR on solubility, dissolution and bioavailability of the drug. That will become of future study target in our workgroup.

The evaluation of the role played by the formulation and packaging in the maintenance of product characteristics for DAR ethanolate tablets provides important data and may be useful for the pre-formulation studies of generic products.

Conclusions

Polymorphic stability studies have provided important information about the solid-state stability of DAR and its formulation. DAR ethanolate showed its polymorphic behavior during this study, confirming what Gyseghem et al. [8] had described. However, the primary container (plastic jar comprising high-density polyethylene) proved capable of protecting the product against polymorphic changes in the evaluated conditions. The coated tablets kept in its container remained the DAR ethanolate crystal structure unchanged. It may be concluded that as important as the formulation or coating is the protection provided by the packaging to the product used today. Thus, the packaging material to be used in the product containing DAR should be chosen carefully.

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