

Full Length Research Paper

Pharmaceutical polymorphisms and its influence on the dissolution profile of two pioglitazone brands

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The aim of this work is to identify the presence of pharmaceutical polymorphisms in two brands of pioglitazone and evaluate their influence on the dissolution of tablets *in vitro*. The presence of polymorphisms was determined by Raman spectrometry and Infrared spectrometry. To relate the presence of these polymorphisms with the dissolution capacity of the drug (bioavailability *in vitro*), the dissolution profile of the drugs was determined through the development and implementation of a high resolution liquid chromatography (HPLC) method using a detector UV-vis. Two displacements were detected in Raman spectroscopy, suggesting an alteration in the crystalline structure of the test drug in relation to the reference drug. Significant differences were also found in the dissolution profile evaluated by the dissolution factor "f2", which could be explained by the presence of these polymorphisms. The presence of pharmaceutical polymorphisms can lead to the alteration of the processes of absorption and metabolism *in vivo* and therefore, alteration in the therapeutic effect.

Key words: Pharmaceutical polymorphism, dissolution, pioglitazone, high resolution liquid chromatography (HPLC), Raman spectrometry.

INTRODUCTION

The absorption of a drug in solid dosage form orally depends on various processes including drug dissolution and transport within the body. The processes involved in the absorption, mainly the release and dissolution are critical to achieve a good therapeutic effect because the bioavailability of a drug depends on the degree and speed of dissolution and its absorption. These processes

depend on the physicochemical characteristics of the drugs, which include the crystalline forms. Defects in the elaboration of medications can alter the crystalline formations that later will alter the absorption processes and, therefore, the efficacy of the medication (De Salvi, 2011). These alterations in the structure of a solid are called polymorphisms. Polymorphism is defined as the

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ability of a solid material to exist in two or more crystalline forms that present different conformational arrangements (Sánchez et al., 2007). More than 600 active ingredients and excipients are known that are used to manufacture medicines which have the ability to acquire different crystalline forms. The formation of different crystalline forms depends on the quality in the manufacturing process of the drug (Islán and Montes, 2006), the temperature used during manufacturing, the use of solvents, the evaporation process and the presence of contaminants, among other factors (Lara and Lopez, 2017). The presence of one or another crystalline form can directly influence the solubility of the drug and it is known that the presence of polymorphic structures in drugs can alter their bioavailability and half-life and therefore have a different therapeutic effect (Hiendrawan et al., 2017; Zhou et al., 2018)). This is why *in vitro* dissolution profiles can help predict the behavior of drugs *in vivo*.

In general, polymorphs can be studied by X-ray diffraction, thermal analysis, vibrational spectroscopy, nuclear magnetic resonance in solid state and microscopy. However, Raman spectroscopy and Infra-red analysis have now been used because they present better resolution (Prohens and Puigjaner, 2007; Du and Xue, 2016). The Raman technique is a type of molecular vibrational spectroscopy which bases its operation on the Raman effect consisting of an inelastic dispersion of a photon by the molecules that make up the material. Raman spectroscopy has great importance in the study of objects of cultural interest thanks to its high specificity, its non-destructive nature on certain materials and micro destructive in others; it is also non-invasive and has an excellent spatial resolution (García, 2013). This technique, in combination with infrared spectrometry, represents an excellent option for the study of the crystalline configuration of drugs.

On the other hand, pioglitazone is a drug belonging to the family of thiazolidinediones used for the control of diabetes mellitus type 2. Of all the drugs that formed this family only pioglitazone remains on the market, although it has already been removed from some countries like France due to its adverse effects, mainly liver damage. However, in Mexico and many other countries it is still manufactured and marketed in different brands. Reports on changes in therapeutic effects due to the presence of polymorphisms in diabetic patients were not found. The aim of the present work was to determine the relationship between the dissolution profile of two pioglitazone brands and the presence of pharmaceutical polymorphisms.

MATERIALS AND METHODS

Reagents

Acetonitrile grade high resolution liquid chromatography (HPLC)

brand J.T. Baker lot 9017-03 C. Distilled water in the same laboratory, CTR brand reagent grade acetic acid and ethyl acetate HPLC brand J.T. Baker lot 604033. The reference listed drug was Pioglitazone hydrochloride in 15 mg tablets of Zactos® manufactured by Takeda - Japan and distributed in Mexico by Eli Lilly, lot C410501C. The test drug was Pioglitazone hydrochloride in 15 mg tablets issued as a generic drug, (lot 15E087). This drug is one of the most consumed by the diabetic population due to its low price. A HPLC reference standard was used, manufactured by Aarti Drugs Limited, Lot PIO / 10100105 ES-DA-022.

Development and validation of the chromatographic method

An Agilent model 1100 chromatograph with UV-vis detector VWD G1314A ($\lambda = 230$ nm) with quaternary pump G1311A and an Agilent Eclipse XDB-C18 4.6x150 mm and 5µm column were used. The methodology used was developed based on modifications made to the work reported by Vertiz et al. (2014). A solution of 0.1% acetic acid and acetonitrile in a 50:50% v/v ratio was used as the mobile phase. The following concentrations were established as working range: 80, 200, 400, 800, 1000, 1300, 1600, 2000 and 2600 ng/ml. The mobile phase was composed of acetic acid (ac) 0.1%/Acetonitrile 50/50% v/v with isocratic elution at a flow of 1.2 ml/min. 50 µl was injected. The validation of the chromatographic method was carried out based on the provisions of the Mexican standard NON-177-SSA1-2013 that includes the following parameters:

- (i) Linearity: three calibration curves were prepared and analyzed into the working range. The average of the Pearson regression coefficient $r \geq 0.99$ was rated as acceptable. Precision: The coefficient of variation -C.V.- (standard deviation / average) was calculated for the analytical responses of each concentration level of the work range. It was rated as acceptable when C.V. $\leq 2\%$. Further, intraday precision was determined by the analysis of 5 repetitions of standard concentration samples known (high and low) on the same day. The interday precision was determined by the analysis of 3 known standard concentration samples (high and low) analyzed in three successive days.
- (ii) Accuracy: Using the precision data (peak areas), the concentration in each level of the curve and standard concentration samples known (high and low) was calculated by the corresponding equation and the Relative Error expressed as Relative Standard Deviation ($RSD = \frac{[\text{Known Concentration} - \text{Calculated Concentration}]}{\text{Known Concentration}}$) was determined and expressed as percentage. The curve was qualified as exact when at least 75% of the levels met criterion $RSD \leq 2.0\%$. If a level failed in this criterion, it was eliminated from the curve and the curve was recalculated.
- (iii) Sensitivity: the limit of quantification (sensitivity) was established as the lowest concentration of the working range (80 ng/ml)
- (iv) Stability of the sample during processing: a series of three samples was analyzed at the beginning and end of the process and the initial and final signals were compared. The samples were rated as stable if the absolute difference of the average of the percentage quantified in the initial and final analysis was $\leq 3\%$.
- (v) Stability in solution: To build calibration curves and quality solutions, a mother solution and secondary solutions were used, which were prepared in mobile phase and stored for not more than a week. To demonstrate its stability, the concentration of the solutions was analyzed at the time of preparation and compared against the concentration analyzed after one week. They were taken as stable if the $RSD \leq 5\%$. All the solutions were stored in refrigeration ($5 \pm 2^\circ\text{C}$).
- (vi) Selectivity: it was verified that there were no interferences in

the analytical answers. In case of interferences, these should not be greater than 3% of the minor response of the working range. (vii) Equipment suitability: For each analytical run, the injector, the pump and the column were evaluated by injecting a solution of known standard concentration in triplicate. The performance of the pump and column was rated as acceptable if the average retention time of the three injections was less than 5%. The injector was rated as acceptable if the average of the analytical responses of the three injections was less than 5%. To evaluate the column, calculate the number of theoretical plates (N) based on the average of the retention times and the peak width at the average height. The column was rated as acceptable when $N \geq 2000$.

(viii) Quality control: A calibration curve was prepared for analytical run. The concentrations of the samples were calculated by the corresponding equation on each work day. Additionally, quality solutions were prepared consisting of three levels of known concentration of reference standard (high, medium and low) prepared in triplicate. These were distributed homogeneously in the analysis lot. Subsequently, the concentration of each of them interpolated in the corresponding calibration curve was calculated. The analytical run was qualified as acceptable when the calibration curve met the linearity criteria and the control samples met the accuracy criteria.

Sample dissolution

A Hanson Research Corp. Model 64-100-121 pallet dissolutor was used. The stirring speed was set at 50 r.p.m. The drugs were dissolved in three different solutions: A) 0.1 N hydrochloric acid (pH 1.2) that simulates gastric conditions. B) Phosphate buffer solution of pH 4.5 and C) Phosphate solution of pH 6.8 simulating intestinal fluid. 6 tablets of each drug were separately dissolved in each of the solutions. An aliquot of 1 ml was taken from time zero to 45 min every 3 min and diluted in a 1:10 ratio to be analyzed in the chromatograph.

Equality criteria

The similarity factor f_2 was used to determine the equality between the dissolution profiles. Both the NOM-177 and the FDA (Guidance Drugs) define f_2 as a transformation of the logarithmic reciprocal square root of the sum of the error and it is an adequate instrument to measure the similarity in the percent dissolution.

$$f_2 = \log \left[\frac{100}{\sqrt{1 + \left(\frac{1}{t}\right) S(Ri - Pi)^2}} \right]$$

t = number of sampling times.

Ri = Average of the dissolved percentage of the reference medicine in the "i" sampling time.

Pi = Average of the dissolved percentage of the test drug in the "i" sampling time.

The drugs have a similar rate of dissolution when the similarity factor $f_2 \geq 50$.

Raman and infrared spectrometry

The Raman and Infrared techniques tend to complement one

another, and are ideal for studying pharmaceutical polymorphisms. Raman is generally better at identifying non-polar functional groups, while infrared behaves better at polar molecular vibrations (Vértiz et al., 2014). Raman spectrometry is based on the inelastic shock of the radiation incident on a molecule, that is, the scattered radiation is of different wavelength than the incident radiation. The radiation consists of a beam of monochromatic light that goes from the UV to the infrared (García, 2013). The vibration of the functional groups of a compound subjected to Raman produces a characteristic spectrum that can be compared with other compounds to verify if they are similar or not (Aubuchon and Gracia-Del Rió, 2018). An Ocean Optics ® model QE65000 was used with a controlled power of 578 mW and an integration time of 2 s. The equipment has a 0.922 intensity laser source at a wavelength of 785 nm and a power of 499 mW with a high sensitivity Hamamatsu S7031-1006 detector. The Spectra Suite ® software was used for signal processing. Samples for Raman analysis consisted of one tablet of each drug extracted from the package and directly subjected to analysis. A sample of the HPLC grade reference standard of purity was also subjected to analysis. The spectra were compared with each other to determine conformational similarities.

On the other hand, infrared (IR) spectrometry is based on the radiation absorbed by the molecules in vibration. An ABB3000 model 3000 medium infrared machine with a range of 450-4000 cm^{-1} was used for the analysis. A tablet of medicine was taken and pulverized in Agate mortar by adding KBr and then compressed to obtain a uniform transparent glass that was subjected to analysis. The spectra of each medication (test and reference) and the reference standard were compared to determine similarities.

RESULTS AND DISCUSSION

Chromatographic method

The method met all the acceptance criteria and was therefore classified as reliable, precise and exact for the established work range. The curves showed that Pearson regression coefficient $r = 0.9955$; Coefficient of variation C.V. = 6.05% and Relative standard deviation R.S.D. = 5.94. The stability was also satisfactory and the samples resisted at least two freeze-thaw cycles.

Dissolution profile

Pioglitazone dissolves better in acid media, so the dissolution profiles in solutions B and C were inconclusive due to the low solubility presented by both brands. However, in the solution, both drugs (test and reference) present a solution of more than 85% in the first 15 min, so they can be considered as rapidly dissolving drugs. However, the similarity factor f_2 was equal to 25.1, which means that there are significant differences between the test and reference medicine. In Figure 1 it can be seen that the test drug has lower dissolving power; the concentrations in the test drug are lower at any time. Additionally, Figure 2 shows the dissolution profiles in the three solutions tested. As can be seen, the solubility of the drug in B and C is very low.

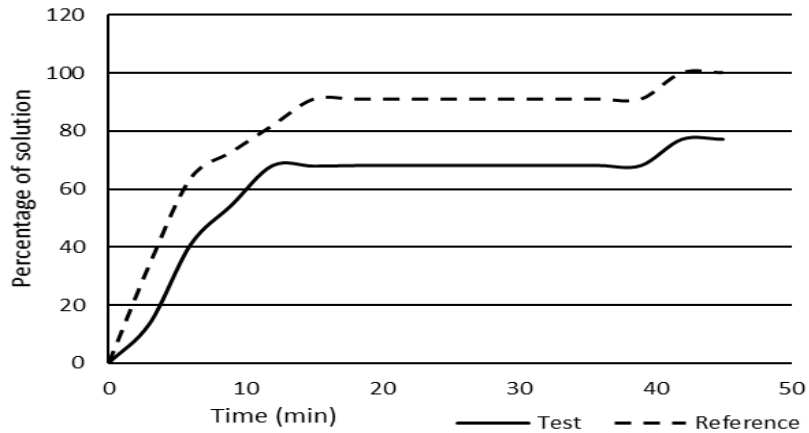


Figure 1. Dissolution profiles of test and reference medicine in solution A.

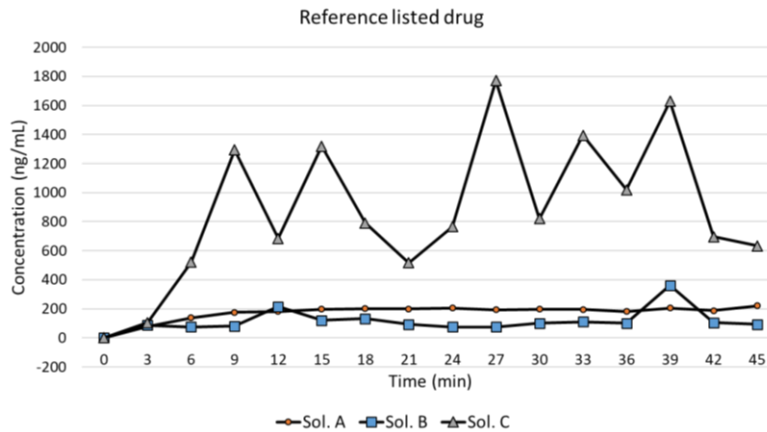


Figure 2. Dissolutions profiles in solutions A, B and C.

Raman spectrometry

The intensity in the signals of the spectra of the test and reference medicine was lower than the intensity observed in the reference substance. This can be attributed to the presence of the excipients present in the drugs and which are not found in the reference substance due to its purity. However, the spectrum shows two anomalies in the dispersion of the response at 1070.13 cm^{-1} and 1311.22 as shown in Figure 3. This response suggests the presence of different configuration between the test and reference substance' that is it suggests the presence of pharmaceutical polymorphism.

In addition, the displacement at 606.73 cm^{-1} presents an intense signal in the reference substance but not in the test drug. On the contrary, in displacements 875.92 , 1070.13 and 1745.49 cm^{-1} the same signals are present in the test and reference medicine, so it could be

concluded that these three functional groups do not differ from each other. Raman spectroscopy is based on the vibration responses of functional groups. Each functional group presents a characteristic displacement. The fact that there are anomalies in these responses suggests the presence of changes in the functional groups in the structural conformation of the molecule. In the infrared analysis, there are no differences in the signals of the test and reference medicines, as shown in Figure 4.

DISCUSSION

Two crystalline forms of pioglitazone hydrochloride are currently known, referred to as Form I and Form II (Tao-Zhao et al., 2013). It has been found that pioglitazone hydrochloride form II is identical to pioglitazone base. However, form I of pioglitazone hydrochloride is a

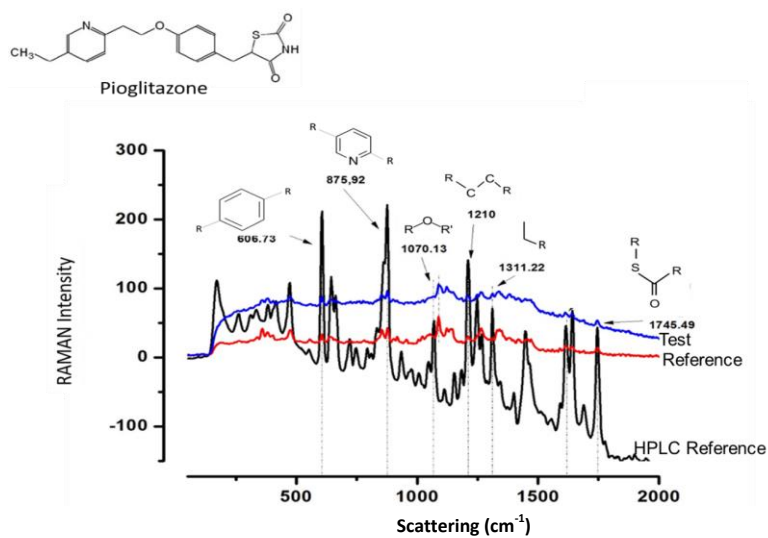


Figure 3. RAMAN spectrometry of the functional groups of two brands of pioglitazone and reference substances.

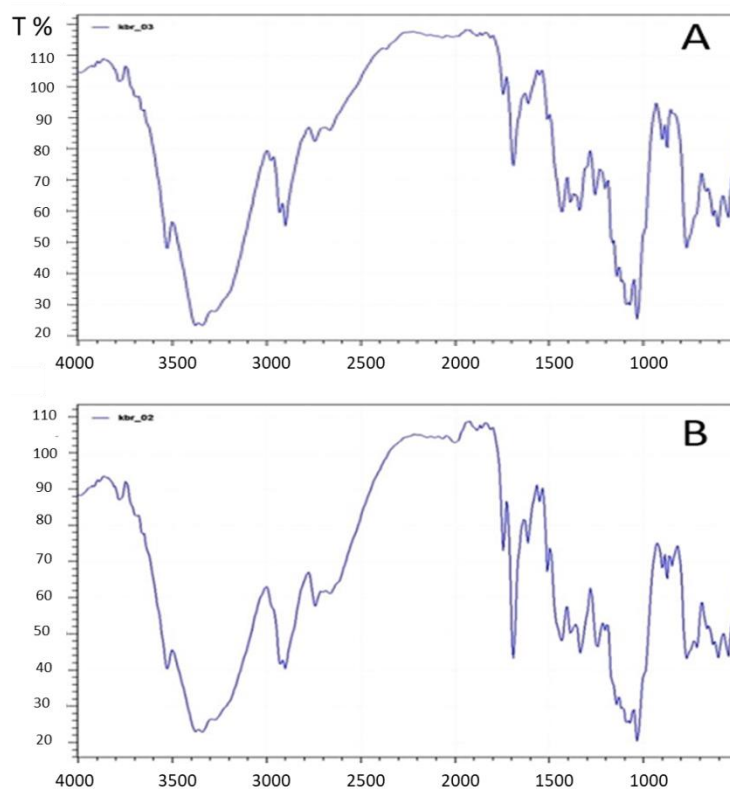


Figure 4. Infrared spectroscopy of the reference drugs (A) and tests (B).

conglomerate that is routinely used in the manufacture of the drug. Even when the two forms could be present in the samples, they interconvert *in vivo*. Until now no

differences have been detected in its pharmacokinetics (Chengcheng and Adam, 2017). The results obtained in our Raman analysis and in the solubility profiles suggest

the presence of polymorphisms or variations in the crystalline conformations of the pioglitazone hydrochloride of the test drug, but they do not seem to have any relation with the common Form I and II of pioglitazone. The formation of crystals during the tableting process can be due to several factors, such as the method used (sublimation, evaporation of the solvent, heat treatment, etc.). The use of different additives during the crystallization of pioglitazone hydrochloride also influences the power of solubility because a crystalline configuration can lead to a greater porosity and decrease in density, increasing solubility, and modifying absorption in the stomach (Sachinkumar et al., 2012).

Many drugs of solid form can exist in various crystalline forms and have variable physicochemical properties. These variations can influence such a way that a medicine considered as bio-interchangeable changes to be non-bio-interchangeable because it presents different crystalline forms than the original medicine (Zhou et al., 2018; Fuentes et al., 2006). When a drug presents polymorphisms it is recommended to choose the formulation that produces the most stable form, however, the question always arises: are all the polymorphisms known? Do we know the most stable form? (Garland, 2007). At present, the dissolution profile is taken as a predictive tool for *in vivo* product development and its biopharmaceutical properties (Gonzalez et al., 2015). Therefore, it is necessary to analyze more closely the crystalline configurations and co-relate them with their solubility to prevent unwanted pharmacokinetics. Rifampicin, for example, has a very variable bioavailability due to the presence of polymorphisms. Because of this, WHO already recommends using only those formulations whose dissolution has been satisfactorily tested. Phenylbutazone, verapamil, nitrofurantoin and ampicillin are other examples (Sharman et al., 2011).

Sugita et al. (2014) indicated that for class II drugs in the Biopharmaceutical Classification System with low solubility and high permeability, the solubility and/or dissolution rate in the gastrointestinal tract are considered the principal factors limiting oral absorption. In this study, particles smaller than 1 mm help better solubility, as presented in the reference listed drug. The use of hydroxypropyl cellulose as part of the excipients helps pioglitazone to have a better solubility. It must be considered then, that apart from the modified structures found in our study, the excipients used in one or the other drugs may also influence the determined solubility profile.

The greater solubility of pioglitazone in acidic media is directly related to its polar structure and its thermodynamic properties. Tao et al. (2013) indicated that the solubility of pioglitazone hydrochloride (Form I) increased in methanol, ethanol, 1-propanol, acetic acid, and *N,N*-dimethylacetamide with increasing temperature, because of the polarity of the solubility system. Finally, it is necessary to highlight that in the infrared analysis no

anomalies were detected between the two drugs analyzed. The reason why the results in Raman and Infrared differ is not very clear and should be the subject of a detailed study in the future.

Conclusion

The RAMAN spectrometry showed at least two anomalies in the configuration of the test drug, which could be related to the difference in the dissolution profile found. This may have consequences on the bioavailability of the drug in patients who are still using pioglitazone hydrochloride for diabetic control.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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