Challenging identification of polymorphic mixture: polymorphs I, II and III in olanzapine raw materials.

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ABSTRACT: Olanzapine (OLZ), a drug for the treatment of schizophrenia, presents in more than 60 crystal forms. Polymorphs I, II and III were reported, however, the preparation conditions for pure II and III have not been reported. Polymorph IV was reported but this form is actually polymorph II described at different temperature. The diversity of solid forms of OLZ, the change in the nomenclature found in the literature and the presence of polymorphic mixture in samples, increase the difficulty for a correct solid state characterization. Therefore, the goal was the polymorphic identification of three OLZ raw materials, highlighting the limitation of conventional techniques (typically used in analytical control) and the necessity to use a combination of advanced ones to solve this challenge. The samples were studied by conventional techniques such as powder X-ray diffraction, thermoanalytical techniques, infrared spectroscopy. In apart from that, synchrotron powder X-ray diffraction (SPXRD) and solid state nuclear magnetic resonance (ss-NMR) were used. All samples were in accordance with the pharmacopoeia criteria. However, the conventional techniques were not specific for the complete polymorphic identification. Therefore, a combination of advanced techniques (SPXRD and ss-NMR) was necessary to identify the mixture of polymorphs (I, II and III) in all samples.

Keywords: olanzapine; polymorphism; X-ray diffraction; thermal analysis; solid state nuclear magnetic resonance.

1. Introduction

Schizophrenia is a severe and chronic mental disorder, unrelated to ethnic or geographical factors and is considered the most disabling psychiatric disorder (Braga et al., 2015). It is a relevant public health problem with enormous economic and social impact (Wannmacher, 2004). Olanzapine (OLZ), a second-generation antipsychotic drug, belongs to the class of thienobenzodiazepines, being indicated for the treatment of schizophrenia (Bhana and Perry, 2001). Its molecular formula is $C_{17}H_{20}N_4S$ and its molar mass is 312.43 g/mol (USP, 2013). The drug appears as a yellow crystalline solid that can be obtained in more than 60 different crystal forms (Bhardwaj et al., 2013). There have being 4 reported anhydrous polymorphs (I, II, III and IV), 3 dihydrates polymorphs (B, D and E), a major hydrate and numerous solvates (Bhardwaj et al., 2013; Kolodziejcki et al., 2011; Polla et al., 2005; Reutzel-Edens et al., 2003). The major hydrate is a metastable crystal form, which contains 2 to 2.5 mols of water and was only observed in wet precipitates of OLZ (Reutzel-Edens et al., 2003).

The U.S. Patent 5,229,382 of 1993 by Eli Lilly, reported the discovery and preparation of OLZ as a novel organic compound (Chakrabarti et al., 1993). Later, in 1998, the same company described the preparation and characterization of a crystal form, named I, produced using the methods described in U.S. Patent 5,736,541.10 (Bunnell et al., 1998). Also, in the same document of 1998, Eli Lilly patented the preparation and use of a new crystalline anhydrous form, named II (Bunnell et al., 1998), already reported in a previously patent of 1997 (Bunnell et al., 1997). According to U.S. Patent 5,736,541 of 1998, polymorph I showed to be metastable and not suitable for commercial use in pharmaceutical formulations since it presents a color change with exposure to air (Bunnell et al., 1998). Color changes could be problematic, especially for use by psychotic patients.

Polymorphs I and II of OLZ were reported in different patents from Eli Lilly. Comparing the nomenclature used in U.S. Pat. 5,703,23211 and in U.S. Pat. 5,736,54110 one can see that polymorph I described in the first one has the same interplanar spacing as polymorph II described in the second. Similarly, polymorph II described in U.S. Pat. 5,703,232 has the same interplanar spacing as I described in U.S. Pat. 5,736,541.

The reversal in nomenclature was also made by Reutzel-Edens and collaborators (2003). Since then, numerous studies reference polymorphs I and II in different ways. In this work, a spreadsheet was developed to standardize the nomenclature used by different authors (Table 1).

Table 1.

Figure 1

Five entries were found using the Cambridge Structural Database (CSD) (Allen, 2002) for anhydrous OLZ (Fig. 1 and Table 2). These entries corresponded to polymorphs I and II. OLZ polymorph I was reported with the codes UNOGIN, UNOGIN01 and UNOGIN03 (Fig. 1 (A), (B) and (C), respectively). The first two were solved at room temperature whereas UNOGIN03 at low temperature. Thakuria and Nangia reported polymorph IV (UNOGIN02) and Bhardwaj and collaborators (2013) reported the same structure at low temperature and referred to it as polymorph II (UNOGIN04). In this case, there is a displacement in the X-ray peaks positions (Fig. 1D and 1E) due to different temperatures that causes a unit cell contraction.

Table 2.

In this study, for comparison of the crystallographic data, polymorph I described by Reutzel-Edens and collaborators and polymorph II described by Thakuria and Nangia (although named as IV) will be used in this paper. The selection was based on data collection at room temperature, closer to the temperature used in the powder X-ray diffraction studies.

A different polymorph was also described in the literature and named as polymorph III, The crystalline structure information of polymorph III has not been reported but there is information about its ss-NMR spectrum (Reutzel-Edens et al., 2003). The preparation of polymorph II and III was reported in this previously mentioned study, however, no conditions were identified that would yield pure polymorphs II or III (Reutzel-Edens et al., 2003).

Polymorphs II and III were described as less stable forms with lower melting points compared to I (melts at 195 °C), with conversion to the stable polymorph at 125 °C and 180 °C, respectively. However, the study did not reported the differential scanning calorimetry (DSC) results and the authors reported that they couldn't prepare pure polymorphs II and III (Reutzel-Edens et al., 2003). A different paper in 2005 (Polla et al., 2005) showed the DSC curves of polymorphs I and II. Both curves presented endothermic peaks in 194 °C related to melting. For polymorph II, a second endothermic event in 177 °C and an exothermal signal were observed (Polla et al., 2005). The same result was observed in a study of 2016, that showed the DSC curves of both polymorphs (Paisana et al., 2016).

Currently, there are numerous techniques available for the solid state analysis of materials of pharmaceutical interest (Prado and Rocha, 2015). However, some of these commonly used methods fail to differentiate polymorphs with similar crystalline structures. Thus, a combination of methods must be used to avoid misinterpretation and wrong identification of different crystalline phases.

Therefore, the goal of this study was to present OLZ characterization results using traditional techniques followed by a more robust evaluation with advanced techniques. This approach highlights the difficulty to understand some kind of systems and the solution using powerful techniques.

2. Materials and methods

2.1. Materials

OLZ related compound A USP Lot GOK232, OLZ related compound B USP Lot GOK318, OLZ manufacturer A (Brazil), OLZ manufacturer B (Portugal), OLZ manufacturer C (India) and OLZ reference standard USP lot FOH200. Reagents from Vetec (Brazil) were: ammonium acetate, sodium acetate anhydrous, acetonitrile HPLC grade, glacial acetic acid, hydrochloric acid, phosphoric acid, disodium EDTA, potassium phosphate monobasic anhydrous, sodium phosphate monobasic, sodium hydroxide, sodium lauryl sulphate, methanol HPLC grade.

2.2. Olanzapine raw material characterization according to pharmacopeia monograph.

The raw materials were analyzed according to USP 36th specifications (USP, 2013).

Identification was performed by comparing the retention times of the samples with an OLZ standard solution (assay method B). The water content was determined by Karl Fischer (method I). It was also determined the residue of ignition. The content of metallic impurities was evaluated according to method II for heavy metals. The quantification of related substances A and B of OLZ and dosing were performed by high performance liquid chromatography (Shimadzu Nexera X2).

2.3. Powder X-ray diffraction (PXRD) and with synchrotron source (SPXRD).

The PXRD analyzes using conventional source were performed in a Bruker D8 Advance diffractometer. Diffraction patterns were collected from 4 to 50°, with 0.02° step and 0.1 second acquisition time using Cu K α radiation ($\lambda = 1.5418 \text{ \AA}$), operating at 40 kV and 40 mA tube voltage and current, respectively.

The SPXRD data were collected at the Brazilian Synchrotron Light Laboratory (LNLS) in XRD1 beamline. The measurements were performed in a Debye-Scherrer geometry, using borosilicate capillaries with $\phi=0.7 \text{ mm}$. The samples were manually deposited inside the capillaries that were mounted in ferromagnetic stainless-steel holders. The system provides a perpendicular position of the capillaries relative to the beam and the spinning of capillaries during the measurements. The X-ray energy used was around 12.0 KeV. Standard SiO₂ sample was measured as reference and the determined wavelength was $\lambda=1.4847 \text{ \AA}$. The diffraction patterns were obtained at room temperature using the MYTHEN 24K system, from Dectris. The program MERCURY (Macrae et al, 2008) was used for the calculation of the theoretical powder patterns from CSD structure data. The structures UNOGIN01 (polymorph I) and UNOGIN02 (polymorph II) were used.

2.4. Scanning electron microscopy (SEM).

Analyses were performed in an FEI microscope (Quanta 40) using 15 KV accelerating voltage. Small amounts of sample were adhered to a piece of double sided adhesive tape supported on a carbon stub. Samples were metalized with a thin layer of gold using a BAL-TEC SCD 005 sputter coating to be evaluated.

2.5. Infrared spectroscopy.

Mid-infrared (FT-IR) analyzes were performed on a Shimadzu spectrophotometer (IRPrestige-2). Potassium bromide pellets were prepared containing approximately 2% of the sample. The spectra were recorded in the range 4000-400 cm^{-1} with resolution of 4 cm^{-1} .

2.6. Differential scanning calorimetry (DSC).

The measurements were performed on an 8500 DSC fitted with an intracooler 2P-cooling unit (PerkinElmer). All measurements were conducted under a nitrogen gas purge at a flow rate of 20 $\text{mL}\cdot\text{min}^{-1}$ over a temperature range to include the melting temperature of the drug (30–260 $^{\circ}\text{C}$). Heating rates of 10 $^{\circ}\text{C}\cdot\text{min}^{-1}$ and 150 $^{\circ}\text{C}\cdot\text{min}^{-1}$ were used. Samples (0.6 to 2.0 mg) were encapsulated into hermetically sealed pans. The instrument was calibrated for temperature and heat flow using indium and zinc as standards. All DSC analyzes were performed in at least a duplicate and the curves presented represent the mean curve.

2.7. Thermogravimetric analysis (TGA).

Thermogravimetric analyzes were performed using a Shimadzu TGA-50 with a nitrogen flow of 50 $\text{mL}\cdot\text{min}^{-1}$. Approximately 5 mg of samples were weighed in a platinum crucible. Heating rate of 10 $^{\circ}\text{C}\cdot\text{min}^{-1}$ was used up to 600 $^{\circ}\text{C}$.

2.8. Hot stage microscopy (HSM).

A Mettler Toledo heating cell (FP82) and a temperature controller Mettler Toledo (SP90) coupled to an optical microscope Olympus BX50 were used. A heating rate of 10 $^{\circ}\text{C}\cdot\text{min}^{-1}$ was used up to 250 $^{\circ}\text{C}$.

2.9. Solid-state nuclear magnetic resonance (ss-NMR).

High resolution ^{13}C ss-NMR spectra were recorded using the ramp CP/MAS sequence with proton decoupling during acquisition. Experiments were performed at room temperature in a Bruker Avance II spectrometer equipped with a 4 mm MAS probe. The operating frequencies for protons and carbons were 300.13 MHz and 75.47 MHz, respectively. Adamantane was used as an external reference for the ^{13}C spectra and to set the Hartmann–Hahn matching condition in the cross-polarization experiments. Spinning rate was 10 kHz. The number of transients for each compound was 1024 to obtain an adequate signal-to-noise ratio. The recycling time, the contact time during CP and the acquisition time were 7 s, 4.0 ms, and 41 ms, respectively. SPINAL 64 sequence was used for decoupling during acquisition with a proton decoupling field H_{1H} satisfying $\omega_{1H}/(2\pi) = \gamma H_{1H}/(2\pi) = 78.2$ kHz (Bennet, 1995). Quaternary carbon edition spectra were recorded for all samples. These spectra were acquired with the non-quaternary suppression (NQS) sequence, where the ^1H and ^{13}C radio frequency (rf) fields are removed during 40 μs after CP and before the acquisition. This delay allows the carbon magnetization to decay because of the ^1H – ^{13}C dipolar coupling, resulting in spectra where CH and CH_2 are substantially removed (Harris, 1994). This experiment allowed us to identify quaternary carbon and methyl group signals.

2.10. Intrinsic dissolution.

Intrinsic dissolution studies were conducted using the stationary disk method in a Distek Evolution 6100 equipment. Approximately 100 mg of the samples were compressed using a mechanical press with constant pressure (1300 psi for 1 minute) producing discs with a surface area of 0.5 cm^2 . The apparatus was immersed in 900 mL of the dissolution medium (HCl 0.1 N pH 1.2) heated to 37 $^\circ\text{C}$ and with a rotation speed of 50 rpm. Aliquots were withdrawn at intervals of 1, 2, 3, 4, 5 and 6 minutes. Obtained samples were analyzed for their absorbance at 259 nm using a Shimadzu UV-1800 spectrophotometer and concentration was determined by

standard curve of OLZ. The intrinsic dissolution rate was determined from the linear regression of the obtained points.

3. Results and discussion

3.1. Raw material characterization according to pharmacopeia monograph.

The infrared spectra obtained for the three manufacturers correspond to the results from the literature for OLZ and will be presented later. The results of pharmacopeia tests (USP, 2013) for all samples are described on Table 3.

The acceptance criteria established in the official compendium is no more than 1.0% of water (USP, 2013). The three samples of OLZ were within the specified limits. However, samples from manufacturers A and B showed higher water content than the value found for the sample C. The limit for heavy metals is 10 ppm (0.001%) (USP, 2013). All samples showed results according to this specification.

Table 3.

For OLZ there are eight organic impurities: imp-1, 2, 3, 4, 5, 6, 7 and 8 (Krishnaia, 2011). The USP test cannot separate them all but monitors two: imp-4 (related compound B) and imp-7 (related compound A). Furthermore, the compendium sets limits for any individual impurity not specified and total impurities (USP, 2013). OLZ A, B and C were analyzed for the presence of related compounds. The results demonstrated that samples B and C were approved. For sample A there was an unspecified impurity with a concentration of 0.9%, higher than the recommended maximum of 0.1% (USP, 2013).

3.2. Non-pharmacopeia raw material characterization by conventional techniques

3.2.1. Powder X-ray diffraction

Fig. 2 presents the diffraction patterns for OLZ samples A, B and C. The results were compared with the calculated diffraction pattern for polymorphs I (Reutzel-Edens et al., 2003) and II (Thakuria and Nangia, 2011). As discussed in the introduction, the crystal structure reported by Thakuria and Nangia, named polymorph IV, is actually II resolved at room temperature. Therefore, this structure was used to simulate the powder pattern of polymorph II.

Figure 2

The powder patterns showed several reflections corresponding to the calculated pattern of polymorph II (Thakuria and Nangia, 2011), such as in 8.9° , 10.4° , 12.9° and 18.4° . Additionally, the reflections in 8.6° , 12.4° , 14.4° and 16.9° seemed to correspond to OLZ polymorph I (Reutzel-Edens et al., 2003) (Fig. 2). Therefore, we concluded that there was a mixture of polymorph II with another phase in all samples, probably I in samples A and B. Additionally, other reflections were observed: the shoulder close to the peak of higher intensity and the reflections demonstrated with the arrows in Fig. 2. This fact indicated the presence of another phase that could not be identified.

According to literature, polymorph I may be obtained directly by crystallization from organic solvents. The other two polymorphs (II and III) are obtained by desolvation. Drastic conditions required for desolvation often result in a contaminated material with the three forms. The literature showed mixtures of polymorphs II and III obtained by the desolvation but none of the two forms were isolated separately (Reutzel-Edens et al., 2003). Polymorph II was also prepared through vapor phase via sublimation and the analysis of the samples revealed a mixture of polymorph II single crystals and polycrystalline polymorph III (Bhardwaj et al., 2013).

Crystallization studies made by Cavallari and collaborators, reproducing the conditions described by Reutzel-Edens and collaborators, failed in obtaining polymorph III. Also, different crystallization experiments were conducted in a study by Bhardwaj and collaborators and similarly pure polymorph III could not be obtained (Bhardwaj et al., 2013).

Considering all the information, the additional reflections observed in PXRD could be from another polymorph. However, these results were not enough to do a complete identification of the solid forms.

3.2.2. Scanning electron microscopy

The literature describes that OLZ form I has flat, plate-like particles of similar length and width while form II has rectangular, long thin blade-like particle (Tiwari et al., 2007). The images obtained by SEM are shown in Fig. 3. Microscopic evaluation performed for the raw materials A and B did not allow the identification of which polymorph was present in the samples because the morphology of the particles might have been changed, probably due to the micronization process to which the raw materials were submitted. Sample C showed particles with a blade-like morphology, as described for polymorph II (Tiwari et al., 2007).

Figure 3

3.2.3. Infrared spectroscopy

Fig. 4 presents the infrared spectra of the samples. Between $3600\text{--}2400\text{ cm}^{-1}$, the stretching vibration of the NH group of OLZ molecule was present as a broad band at 3220 cm^{-1} . Previously studies demonstrated the occurrence of a shift of 19 cm^{-1} in the NH stretching band toward higher wavenumbers for polymorph II and a reduction in the width from 83 cm^{-1} (polymorph I) to 53 cm^{-1} (polymorph II) (Ayala et al., 2006). The enlargement of the NH stretching band and its shift to smaller wavenumbers suggest the participation of this group in hydrogen bonds. Wider bands shifted to lower wavenumbers means stronger hydrogen bonds (Silverstein et al., 1994). Consequently, the infrared from the literature indicates the participation of the NH group in stronger hydrogen bonds in polymorph I. This fact suggests higher stability for polymorph I (Polla et al., 2005; Ayala et al., 2006). All OLZ raw materials from our study showed the absorption band related to NH stretching in 3229 cm^{-1} .

Figure 4

The literature also described, in the stretching region of the double bonds, the infrared spectrum of polymorph II with a characteristic band at 1601 cm^{-1} that is not present in the spectrum of polymorph I (Ayala et al., 2006). OLZ samples analyzed in our study showed this band at 1601 cm^{-1} .

Thus, the results of infrared spectroscopy indicated that samples A, B and C presented the same crystal form. The spectra are similar to the one of polymorph II (Ayala et al., 2006). However, it is not possible to ensure that polymorph I is not present in the samples, since infrared can not provide adequate resolution and detection limit for the studied samples

3.2.4. Differential scanning calorimetry

Fig. 5 presents the DSC curves for samples A, B and C of OLZ at $10^\circ\text{C.min}^{-1}$. In order to better visualize and separate the thermal events, besides the traditional heat flow signal, the heat flow derivative was studied in the all DSC curves. Derivative curves (DDSC) are easily obtained from the heat flow curve via a mathematical algorithm. They can aid with the resolution of data, particularly when overlapping peaks are concerned. The second derivative produces a maximum or minimum for each inflection of the original curve and shoulders in the original curve show up as peaks (Gabbot, 2008).

All OLZ samples had different thermal behaviors and in all DSC curves (Fig.5a, b and c) it was possible to observe the presence of an endothermic peak at $194\text{-}195^\circ\text{C}$, indicating melting of one polymorph.

The literature reported polymorphs I and II as a dimorphic monotropic system, with an irreversible transition from II to I (Polla et al., 2005). Polymorph II showed an endothermic event at temperatures around 180°C which the authors referred to as melting of the less stable form, quickly followed by recrystallization of the drug in its most stable form (polymorph I) (Paisana et al., 2016; Polla et al., 2005; Tiwari et al., 2007). Combining the DSC results with

other techniques such as PXRD with variable temperature, the authors reported the presence of polymorph I coexisting with II, in temperatures from 160 to 180 °C (Polla et al., 2005). For polymorph I, the endothermic peak observed at temperatures around 194 °C was related to melting (Polla et al., 2005).

The studies of the thermal behavior of the OLZ described in the literature and previously mentioned (Paisana et al., 2016; Polla et al., 2005) presented the DSC curves as being for the pure polymorphs I and II. However, with a careful analysis of their results, we observed that the sample named as polymorph II presented a peak (close to 10.8°) in the PXRD pattern that was not related to polymorph II and that had already been assigned as a peak related to polymorph III (Bhardwaj et al., 2013; Reutzel-Edens et al., 2003). In fact, that is a recurring problem in the literature (Tiwari et al., 2007) and it turns out that the DSC curve shown for the polymorph II is actually related to a mixture of phases. No study was found that actually presented the thermal behavior of pure polymorph II.

Figure 5

For sample A (Fig. 5a) there are three endothermic events, supported by the DDSC curve. It is difficult to say precisely what happened to the sample at these temperatures. However, as described in the literature (Cavallari et al., 2013), the first endothermic peak at 145°C can be related with the process of desolvation. The second, at 173 °C, can be melting of polymorph II, although it occurs at a slightly lower temperature than the temperatures described in the literature (181-185 °C) (Paisana et al., 2016; Polla et al., 2005; Tiwari et al., 2007). The well-defined peak at 194 °C ($\Delta H = 205 \text{ J.g}^{-1}$) can be associated with melting of polymorph I, when comparing our results with the literature (Paisana et al., 2016; Polla et al., 2005; Tiwari et al., 2007). No exothermic signal was seen.

Fig. 5b shows the presence of a single well defined endothermic for sample B. As mentioned before, the peak in 194 °C ($\Delta H = 107 \text{ J.g}^{-1}$) may be associated with melting of polymorph I. No other polymorph was observed in this DSC curve. However, there was a disturber in the

baseline at 112 °C, also confirmed by the disorders in the DDSC in which according to the literature can be associated with the desolvation process of the OLZ (114-145 °C) (Cavallari et al, 2013).

Fig. 5c shows the curve for sample C. Comparing our results with the literature (Paisana et al., 2016; Polla et al., 2005; Tiwari et al., 2007), the first endothermic event can be related the melting of polymorph II at 180 °C. The endothermic peak is followed by the exothermic one; this can be associated to recrystallization to another polymorph, at 182 °C. Finally, it was observed an endothermic event that can be related to melting of this other polymorph at 195 °C ($\Delta H=322 \text{ J.g}^{-1}$). Also, in the DDSC curve of sample C a small endothermic peak at 177 °C appeared; this event is not mentioned in the literature.

Correlating our results with the studies previously published (Paisana et al., 2016; Polla et al., 2005; Tiwari et al., 2007), the thermal behavior of sample B was consistent with polymorph I. Samples A and C curves resembled the ones described for polymorphs I and II. It should be remembered that, as already written, we found that there is no DSC curve for pure polymorph II in the literature, although the authors described as being. The DSC results of our OLZ samples are partially in agreement with PXRD results that showed the presence of mixtures containing polymorph II in all the samples. The presence of polymorph II in sample C can be supposed by the transition to polymorph I after heating above 180 °C; however, it is uncertain to know by DSC whether polymorph I existed previously with polymorph II in the original sample or whether it was crystallized in the exothermic transition during heating.

Thus, the effect of heating rate on the thermal profile of OLZ was studied using high speed DSC to determine whether increasing the rate could alter the kinetics of the polymorph transition and if it is possible to identify the presence of polymorph III.

Besides that, analyses performed at high heating rates have as function to verify whether or not the phenomenon of recrystallization of some polymorphic form of olanzapine occurs without influence of the slow heating rate ($10^{\circ}\text{C.min}^{-1}$) usually used in DSC. It is known that the use of slow heating rates may favor the formation of polymorphic forms that do not actually exist in

the sample but were formed during the DSC analysis. One way to inhibit this effect is by using high heating rates (McGregor, et al., 2004).

Fig. 6a presents the DSC curve of OLZ sample A obtained at high heating rate. Significant changes in the thermal profile can be observed when the heating rate is increased from 10 °C.min⁻¹ to 150 °C.min⁻¹. Sample A showed only endothermic events and no exothermic events were observed. An endothermic deviation of the baseline at 85 °C could be seen and can be related to desolvation. Besides that, DSC curve showed two overlapping endotherms related to the melting of probably two different polymorphs (one with melting at 194 °C (polymorph II or III) and another at 206 °C (polymorph I), confirming that sample A is a mixture of at least two polymorphs of OLZ. It should be remembered that in high heating rate mode, the thermal events can be shifted to higher temperatures due to the effect of thermal inertia (Poel and Mathot, 2006).

The analysis of sample B (Fig. 6b) revealed the existence of two consecutive thermal events: the first is an endothermic peak at 189 °C (melting of polymorph II or III, as described by HSM) and another endothermic peak at 207 °C (melting of polymorph I). Thus, we could say that sample B consisted of mixture at least two polymorphs (II or III and I) which could not be detected in a conventional DSC analysis (Fig. 5b).

Figure 6 (color)

Fig. 6c shows the DSC and DDSC curves of sample C performed at 150 °C.min⁻¹. We can be observed minor deviations of the baseline at 69, 145 and 175 °C. The first deviation can be related to desolvation and the last can be the melting of polymorph II or III. We also observed that the peak at 197 °C is related with the melting of polymorph I. We could affirm that sample C is the mixture of polymorphs.

With high heating rates, we observed that samples A, B and C consist of a mixture at least two crystalline forms. Besides that, the DSC results at high heating rate confirm the PXRD results suggesting that the three samples studied presented mixtures of polymorphs.

With these results, we showed that DSC at high heating rate is not meant to replace the conventional DSC but should be seen as a complementary technique to obtain others information about the samples.

3.2.5. Thermogravimetric analysis

The TGA curves obtained for sample A is shown in Fig. 7. The curves of the other samples showed the same pattern but varied in the mass loss: 31.08%, 40.86% and 28.13% at 258 °C and 33.89%, 45.54% and 17.74% at 510 °C for samples A, B and C, respectively.

Figure 7 (color)

All samples showed weight loss after the endothermic event observed in the DSC curve, confirming that the endothermic event did not refer to decomposition. The weight loss in TGA is related to the degradation of OLZ.

3.2.6. Hot stage microscopy

Hot stage microscopy is used in the pharmaceutical industry as a complementary technique (Vitez, 1998). High speed DSC and PXRD results suggested that the three samples presented mixtures of polymorphs. Thus, HSM was used (Fig. 8, 9 and 10).

For sample A, in temperatures between 160-172 °C a part of the sample melts (blue) and another one goes through a phase transition (red). At temperatures below 190 °C (Fig. 8), it can be noted the presence of numerous translucent larger crystals, which only melt at temperatures from 190 to 194 °C. These large crystals are not present in the initial raw material, they were formed in the phase transition at 160 °C.

Figure 8 (color)

These data confirm that sample A is a mixture of polymorphs. In addition, we observed that one polymorph (II or III) goes through a solid-solid transition to polymorph I (red). At the same time, the melting of the other part of the sample (blue) indicates the presence of a different polymorph (II or III, however, being the polymorph different from the previous transition). These two events (solid-solid transition and melting of a different polymorph) occur at close temperatures and it was difficult to see in conventional DSC.

Figure 9 (color)

A similar behavior was observed for sample B; melting of part of the sample occurred at temperatures around 160 °C and there were a presence of crystals melting only at temperatures above 190 °C (Fig. 9). At 160 °C it was also possible to observe the formation of larger crystals that melted at 190 °C.

Sample C showed melting of the first crystalline form at 180 °C (Fig. 10). As for the previous samples it is possible to observe the presence of polymorph I crystals that were formed at 180 °C. Polymorph I melted at 194 °C, through a solid-solid transition. Combining the results of DSC with HSM, we can suggest that sample C presents higher content of the polymorph that first melts (II or III).

Figure 10 (color)

With HSM we concluded that all samples presented polymorphs II and III and that the thermal events described before in the DSC from the literature (Polla et al., 2005) as melting of polymorph II that crystallizes in I is actually melting of one polymorph (II or III) that occurs in temperatures close to a solid-solid transition of another polymorph (II or III) to I (exothermic event). We can affirm that because, as said before, the samples from the literature were not pure polymorph II.

Also, with HSM and with conventional DSC it was not possible to determine if polymorph I was present in the initial samples. However, due to lower melting enthalpy of polymorph I in sample C (Figure 5c), it is reasonable to say that if present, polymorph I was in a lower content in this sample.

3.2.7. Intrinsic dissolution

The intrinsic dissolution graph obtained for the OLZ samples is shown in Fig. 11. The samples showed a high dissolution in HCl 0.1 N and therefore the collection times were in the first minutes of dissolution. In this medium, the intrinsic dissolution rates (IDRs) were $3,359 \pm 0,147$, $3,123 \pm 0,479$ and $3,472 \pm 0,098$ for samples A, B, and C, respectively.

Intrinsic dissolution test is known as a technique that can differentiate between solid forms (Prado and Rocha, 2015), however the similar IDRs for our OLZ samples indicate that this method was not adequate to show differences related to polymorphism between the samples. Also, considering that the samples showed mixtures of polymorphs, it becomes difficult to correlate dissolution with each individual solid form of OLZ.

Figure 11

3.3. *Non-pharmacoepia raw material characterization by non-conventional techniques*

3.3.1. Synchrotron X-ray powder diffraction

X-ray diffraction patterns with synchrotron radiation were collected at room temperature (Fig. 12). OLZ polymorph I and II showed reflections in according with the calculated values for UNOGIN01 and UNOGIN02, respectively (Table 4)

The comparison enabled the identification of OLZ polymorph II in the three samples (Fig. 12b). An experimental set of 2θ values were identified: 8.65° , 12.44° , 17.71° , 18.90° , 21.02° , 22.41° , 22.88° and 23.22° . These values are in according with the calculated values for UNOGIN02. OLZ polymorph I was detected just in samples A and B; an experimental set of reflections were

identified with calculated X-ray diffraction pattern using UNOGIN01 (Figure 12). The comparison enabled the identification of OLZ polymorph II in the three samples (Figure 12) according with calculated X-ray diffraction pattern of UNOGIN02. Furthermore, a distinguished new phase was detected in all three samples (Figure 12b, reflections indicated by an asterisk). This set of reflections not identified as corresponding for polymorphs I and II, could correspond to polymorph III (Table 4).

Figure 12

The structure of OLZ polymorph III and the PXRD pattern were not been reported yet, since this polymorph has never been successfully isolated. The presence of polymorph III in all samples could be confirmed using a NMR analysis.

3.3.2. Solid State NMR.

The ^{13}C CP/MAS ss-NMR spectra are shown in Fig. 13 and 14. In a recent publication, the ^{13}C CP/MAS spectra for the three polymorphs of OLZ were reported (same carbon numbering for OLZ molecule is used here) (Reutzel-Edens et al., 2003). Each polymorph presented a unique spectrum, which allows the identification of each form in a mixture. The ^{13}C spectra for the samples studied in our work (Fig. 13) presented resonances belonging to different polymorphs of OLZ, indicating that the samples are mixtures in different amounts of the three reported polymorph of OLZ. Fig. 13a and Fig. 14a displays the ^{13}C spectrum of sample A. In the low ppm region (Fig. 14a), signals at 14.9, 16.8 and 17.2 ppm correspond to the methyl carbons of polymorphs III, I and II respectively. The presence of the three polymorphs can be also seen in the region of 150-160 ppm. These signals correspond to the carbon C10a of each polymorph. The splitting of the C10a resonances is due to the residual dipolar coupling to the bonded quadrupolar ^{14}N nuclei.

Figure 13

Fig. 14b displays the ^{13}C spectrum for sample B. Under similar considerations, it can be seen the presence of the three polymorphs of OLZ. The ^{13}C ss-NMR spectrum for sample C is shown in Fig. 14c. Comparing the chemical shift of the signals with those presented in the literature, it is evident that only polymorphs II and III are present in this mixture. This fact could also be confirmed by inspection of the ^{13}C spectrum in Fig 13c where only two resonances appear in the low ppm region at 14.9 and 17.2 ppm. Though CP/MAS is a non-quantitative technique, comparing ^{13}C spectra for sample A and sample B we can conclude that the percentage of polymorph I is higher in sample A than in sample B.

The ss-NMR data are in good agreement with synchrotron X-ray diffraction patterns, confirming the presence of a mixture of polymorphs in all the samples.

Figure 14

4. Conclusion

Three batches of OLZ raw materials that could be used in the pharmaceutical development were studied according to pharmacopeia and non-pharmacopeia criteria. Regarding the solid state characterization from conventional techniques, PXRD and infrared showed that the three samples appeared to contain form II of OLZ. However, by PXRD and high speed DSC we verified the presence of mixtures of phases. This work also made an alert about the literature information regarding the thermal profile of OLZ polymorphs. Unfortunately, it is almost impossible to crystallize pure polymorph II without impurities of polymorph III or/and I thus, care must be taken in relation to the purity of the samples and their corresponding thermal profiles. With HSM analyzes we observed a solid-solid transition of a polymorph (II or III) to I occurring at temperatures very close to the melting of another polymorph (II or III). Finally, it was highlighted in this work that the conventional techniques allowed an incomplete solid state characterization, since it was impossible to determine the polymorphs in the raw material. Thus, advanced techniques were used, SPXRD, high speed DSC and ss-NMR. With SPXRD and ss-

NMR it was possible to do a complete identification of the polymorphic mixtures and to determine the presence of polymorph III, still poorly described in the literature. In cases of challenging identifications of polymorphic mixture, non-conventional techniques are necessary.

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Tables

Table 1. References of polymorphs I and II of OLZ described in the literature.

Reference	Name used in the literature	
	I	II
Patent US5,703,232, 1997	Stable	Metastable
Patent US5,736,541, 1998	Metastable	Stable
Reutzel-Edens et al. 2003	Stable	Metastable
Polla et al. 2005	Stable	Metastable
Ayala et al. 2006	Stable	Metastable
Tiwari et al. 2007	Metastable	Stable
Wawrzycka-Gorczyca et al. 2007	Metastable	Stable
Cavallari et al. 2013	Stable	Metastable
Bhardwaj et al., 2013	Stable	Metastable
Paisana et al. 2016	Stable	Metastable

Table 2. Structures present on CSD for pure OLZ.

Ref. code	UNOGIN	UNOGIN01	UNOGIN02	UNOGIN03	UNOGIN04
Polymorph	I	I	IV	I	II
Space group	P21/c	P21/c	P21/c	P21/c	P21/c
Number	14	14	14	14	14
a (Å)	10.388(3)	10.383(1)	9.913(<1)	10.341(1)	9.854(1)
b (Å)	14.839(4)	14.826(3)	16.533(1)	14.521(2)	16.314(2)
c (Å)	10.567(3)	10.560(8)	9.999(<1)	10.531(1)	9.975(1)
α (Å)	90	90	90	90	90
β (Å)	100.64(3)	100.62	98.02(<1)	90	98.30(<1)
γ (Å)	90	90	90	100.29(<1)	90
Volume (Å ³)	1600.871	1597.765	1622.735	1555.988	1586.878
Z	4	4	4	4	4
Z'	1	1	1	1	1
R-factor (%)	3,33	4,3	4,66	3,34	5,42
Temp (K)	Room temp 283-	Room temp 283-	Room temp 283-	123	123

	303	303	303		
Density	1.296	1.299	1.279	1.334	1.308
Reference	Wawrzycka et al., 2004	Reutzel-Edens et al. 2003	Thakuria and Nangia, 2011	Bhardwaj et al., 2013	Bhardwaj et al., 2013

Table 3. Results of pharmacopeia evaluations of OLZ samples from different manufacturers.

Property	Manufacturer		
	A	B	C
Water content (%)	0.744	0.990	0.187
Residue of ignition (%)	0	0.05	0.07
Assay (%)	98.98	98.10	99.84
Related substance A (limit of 0.1%)	0	0	0
Related substance B (limit of 0.1%)	0	0	0
Any individual impurity (limit of 0.1%)	0.9	0	0
Total of impurities (limit of 0.4%)	0.9	0	0

Table 4. D-spacing of olanzapine polymorphs I, II and III.

D-spacing (Å)		
Polymorph I	Polymorph II	Polymorph III
10.20	9.83	9.97
7.07	6.85	8.18
6.09	4.81	6.21
6.04	4.52	5.91
5.19	4.15	4.64
4.97	4.12	4.61
4.46	4.08	3.76
4.12	4.06	3.57
3.98	3.82	
3.71	3.74	
3.52	3.69	

3.49

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Fig. 1. Calculated diffraction patterns for OLZ polymorphs I and II. A) UNOGIN (polymorph I), B) UNOGIN01 (polymorph I), C) UNOGIN03 (polymorph I), D) UNOGIN02 (polymorph II and E) UNOGIN04 (polymorph II at low temperature).

Fig. 2. PXRD patterns of OLZ A, B and C and the calculated ones for polymorphs I (UNOGIN01) and II (UNOGIN02).

Fig. 3. SEM photomicrographs of OLZ (a1) and (a2) A with 1000x and 2000x magnification, respectively; (b1) and (b2) B with 2000x and 12000x, magnification respectively; (c1) and (c2) C with 250x and 2000x magnification, respectively.

Fig. 4. Middle infrared spectra of OLZ A (a), B (b) and C (c).

Fig. 5. DSC (red) and DDSC (black) curves for OLZ (a) A, (b) B and (c) C samples at a heating rate of 10 °C.min⁻¹.

Fig. 6. DSC curves for OLZ (a) A, (b) B and (c) C samples at a heating rate of 150 °C.min⁻¹.

Fig. 7. TGA curve of OLZ sample A.

Fig. 8. Hot stage microscopy of OLZ sample A, 200 x, heating rate of 10 °C.min⁻¹.

Fig. 9. Hot stage microscopy of OLZ sample B, 200 x, heating rate of 10 °C.min⁻¹.

Fig. 10. Hot stage microscopy of OLZ sample C, 200 x, heating rate of 10 °C.min⁻¹.

Fig. 11. Intrinsic dissolution studies of OLZ samples A (□), B (▲) and C (●) in HCl 0.1 N.

Fig. 12. Identification of crystalline phases present in 3 samples of OLZ and calculated X-ray patterns of OLZ polymorph I (UNOGIN01) and polymorph II (UNOGIN02). (a) Experimental synchrotron X-ray diffraction patterns and (b) a magnification showing the phases identified in each sample and the indication of reflections (*) of polymorph III.

Fig. 13. ^{13}C ss-NMR spectra of OLZ samples a) A, b) B and c) C.

Fig. 14. High and low ppm region of ^{13}C ss-NMR spectra of samples a) A, b) B and c) C, indicating the presence of the different polymorphs of OLZ.

Figure 1

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 11

Figure 13