A Discriminating Dissolution Method for Glimepiride Polymorphs

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ABSTRACT: Glimepiride, an oral antidiabetic drug, is practically insoluble in water and exists in two polymorphic forms, I and II, of which form II has higher solubility in water. Because the dissolution rate of drugs can depend on the crystal form, there is a need to develop discriminating dissolution methods that are sensitive to changes in polymorphic forms. In this work, a dissolution method for the assessment of 4 mg glimepiride tablets was developed and validated. The optimal dissolution conditions were 1000 mL of phosphate buffer (pH 6.8) containing 0.1% (w/v) of sodium dodecyl sulfate as the dissolution medium and a stirring speed of 50 rpm using a paddle apparatus. The results demonstrated that all the data meet the validation acceptance criteria. Subsequently, tablets containing forms I and II of glimepiride were prepared and subjected to dissolution testing. A significant influence of polymorphism on the dissolution properties of glimepiride tablets was observed. These results suggested that the raw material used to produce glimepiride tablets must be strictly controlled because they may produce undesirable and unpredictable effects. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:794–804, 2012

Keywords: glimepiride; dissolution rate; HPLC; microscopy; polymorphism of pharmaceuticals; X-ray diffractometry; infrared spectroscopy; differential scanning calorimetry

INTRODUCTION

In vitro dissolution tests for immediate-release solid oral dosage tablets are extremely important, as these tests are crucial for assessing the quality of all drug product lots and can guide the development of new formulations.[1]

In vitro study of dissolution is an alternative to bioequivalence studies. Dissolution tests can, therefore, provide evidence for similarities and differences between medicinal formulations.[2] From a quality assurance point of view, a more discriminating dissolution method is preferred because the test will indicate possible changes in the quality of the product before *in vivo* performance is affected.[3]

For drugs with poor water solubility, there is some difficulty in selecting an appropriate dissolution medium with discriminating power. The choice of a medium capable of discriminating among critical manufacturing variables is crucial in such cases.[4] Among these critical variables, the possible presence of different polymorphic forms deserves to be investigated. Polymorphic forms have been shown to influence the solubility and, therefore, the dissolution rate of numerous drugs.[5]

Therefore, there is a real need to develop discriminating dissolution tests for drugs with poor water solubility, which can discriminate between critical manufacturing variables such as polymorphism in pharmaceutical solids.

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Glimepiride (CAS 93479-97-1), chemical name 1-[[4-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamide) ethyl] phenyl]sulfonyl]-3-(*trans*-4-methylcycloheyil) urea,[6] is an oral antidiabetic drug in the sulfonylurea class, which is widely used in the treatment of type 2 diabetes.[7] It is a white to yellowish white, crystalline, odorless powder and its molecular formula is $C_{24}H_{34}N_4O_5S$. Glimepiride has a molecular weight of 490.62, is sparingly soluble in water, and is classified as a class II drug according to the Biopharmaceutics Classification System.[8] There are two polymorphs of this molecule reported in the literature, form I and form II, of which form II has about 3.5-fold higher solubility than that of form I.[9]

There is a growing number of studies describing the determination of glimepiride concentration in biological fluids[10–18] and pharmaceutical formulations[19–30] using a variety of methods. However, there is no dissolution method for glimepiride tablets in the literature.

In light of the considerations outlined above, this work aims to develop and validate a discriminating dissolution method for glimepiride polymorphs.

EXPERIMENTAL

Chemical and Reagents

All reagents used were of analytical grade. Sodium hydroxide, disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate monohydrate ($NaH_2PO_4{\cdot}H_2O$), ethanol, chloroform, ammonium acetate, citric acid, glacial acetic acid, sodium dodecyl sulfate (SDS), and hydrochloride acid were purchased from Vetec. (Rio de Janeiro, Rio de Janeiro, Brazil). Methanol was of high-performance liquid chromatography (HPLC) grade and was acquired from Sigma–Aldrich (St. Louis, Missouri). HPLC-grade water, used as the mobile phase, was prepared by Milli-Q reverse osmosis and met the United States Pharmacopoeia (USP) requirements. Glimepiride reference substance (assigned purity of 100.12%) was supplied by Zhejiang Xianju Huakang Pharmaceutical & Chemical Company, Ltd. (Xianju, Zhejiang, China). A glimepiride reference product (Amaryl tablets; Sanofi-Aventis, Suzano, São Paulo, Brazil), which is claimed to contain 4 mg of the active component, was purchased from a local market. The placebo mixtures with the same composition as the pharmaceutical formulations were prepared in the laboratory using the following pharmaceutical grade excipients: lactose hydrous, SDS, sodium starch glycolate, povidone, microcrystalline cellulose, magnesium stearate, and lake indigo carmine.

Equipment

Dissolution tests were performed in an Electrolab TDT-08 L multi bath ($n = 8$) dissolution test system (Electrolab, Mumbai, Maharashtra, India) in accordance with the USP general method.[31]

The HPLC equipment used was a Shimadzu series LC-10A (Shimadzu, Kyoto, Quioto, Japan) consisting of an LC AVP pump, a CLASS-VP 5.02 integration system, a DGU-14 A degasser, a 7725i manual injector with a 20 μL loop, a SPD-10AVP integrated ultraviolet detector, a FCV-10ALVP valve, a CTO-10AVP column oven, and a SCL-10 AVP controller. The separation was performed on a Waters Symmetry C-18 column ($4.6 \times 250\ mm^2$, 5 μm; Waters, Milford, Massachusetts).

Powder X-ray diffraction (PXRD) of samples was performed on a Rigaku ultima IV X-ray diffractometer (Rigaku Company, Ltd., Tokyo, Honshu, Japan).

Infrared (IR) spectra were obtained using a Prestige-21 Fourier transform (FT) IR spectrophotometer (Shimadzu, Tokyo, Honshu, Japan).

Differential scanning calorimetry (DSC) was performed using a Mettler Toledo instrument model DSC 1 STARe system (Mettler–Toledo, Barueri, São Paulo, Brazil).

Scanning electron microscopy (SEM) photographs were taken with a Jeol scanning electron microscope model JSM 7500F (Jeol, São Paulo, São Paulo, Brazil).

The following equipments were also used: a single rotary tablet compression machine (Lemaq model LM08B; Lemaq, Diadema, São Paulo, Brazil), a digital pH meter (Marconi model PA 200; Marconi, Piracicaba, São Paulo, Brazil), an ultrasonic bath (Unique model USC2800A; Unique, Indaiatuba, São Paulo, Brazil), and an analytical balance (Kern model 410; Kern & Sohn GmbH, Balingen, Zollernalbkreis, Germany).

Solutions

All dissolution media used in this study were degassed at 41°C in an ultrasonic bath for 30 min prior to use.

A stock standard solution containing 200 mg L^{-1} of glimepiride was prepared by accurately weighing 10 mg of the glimepiride reference substance and then transferring it to a 50-mL volumetric flask and adding 40 mL of methanol. The flask was sonicated for 5 min and the remaining volume was filled with methanol. Working standard solutions were prepared immediately prior to use by appropriately diluting the corresponding stock solutions of glimepiride with the dissolution medium.

Sample solutions were prepared by placing one tablet in a vessel containing the dissolution medium (1000 mL) at a temperature of 37 ± 0.5°C. Samples were collected at the end of a specified time and filtered using a quantitative VETEC filter paper (Vetec). For the HPLC analysis, samples were injected directly into the HPLC system.

Sink Conditions

To establish sink conditions, the solubility of the drug was tested using 4 mg of glimepiride in 333 mL of 0.1 mol L^{-1} HCl, 0.01 mol L^{-1} HCl, acetate buffer (pH 4.5), phosphate buffer (pH 6.8), and phosphate buffer (pH 6.8) containing various SDS concentrations [0.1% (w/v), 0.5% (w/v), 1.5% (w/v), 2% (w/v), and 2.5% (w/v)].

Optimization of the Dissolution Method

The dissolution experimental conditions were established by subjecting a 4 mg tablet reference product to the following dissolution media conditions: phosphate buffer (pH 6.8), phosphate buffer (pH 6.8) containing 0.1% (w/v) of SDS, and phosphate buffer (pH 6.8) containing 0.5% (w/v) of SDS as the dissolution media. After determining the optimum dissolution medium, the paddle and basket stirring apparatus was tested at stirring speeds of 50, 75, and 100 rpm. A volume of 1000 mL was used in the vessels and the temperature was kept constant at 37 ± 0.5°C. A sample (10 mL) was withdrawn from the dissolution medium at 5, 10, 15, 20, 30, and 60 min; the withdrawn volume was immediately replaced, and the withdrawn sample was analyzed using an HPLC method and the data were employed in the acquisition of dissolution profiles. Six samples were assayed.

Chromatographic Conditions

The chromatographic conditions were based on the previous work in which an HPLC method for the quantitation of glimepiride in tablets was developed and validated.[32] The mobile phase was composed of 27.5 mmol L^{-1} potassium phosphate buffer (pH 6.5)/methanol [34:66 (v/v)] and was eluted at a flow rate of 1 mL min^{-1}. The mobile phase was filtered under vacuum through a 0.45-μm modified hydrophilic polytetrafluoroethylene membrane and degassed ultrasonically for 30 min prior to use. Peak areas (in volts) were used as the measured analytical response, with detection at 228 nm and an injection volume of 20 μL. The column was maintained at controlled room temperature (25°C). All analyzed solutions were filtered through a 0.45-μm Millex-LCR filter (Millipore, São Paulo, São Paulo, Brazil) prior to injection into the column.

Validation of the Dissolution Test

After dissolution test optimization, the assay was validated in accordance with the International Conference on Harmonisation guidelines[33] through the analysis of selectivity, linearity, precision, accuracy, detection limit (DL), quantitation limit (QL), and robustness parameters.

Selectivity

For the determination of selectivity, a placebo sample containing the same excipient composition as the 4 mg tablet reference product was prepared. The placebo sample was then transferred into a vessel containing 1000 mL of phosphate buffer (pH 6.8) with 0.1% (w/v) of SDS at 37 ± 0.5°C and stirred for 60 min at 150 rpm using a paddle apparatus. Aliquots were collected and the interference of the placebo mixture of each formulation was evaluated by the HPLC method.

Linearity

Linearity was assessed by the analysis of standard solutions in triplicate prepared on three consecutive days for concentrations of 1, 2, 3, 4, 5, and 6 mg L^{-1} of glimepiride in phosphate buffer (pH 6.8) containing 0.1% (w/v) of SDS. Linearity was evaluated by linear regression analysis, which was calculated by the least squares regression method. The acceptance criterion was a correlation coefficient of 0.999 or greater and a relative standard deviation (RSD) of each point (n = 5) of less than 2%.

Accuracy and Precision

The accuracy of the method was evaluated through a recovery test of known amounts of glimepiride reference substance added to the placebo. A stock solution containing 400 mg L^{-1} of glimepiride was prepared in methanol. Aliquots of 5, 10, and 15 mL of this solution were added to vessels containing the dissolution medium for a total volume of 1000 mL (final concentrations were 2, 4, and 6 mg L^{-1}, which corresponded to 50%, 100%, and 150% of the target concentration). Placebo samples containing the same excipient composition as the 4 mg tablet reference product were then transferred to each vessel. The samples were maintained at 37 ± 0.5 °C and stirred at 50 rpm for 1 h. Subsequently, aliquots of each vessel were collected and analyzed. These studies were performed in triplicate. The percent recoveries were calculated according to Eq. 1:

$$R(\%) = \frac{(C_{\text{measured}})}{(C_{\text{added}})} \times 100\% \quad (1)$$

where C_{measured} is the sample concentration obtained from the HPLC method at each concentration level, and C_{added} is the theoretical concentration at each concentration level. The acceptance criterion was recovery between 95% and 105%.

For intraday precision studies, the same solutions used in the accuracy test were analyzed to ensure the precision of the method. Interday precision was examined by repetition of the described procedure on two different days by two analysts. The acceptance criterion was an RSD of 5% or less.

Detection Limit and Quantitation Limit

The DL and QL of the methods were obtained from Eq. 2 and Eq. 3:

$$DL = 3(SD/a) \quad (2)$$

$$QL = 10(SD/a) \quad (3)$$

where SD is the intersection standard deviation and a is the slope of the calibration curves obtained in the linearity study.

Robustness

The robustness of the method was evaluated by analyzing the data acquired by varying seven factors according to Youden and Steiner's robustness test.[34] This study was performed to determine how the proposed method would be affected by variations in experimental conditions. The percentage drug release (4 mg tablet reference product) was calculated for each experiment by comparing the area of the sample peak with the area obtained from the glimepiride standard solution of 4 mg L^{-1} (section *Solutions*). The following factors were probed: the time of dissolution test, pH of the dissolution medium, temperature of the dissolution medium, dissolution medium degassing, stirring speed, sample filtration, and exposure to light. The factors' nominal values were denoted by A, B, C, D, E, F, and G, and their alternative values were denoted by the corresponding lower case letters a, b, c, d, e, f, and g. A total of eight experiments were conducted, as indicated in Table 1. From the obtained results, the effect of each factor was estimated by obtaining the difference between the average values of the four analyses that had a nominal value (upper case letter) and the four analyses with an alternative value (lower case letter). For example, the effect of the time of dissolution test was obtained from $[(1/4)(92.2 + 93.3 + 89.5 + 77.7)] - [(1/4)(87.8 + 95.2 + 93.8 + 84.8)] = -2.22$. From the eight observed results (Table 1), the standard deviation was calculated. Effect values greater than the criterion $s\sqrt{2}$ (standard deviation multiplied by the square root of two) were considered significant, and the method was shown to be sensitive to changes in the factor of interest.

Study of the Dissolution Behavior of Glimepiride Polymorphic Forms in Tablets

Preparation of Crystal Forms

Form I was prepared in the same manner as previously reported.[9] Typically, crystals were grown by diffusing ethanol into a chloroform solution of glimepiride at room temperature. Form II was crystallized by dissolving glimepiride in an EtOH–H_2O (1:1) solution up to saturation in a water bath for 1 h at 85°C. The solution was maintained at controlled room temperature (25°C) for 12 h (overnight) and was then maintained in a refrigerator at 5°C for 1 week. The crystals were collected by filtration and dried at 60°C in vacuum.

XRD, FTIR, DSC, and SEM Studies

X-ray measurements were carried out at an ambient temperature under the following conditions: graphite monochromated Cu-Kα radiation ($\lambda = 1.542$ Å), voltage of 40 kV, current of 30 mA, and scan rate of 1°C/min between 3° and 40° of the 2θ range. FTIR spectroscopy data were obtained in the spectral range of 3500 to 400 cm^{-1} at an ambient temperature. Samples were prepared using a KBr disc. DSC analyses were carried out with a temperature ramp from 25°C to 250°C at 2°C min^{-1}. SEM photographs were observed for morphological characteristics of the crystals.

Preparation of Glimepiride Tablets

To evaluate the influence of polymorphism on tablets containing glimepiride, two batches were prepared: batch 1 (containing the polymorphic form I) and batch 2 (containing the polymorphic form II). First, a placebo mixture was prepared by accurately weighing out the following ingredients: lactose hydrous (80%), SDS (5%), sodium starch glycolate (4%),

Table 1. Factors and Their Levels from the Robustness Studies and Experimental Conditions According to Youden and Steiner's Robustness Test[34]

Selected Factor	Nominal Conditions	Alternative Conditions	Experimental Condition							
			1	2	3	4	5	6	7	8
Time of dissolution test	60 min (A)	55 min (a)	A	A	A	A	a	a	a	a
Dissolution medium pH	6.8 (B)	6.6 (b)	B	B	b	b	B	B	b	b
Stirring speed	50 rpm (C)	45 rpm (c)	C	c	C	c	C	c	C	c
Dissolution medium temperature	37°C (D)	35°C (d)	D	D	d	d	d	d	D	D
Dissolution medium degassing	Yes (E)	No (e)	E	e	E	e	e	E	e	E
Samples filtration	Yes (F)	No (f)	F	f	f	F	F	f	f	F
Exposure to light	Yes (G)	No (g)	G	g	g	G	g	G	G	g
Observed results (%)			92.2	93.3	89.5	77.7	87.8	95.2	93.8	84.8

Upper case letters represent nominal conditions and lower case letters represent alternative conditions.

povidone (0.4%), microcrystalline cellulose (10%), magnesium stearate (0.4%), and lake indigo carmine (0.2%). These excipients were mixed in a plastic bag by vigorous shaking. Subsequently, 40 mg of each polymorphic form was individually added to two separate portions of 1.665 g of the placebo mixture. Finally, the two batches were prepared in a similar manner by direct compression using a single rotary tablet compression machine.

Dissolution Profiles Comparison

The two batches prepared according to the previous section were subjected to dissolution test under optimal conditions. The following procedure was utilized for the dissolution experiments: phosphate buffer (pH 6.8) containing 0.1% (w/v) of SDS as the dissolution media and a paddle stirring apparatus at a rate of 50 rpm. Ten milliliters of the dissolution medium (controlled at $37 \pm 0.5^{\circ}$C) was sampled after 5, 10, 15, 20, 30, and 60 min followed by immediate replacement of the withdrawn volume, and the sample was analyzed by the HPLC method. Six samples were assayed for each product. The similarity of the dissolution profiles was then determined by the difference factor ($f1$) and similarity factor ($f2$) calculated from Eq. 4 and Eq. 5 as follows:

$$f1 = \left[\left(\sum_{t=1}^{n} |R_t - T_t|\right)\right] \Big/ \left[\left(\sum_{t=1}^{n} |R_t|\right)\right] \chi 100 \quad (4)$$

$$f2 = 50 \times \log\left\{\left[(1 + 1/n)\sum_{t=1}^{n} (R_t - T_t)^2\right]^{-0.5} \chi 100\right\} \quad (5)$$

where R_t is the percentage of drug dissolved from batch 1 at each time point, T_t is the percentage of the drug dissolved from batch 2 at each time point, and n is the number of sampling time points.

For curves to be considered similar, $f1$ values should be close to 0 and $f2$ values should be close to 100. Generally, if $f1$ values up to 15 (0–15) and $f2$ values greater than 50 (50–100), equivalence of the two curves is ensured.

Evaluation of Tablet Relative Density

The relative densities of three tablets selected at random were evaluated for each batch using Eq. 6:

$$\mathrm{RD} = m/\pi r^2 t \rho_g \quad (6)$$

where RD is tablet relative density, m is the tablet weight in grams, r is the tablet radius in centimeters, t is the tablet thickness in centimeters, and ρ_g is density of the granules in gram per cubic centimeter.

The averages of the tablet relative densities of the batches were statistically compared by analysis of variance at a 0.05 significance level.

RESULTS AND DISCUSSION

Dissolution Test Optimization

In general, mild conditions should be maintained during the dissolution testing to allow maximum discriminating power.[3] In this study, the dissolution test was optimized in terms of the dissolution medium, the nature of the stirring apparatus, and the rotation speed in order to obtain a slow dissolution of the reference product, which certainly will result in a test suitably discriminative. In addition, we seek a dissolution test that can meet the specifications of the US Food and Drug Administration,[1] which states that only one measurement should be considered after 85% dissolution for comparison of the dissolution profiles using the simple model-independent approach.

With respect to the dissolution medium, the sink condition tests showed precipitation of glimepiride reference substance in 0.1 and 0.01 mol L^{-1} HCl, acetate buffer (pH 4.5), phosphate buffer (pH 6.8), and phosphate buffer (pH 6.8) containing 0.1% (w/v) of SDS. These media were then initially discarded and the dissolution tests for the glimepiride reference product were performed in a phosphate buffer (pH 6.8) containing 0.5% (w/v) of SDS, using a paddle apparatus at a stirring speed of 50 rpm (Fig. 1). From the results obtained in this study, we can conclude that the phosphate buffer (pH 6.8) containing 0.5% (w/v) of SDS causes rapid dissolution (100% in 15 min). Thus, although the phosphate buffer (pH 6.8)

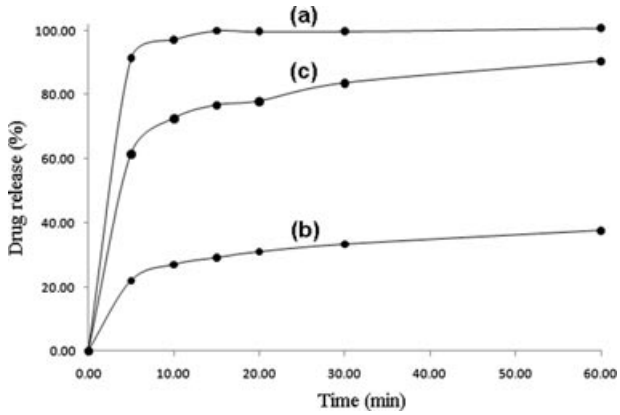

Figure 1. Dissolution profiles of 4 mg tablet reference product using a paddle at 50 rpm. (a) Phosphate buffer (pH 6.8) containing 0.5% sodium lauryl sulfate as the dissolution medium, (b) phosphate buffer (pH 6.8) as the dissolution medium, and (c) phosphate buffer (pH 6.8) containing 0.1% sodium lauryl sulfate as the dissolution medium. Samples were analyzed by high-performance liquid chromatography.

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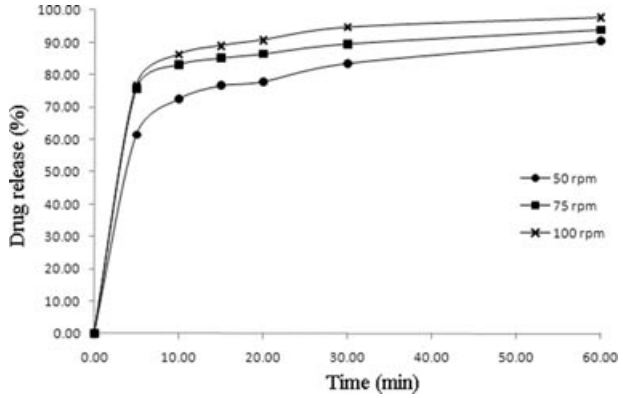

Figure 2. Effect of the rotation speed of the paddles on the dissolution profiles of 4 mg tablet reference product. Phosphate buffer (pH 6.8) containing 0.1% sodium lauryl sulfate was used as the dissolution medium. Samples were analyzed by high-performance liquid chromatography.

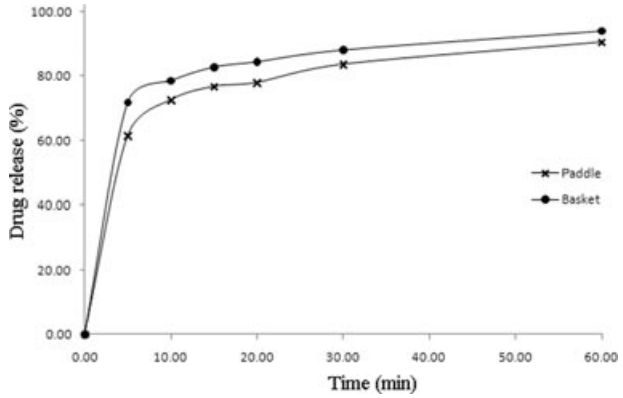

Figure 4. Effect of the type of dissolution apparatus at 50 rpm on the dissolution profiles of 4 mg tablet reference product. Phosphate buffer (pH 6.8) containing 0.1% sodium lauryl sulfate was used as the dissolution medium. Samples were analyzed by high-performance liquid chromatography.

did not ensure a sink condition, it was tested in order to obtain a dissolution profile with slower release. Figure 1 shows that phosphate buffer (pH 6.8) is not an "ideal" dissolution medium because no effective release of glimepiride was observed in this medium. Subsequently, the phosphate buffer (pH 6.8) containing 0.1% (w/v) of SDS was tested. As can be seen in Figure 1, this medium is "ideal" because this condition allows slow glimepiride release, which probably will result in a test suitably discriminative.

The effect of the rotation speed of the paddles (Fig. 2) and baskets (Fig. 3) on the dissolution profile of glimepiride tablets was examined at 50, 75, and 100 rpm. The experimental results showed a faster dissolution rate at 75 and 100 rpm for both apparatuses. Therefore, a rotation speed of 50 rpm was selected for further experiments to have a maximum discriminatory power for glimepiride polymorphic forms.

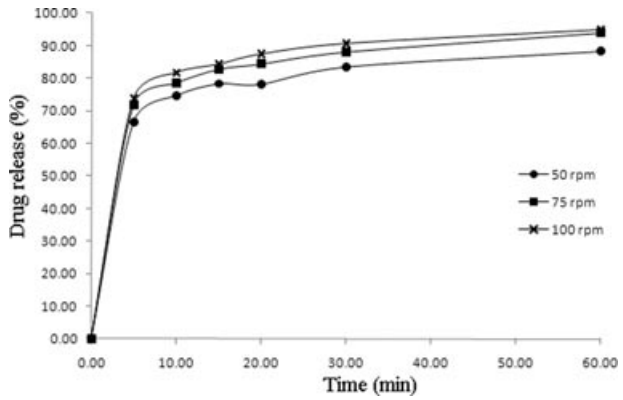

Figure 3. Effect of the rotation speed of the baskets on dissolution profiles of 4 mg tablet reference product. Phosphate buffer (pH 6.8) containing 0.1% sodium lauryl sulfate was used as the dissolution medium. Samples were analyzed by high-performance liquid chromatography.

In the experiments that evaluated the stirring apparatuses, it was observed that the dissolution rate was lower when the paddle was used (Fig. 4). Therefore, this apparatus at a speed of 50 rpm was selected for further experiments to have a maximum discriminatory power of the method.

Thus, the established dissolution conditions for comparison of the dissolution profiles of 4 mg glimepiride tablets were 1000 mL of phosphate buffer (pH 6.8) containing 0.1% (w/v) of SDS at 37°C as the dissolution medium and a paddle at a stirring a speed of 50 rpm as the apparatus.

Method Validation

Selectivity

No interfering peaks from the placebo formulation were observed at the same retention time as for glimepiride, which demonstrates the selectivity of this method (Fig. 5).

Linearity

For linearity studies, the calibration equation and correlation coefficient obtained were $y = 31595x + 497$ and 0.99987, respectively. Therefore, the response to the drug was linear in the concentration range of 1–6 mg L^{-1}.

Accuracy and Precision

The accuracy was demonstrated by the recovery of known amounts of glimepiride from the dissolution vessels. As shown in Table 2, the mean percentage of recovery was in accordance with fixed limits from 95 up to 105, confirming the accuracy of the method.

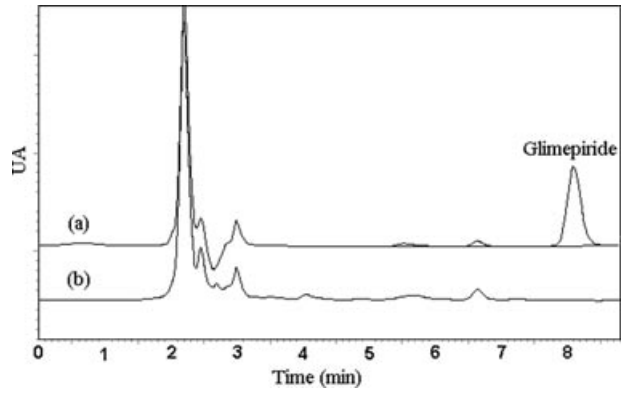

Figure 5. (a) Chromatogram of glimepiride at 4 mg L^{-1} in phosphate buffer (pH 6.8) containing 0.1% of SDS and (b) chromatogram of placebo formulation submitted under dissolution conditions described in the section *Selectivity* for selectivity studies. UA = Units of Absorbance.

Table 2. Accuracy Studies

Concentration Added (mg L^{-1})	Concentration Found (mg L^{-1})	Recovery Rate ± RSD (%) ($n = 3$)
2	1.9857	99.29 ± 0.42
4	4.0095	100.24 ± 0.58
6	5.8706	97.84 ± 0.46

RSD, relative standard deviation.

The values of the precision studies are shown in Table 3. The % RSD results of intraday and interday precision were within 5%, confirming the excellent precision of the assay.

Detection Limit and Quantitation Limit

The DL and QL values were found to be 0.042 and 0.142 mg L^{-1}, which demonstrates that the analyses are performed in a region above these values.

Robustness

From the results demonstrated in Table 1, the effect of each factor and the standard deviation of the eight observed results were calculated according to the section *Robustness*. An effect value higher than the criterion $s\sqrt{2}$ means that this effect is significant and the method is sensitive to the studied factor. The results are showed in Table 4. As observed, all effect values are smaller than the criterion $s\sqrt{2}$. Therefore, the method was considered fairly robust to all factors considered in this study.

Table 3. Precision Studies

Concentration Added (mg L^{-1})	Intraday (RSD)	Interday (RSD)
2	0.42 ($n = 3$)	2.06 ($n = 6$)
4	0.58 ($n = 3$)	2.86 ($n = 6$)
6	0.46 ($n = 3$)	1.66 ($n = 6$)

RSD, relative standard deviation.

Table 4. Robustness Test Results

Factor	Effect
Time of dissolution test	–2.22
Dissolution medium pH	5.70
Stirring speed	3.11
Dissolution medium temperature	3.47
Dissolution medium degassing	2.29
Sample filtration	–7.32
Exposure to light	0.87
$s\sqrt{2}$	8.23

Study of the Dissolution Behavior of Glimepiride Polymorphic Forms in Tablets

Figure 6 shows the PXRD pattern of crystals crystallized from a chloroform/ethanol system (form I) and from an ethanol/water system (form II). These patterns were in agreement with those previously demonstrated in the literature.[9]

Figure 7 shows the FTIR spectra of form I and form II of glimepiride. The major difference in the IR spectra between form I and form II was similar to

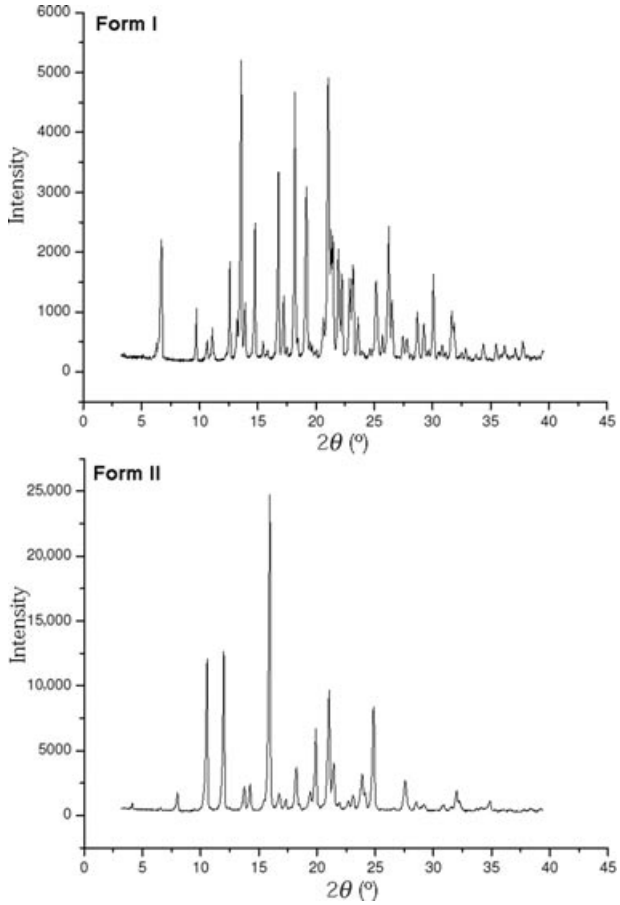

Figure 6. Powder X-ray diffraction of forms I and II of glimepiride.

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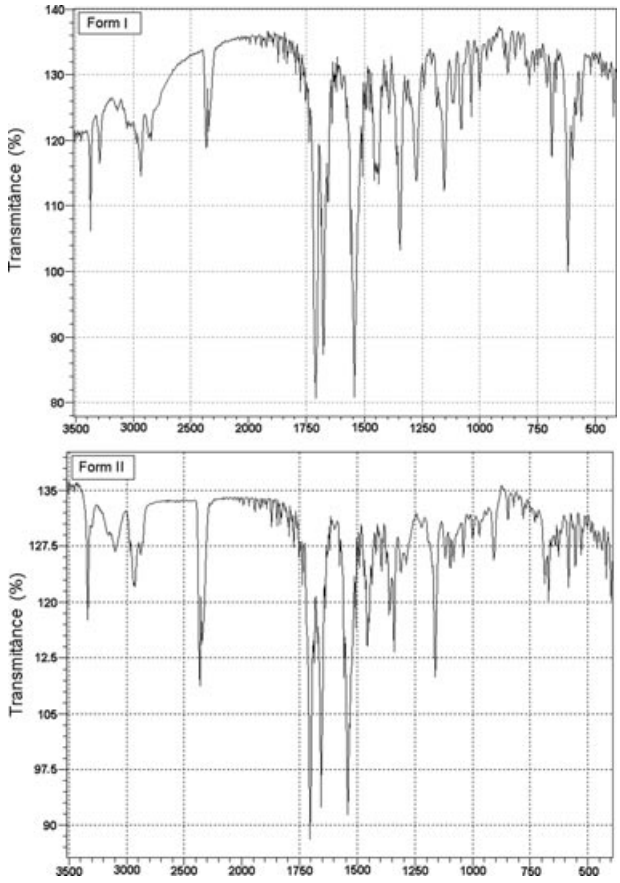

Figure 7. Fourier transform infrared spectra of form I and form II of glimepiride.

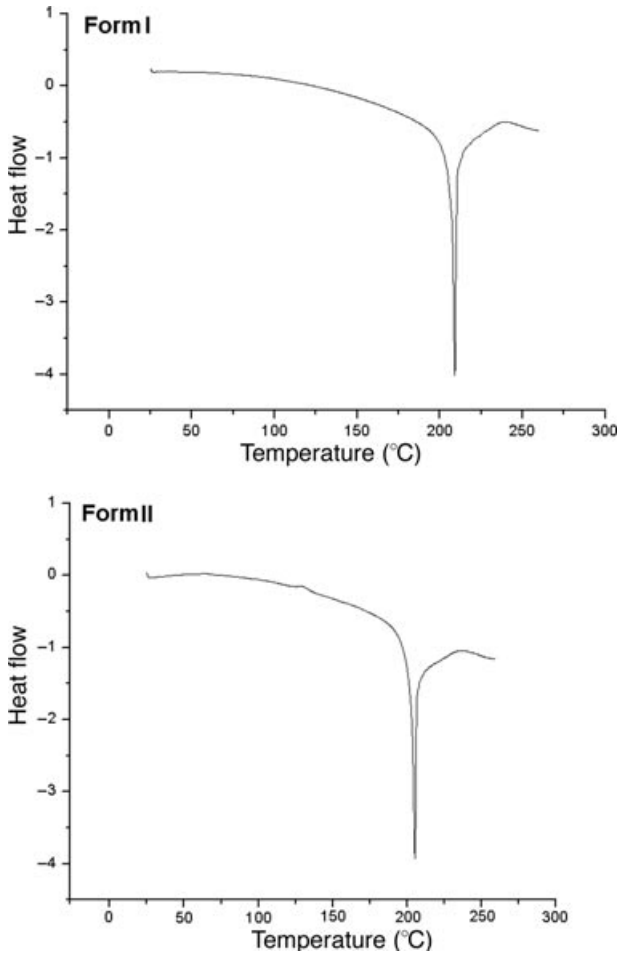

Figure 8. Differential scanning calorimetry of form I and form II of glimepiride.

those demonstrated by Endo et al.[9] A broad band near 3100 cm^{-1} was observed only in form II, which was attributed to an intermolecular hydrogen bonding of the sulfonamide N-H group.

Figure 8 shows the DSC of form I and form II of glimepiride. Both forms showed endothermic peaks above 200°C corresponding to the melting points. As previously demonstrated,[9] only form II showed an exothermic peak around 140°C, which was attributed to the polymorphic transition from form II to form I.

Therefore, the results of PXRD, FTIR, and DSC analyses showed that both forms were successfully crystallized in this work.

Subsequently, tablets containing forms I and II of glimepiride were prepared and subjected to the dissolution test under optimal conditions. The results are depicted in Figure 9. Finally, a comparison of the dissolution profiles of these two batches was conducted. The results of $f1$ and $f2$ were 61.40 and 7.06, respectively. These results demonstrate the significant influence of polymorphism on the dissolution properties of tablets containing glimepiride and show the discriminatory power of the proposed method.

In order to verify whether the dissolution rate differences are due to the different solubilities of the polymorphs and are not due to different states of compaction of the tablets, the RD of each batch was calculated according to the section *Evaluation of Tablet*

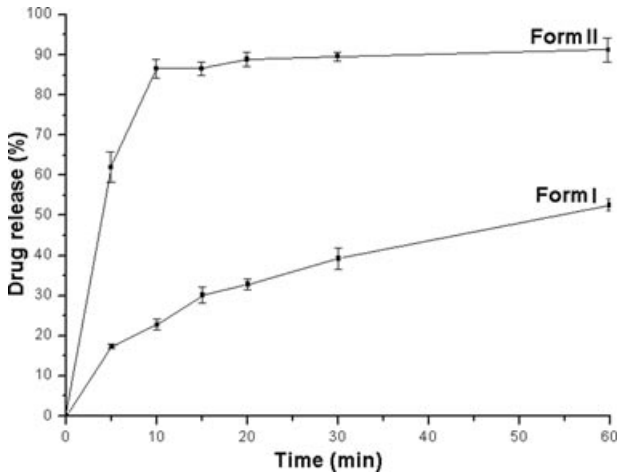

Figure 9. Dissolution profiles of tablets containing forms I and II of glimepiride. Error bars represent confidence intervals at 95%.

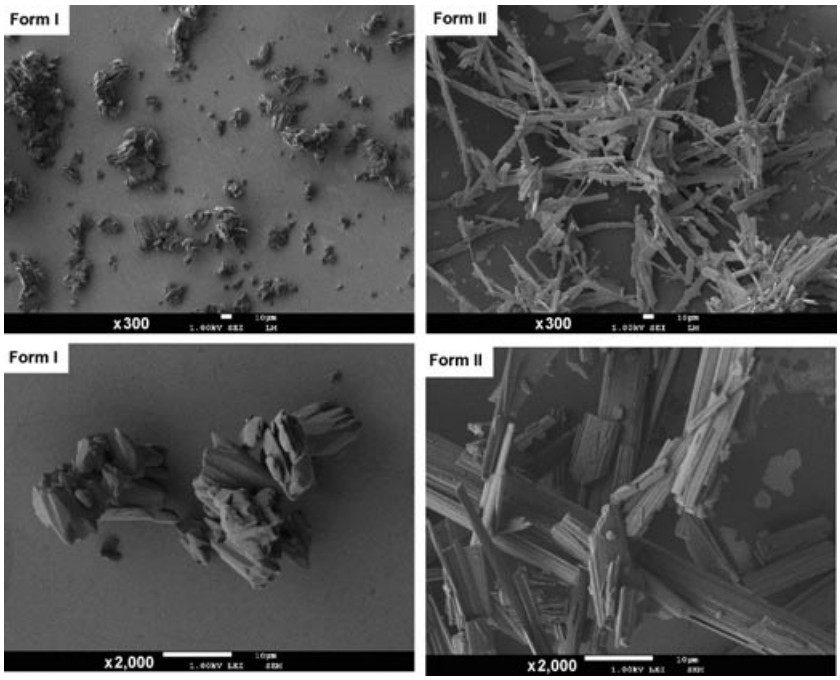

Figure 10. Scanning electron microscopy of form I and form II of glimepiride.

Relative Density. The average tablet relative densities for batch 1 and batch 2 were 0.548 and 0.559, respectively. *These values were not statistically* different ($p > 0.05$).

As the dissolution rate is inversely proportional to the particle size,[35] we performed SEM studies of the two polymorphs used in the tablet batches in order to check whether there was an influence of particle size on the dissolution properties of glimepiride. As shown in Figure 10, there was no clear difference between the particle sizes of the polymorphs. This result confirms that the differences in the dissolution rates of tablets containing glimepiride were due to polymorphism.

Unfortunately, it was not possible to identify the polymorphic form present in the reference product using PXRD, FTIR, and DSC techniques because the amount of glimepiride as a fraction of the total weight of the tablet was very small (about 2.35%).

United States Pharmacopoeia and European Pharmacopoeia do not mention which polymorph is preferred to be present in tablets. Therefore, both forms are acceptable. However, as demonstrated, the use of a polymorphic form other than the one used during the formulation development process will significantly alter the dissolution properties of glimepiride tablets. This is relevant because glimepiride is an oral antidiabetic agent, which acts by stimulating insulin secretion. Therefore, a significant decrease in the dissolution of this drug can harm treatment and a significant increase can cause hypoglycemic effect. For this reason, our findings suggest that the glimepiride polymorphic form in the raw material must be strictly controlled by using techniques presented in this paper (PXRD, FTIR, and DSC). Nevertheless, as control of the glimepiride polymorphic form in tablets is not possible (as mentioned in the above paragraph), the dissolution test proposed in this work is an important tool to detect changes in the dissolution properties of glimepiride tablets, which may occur due to polymorphism.

CONCLUSION

In this work, a dissolution test for glimepiride was developed and fully validated. The established dissolution conditions were 1000 mL of phosphate buffer (pH 6.8) containing 0.1% (w/v) of SDS at $37 \pm 0.5^{\circ}$C as the dissolution medium and a paddle as the stirring apparatus at a speed of 50 rpm. The validation results demonstrate that all data meet the acceptance criteria.

The test developed in this work is adequate to discriminate glimepiride polymorphic forms in tablets. Moreover, due to the significant difference between the dissolution profiles of the two glimepiride polymorphs in tablets, it is suggested that the raw material used to produce glimepiride tablets be strictly controlled to avoid changes in the dissolution rate of these tablets.

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