Drug Development and Industrial Pharmacy, 2011; 37(6): 727–736
© 2011 Informa Healthcare USA, Inc.
ISSN 0363-9045 print/ISSN 1520-5762 online
DOI: 10.3109/03639045.2010.538061

informa
healthcare

RESEARCH ARTICLE

Strategies to improve dissolution and oral absorption of glimepiride tablets: solid dispersion versus micronization techniques

Xiao Ning[1,2], Jin Sun[1], Xiaopeng Han[1], Yue Wu[1], Zhongtian Yan[1], Jihong Han[3], and Zhonggui He[1]

[1]Department of Pharmaceutics, School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, P. R. China, [2]National Institute for the Control of Pharmaceutical and Biological Products, Beijing, P. R. China, and [3]Institute for Science and Technology in Medicine and School of Pharmacy, Keele University, Staffordshire, UK

Abstract

The objective of this study is to compare two different dissolution-enhancing strategies, solid dispersion (SD) and micronized techniques, for improving oral absorption of poorly soluble glimepiride, and to decide which strategy is suitable for its solubilization. The formulation of glimepiride SD was prepared by a solvent-evaporation process with povidone k-30 (PVPk30) at a weight ratio of 1:9 (drug:carrier). The other was prepared via a modified micronization technique, where glimepiride was premilled together with lactose and Lutrol F68 until the milled material passes through a 500 mesh ASTM sieve (30 μm). The dissolution results indicated that the two techniques were both capable of enhancing the dissolution rate and extent of glimepiride. The release profiles of the two prepared products were similar to the marketed product (Amaryl®) in various types of dissolution media. Furthermore, the oral bioavailability was evaluated for the three formulations in fasted beagle dogs. Statistical analysis indicated that there were no significant differences in pharmacokinetic parameters among the two prepared formulations and a marketed product, especially for AUC_{0-36}, C_{max}, and T_{max}. The dissolution parameters (D_{10} and AUC_{0-20}) in Tris buffer demonstrated the good *in vitro/in vivo* relationship with T_{max} values for the three formulations. In conclusion, our studies confirmed that both SD and micronization techniques were capable of improving dissolution and oral absorption of glimepiride tablets to a similar extent as the marketed product, and the three glimepiride tablets were bioequivalent in the case of the rate and extent of absorption in dogs.

Keywords: Solid dispersion, micronization, glimepiride, in vitro dissolution, in vivo absorption

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/30/12
For personal use only.

Introduction

For highly lipophilic compounds, it has been generally hypothesized that the uptake across the gut membrane is efficient, resulting in sink conditions in the gut lumen, where the drug dissolves. In such case, the rate and extent of absorption from the gastrointestinal tract (GIT) are usually controlled and limited by its dissolution process. Thus, dissolution rate and extent has presented a great challenge to the development of suitable formulations for oral administration of poorly water-soluble drugs. With the recent advent of high-throughput screening of potential therapeutic agents, the number of poorly soluble drug candidates has increased rapidly and the development of oral delivery formulation for poorly soluble drugs is now one of the most frequent and greatest challenges to formulation scientists in the pharmaceutical industry.

The most attractive option for increasing drug dissolution is to improve the solubility through formulation approaches. Solid dispersion (SD) method is one of the most commonly employed pharmaceutical approaches to achieve the effect. Several methods can be employed to manufacture a SD, such as melting[1–3], dissolution in a solvent[4], or spray drying[5], depending on the characteristics of the drug and carrier. In the present study, SDs with povidone k-30 (PVPk30) were prepared using solvent-evaporation method. PVP belongs to a class of

Address for Correspondence: Jin Sun, Department of Pharmaceutics, School of Pharmacy, Shenyang Pharmaceutical University, No. 103 of Wenhua Road, Shenyang 110016, P. R. China. E-mail: sunjin66@21cn.com; hezhonggui@gmail.com

(Received 03 August 2010; revised 31 October 2010; accepted 01 November 2010)

RIGHTSLINK

water-soluble polymers, which has been used as a carrier to increase the dissolution rate of various poorly water-soluble drugs, such as ofloxacin[6], glibenclamide[7], and carbamazepine[8]. From a chemical point of view, PVP is a polymeric lactam with an internal amide bond, which contains a highly polar amide group that confers hydrophilic and polar-attracting properties. It is interesting that it has biological structural features similar to those of proteins, and has a great potential for applications in the medical domain.

Micronization technique is also a very promising approach to enhance dissolution rate and bioavailability of poor water-soluble drugs. It is well-known that particle size can affect the solubility of poorly soluble solutes. In particular, a reduction in particle size may improve the dissolution rate as a result of increased surface area in contact with the aqueous medium. Attempts have been made to modify the dissolution characteristics of fenofibrate[9], phenacetin[10], and oxcarbazepine[11] by reducing particle size. However, a problem associated with the milling of a pure active ingredient is the formation of agglomerates. An alternative approach, adding suitable excipients when milling, has been adopted to address this problem in the present study. Other possible methods include the use of amorphous forms to increase drug solubility, the reduction of particle size to expand surface area for dissolution, and the reduction of interfacial tension with the aid of a water-soluble carrier.

Glimepiride, 1-[[*p*-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxami-do) ethyl]phenyl]sulfonyl]-3-(trans-4-methylcyclohexyl) urea, is a new oral sulfonylurea hypoglycemic agent for the treatment of non-insulin dependent (type II) diabetes mellitus. It causes hypoglycemia by stimulating release of insulin from pancreatic β cells and by increasing the sensitivity of peripheral tissue to insulin. It also promotes the movement of sugar from the blood into the cells that need it. Glimepiride is practically insoluble in water (2.7×10^{-4} mg/mL at 25°C) and belongs to "Class II" drugs in the Biopharmaceutical Classification System[12]. It is likely to display low and irregular bioavailability following oral administration due to the low solubility[13,14].

It is reported that the product Amaryl produced by the Beijing Sanofi-Aventis, in comparison with the traditional glimepiride tablets, showed significant improvement in both *in vitro* dissolution and *in vivo* bioavailability[15]. The tablet weight of Amaryl was 87 mg, and each tablet contained 1 mg glimepiride. But regretfully, the manufacturer has not yet reported its formulation and preparation technique in public.

In this work, we intended to develop two formulation tablets via different dissolution-enhanced strategies, SD versus micronization, for the delivery of poorly soluble glimepiride via oral route. With the commercial available product Amaryl being the reference, the physicochemical and pharmacokinetic characteristics of the above two formulations were carried out and compared in order to decide which approach is better to prepare glimepiride tablets.

Materials and methods

Materials

Glimepiride was purchased from Chongqing Kangkeer Pharm. Co. (Chongqing, China). Amaryl® 1 mg tablets were purchased from Aventis Pharma S.P.A. (Beijing, China). Povidone k-30 (plasdone k-30) was obtained from ISP Japan (Tokyo). PVPP (Polyplasdone® XL, USP grade) was a gift from ISP Japan (Tokyo). FLOWLAC® 100 (lactose monohydrate) was a gift from Meggle GmbH (Wasserburg, Germany). Avicel® PH 101 (MCC, microcrystalline cellulose) was purchased from Asahi Kasei (Tokyo, Japan). Lutrol F68 was kindly provided by BASF (Ludwigshafen, Germany). Pregelatinized starch, sodium carboxymethyl starch, and magnesium stearate were bought from Anhui Shanhe Pharm. S.P.A. (Anhui, China). HPLC-grade methanol was purchased from Fisher Scientific (Pittsburgh, PA). Water was prepared using an EASYPURE®αRF/UV ultrapure water system (Barnstead international Co., Boston, MA). All other materials were of analytical grade and used as received.

Methods

Developments of the SD tablets (Formulation 1)

Preparation of physical mixture Glimepiride and PVPk30 (passed through 100-mesh sieve, respectively) were accurately weighed and mixed in a sealed polyethylene bag by hand for 10 min to obtain a homogeneous physical mixture (PM). The resulting mixtures were stored in a desiccator at room temperature until further evaluation.

Preparation of SD by solvent evaporation Accurately weighed amounts of glimepiride alone, PVPk30 alone, and a series of mixtures of polymer and drug having a final carrier–drug weight ratio ranging from 1:3 to 1:12 were dissolved at 40°C in minimum amount of CH_2Cl_2. The solvent was evaporated under vacuum at 40–50°C. Desiccation was completed in a vacuum oven until constant weight was achieved and the resulting solids were pulverized. The dried powder was then passed through a 100-mesh sieve and stored in a desiccator until further evaluation.

Differential scanning calorimetry analysis Differential scanning calorimetry (DSC 60, Shimadzu) was used to characterize the thermal properties of the drug, polymer, PMs, and SD. Ultrahigh purity nitrogen was used as the purge gas at a flow rate of 150 mL/min. Samples weighing 10 ± 5 mg were crimped in hermetic aluminum pans with lids and then analyzed using a heating rate of 10°C/min. The thermograms were recorded from 10°C to 250°C.

X-ray powder diffraction analysis X-ray powder diffraction analysis (XRPD) was performed using a D/Max-2400

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/30/12
For personal use only.

RIGHTSLINK

diffractometer (Rigaku Instrument, Japan). The samples were exposed to Cu–Kα radiation under 40 kV and 60 mA over the 2θ range from 3 to 45° in increments of 4°·per minute.

Tableting condition of Formulation 1 The SD with lactose, sodium carboxymethyl starch, PVPP, and magnesium stearate was directly compressed to tablets (90 mg) by a tablet-hitting pressure displacement system equipped with a flat-faced punch (6 mm diameter) (TDP single-punch tablet press, Shanghai Pharmaceutical Factory, China).

Preparation of micronized power tablets (Formulation 2)

Preparation of micronized PM Co-grinding of glimepiride, Lutrol F68, and lactose (1:1:53, w/w/w) in a ball mill until the milled material passes through 200, 300, and 500 mesh ASTM sieves, respectively.

Tableting condition of Formulation 2 The micronized mixture with MCC, sodium carboxymethyl starch, PVPP, and magnesium stearate was compressed to tablets (90 mg), by a tablet-hitting pressure displacement system equipped with a flat-faced punch (6 mm diameter) (TDP single-punch tablet press, Shanghai Pharmaceutical Factory, China).

Solubility study

Excess samples equivalent to 1 mg glimepiride was added to 10 mL phosphate buffer of pH 7.8. The resulting suspensions were sonicated for 30 min and then shaken at 37°C for 24 h in a thermostatically controlled air bath, since these conditions were previously shown to be sufficient for achieving equilibrium solubility without any extra solubilization factor. Samples were then passed through a 0.45 μm Millipore membrane filter, and the filtrates were suitably diluted and analyzed using HPLC. The HPLC method is described in the dissolution testing section.

In vitro dissolution test

The dissolution test was carried out at 37 ± 0.5°C in 100 mL of dissolution medium using a dissolution apparatus (ZRS-8G Test Dissolution Tester, China). The test was performed according to dissolution test method 2 as described in the Chinese Pharmacopoeia 2005 with a paddle rotation speed of 75 rpm. The dissolution media used contained water, phosphate buffer of pH 6.8, 7.2, 7.8, and Tris solution (pH 10.4). The prepared tablets (Formulation 1 and Formulation 2) and marketed Amaryl® weighed to be equivalent to 1 mg of drug were added to the dissolution apparatus, respectively, and 2 mL of test fluid was withdrawn after 5, 10, 20, 30, 45, and 60 min, and fresh dissolution medium was simultaneously replenished in the apparatus to maintain a constant total volume. The withdrawn samples subsequently were filtered through a 0.45-μm Millipore filter and assayed for the dissolved drug concentration by HPLC. Each release test was carried out in the parallel six times.

The HPLC system consisted of an LC-10A HPLC pump, a SPD-10A VP UV–VIS detector set at 228 nm, and an ANASTAR interface (Tianjin, China). UV signals were monitored and peaks integrated using ANASTAR HSM software. A calibration was previously performed and it was confirmed that excipients produced no absorption signal at this wavelength across a wide range of concentrations. Also, there was no interference in UV absorption between glimepiride and these excipients. Chromatographic separations were performed at room temperature using a C_{18} column (Hypersil BDS Silica, 4.6 mm × 150 mm, 5 μm; Phenomenex, China) guarded with a refillable precolumn (C_{18}, 2.0 mm × 20 mm; Alltech Associates, Inc., IL, USA) and a flow rate of 1.0 mL/min. The mobile phase consisted of methanol:0.01 M monoammonium phosphate buffer solution (pH 3.5) (70:30, v/v) and was filtered through a 0.45-μm membrane filter and degassed by ultrasonication before use. These conditions resulted in a typical elution time for glimepiride of 7.8 min.

To evaluate the dissolution behavior of test preparations and marketed tablets in various medium simulating different physiology pH, dissolution was performed in HCl of pH 1.2, water, phosphate buffer of pH 6.8, 7.2, and 7.8. Similarity index was introduced by Moore and Flanner in 1996[16] to determine similarity of two dissolution profiles and is defined as follows:

$$f_2 = 50 \lg \left\{ \left[1 + (1/n) \sum_{t=1}^{n} (R_t - T_t)^2 \right] \right\}^{-0.5}$$

where n is the sample number, and R_t and T_t are the percentages of the reference and test drug release, respectively, at different time intervals t. The f_2 value is between 0 and 100. The value is 100 when the test and the reference profiles are identical and approaches zero as the dissimilarity increases, but because f_2 is a log function small differences in profile lead to a large drop in f_2. If f_2 of two dissolution drug release profiles is between 50 and 100, then these two drug release profiles are similar. Value under 50 indicates differences between the release profiles[17].

In vivo bioavailability studies

Experimental design Beagle dogs were purchased from General Hospital of Shenyang Military Region (China) and were given a normal standard chow diet and free access to water. Animals were housed in laminar flow house maintained at 22 ± 2°C, 50–60% relative humidity under a 12-h light:12-h dark cycles throughout the experiment. Additionally, the dogs were kept in these facilities for at least 1 week prior to these experiments, and the studies were performed in accordance with the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology of US in July 1989 and revised in March 1999.

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/30/12
For personal use only.

© 2011 Informa Healthcare USA, Inc.

RIGHTSLINK

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/30/12
For personal use only.

The experiment involves fasting, single dose with the three different preparations with washout period in between. Six dogs were used for each treatment group. The dogs (13.1 ± 1.2 kg) were fasted for 24 h prior to experiments and the preparations (Formulation 1, Formulation 2, and reference product) containing 3 mg of drug were administered in the morning and a standard lunch was given 4 h after dosing. Clinical recommendation of human (50 kg) daily doses is 1 mg glimepiride. The animal dose was obtained by extrapolation of human daily dose based on body weight. Blood was withdrawn via cannulated needle from front legs. Four milliliters of blood were collected in heparinized tubes immediately prior to dosing (time zero) and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 9.0, 12.0, 24.0, and 36.0 h after dosing. The plasma was obtained by the centrifugation of blood at 3000 rpm for 20 min and then kept frozen at–20°C until analysis. The concentrations of glimepiride in plasma were determined by ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS).

Determination of glimepiride in dog plasma A selective, rapid, and sensitive UPLC-MS/MS method was developed for the quantification of glimepiride in dog plasma. With glipizide as an internal standard, sample pretreatment utilized a simple precipitating protein. A methanol solution of internal standard (400 ng/mL) 50 μL and 50 μL methanol were added to 50 μL plasma. After vortex-mixing for 3 min, the samples were centrifuged at 15,000 rpm for 10 min. and the supernatant was collected and centrifuged under the same conditions. The separation was carried out on an ACQUITY UPLCTM BEH C_{18} column (50 mm × 2.1 mm, 1.7 μm; Waters Co., Milford, MA) with water (containing 0.3% formic acid) and acetonitrile as the mobile phase at a flow rate of 0.2 mL/min. The detection was performed by a Waters Tandem Quadrupole (TQ) Detector (Waters). The mass spectrometer was operated with electrospray ionization (ESI) interface in positive ionization mode and with multiple-reaction monitoring mode. The selected reaction monitoring (SRM) of glimepiride and the internal standard were m/z 491 → 352 (glimepiride) and m/z 446.3 → 312.2 (glipizide), respectively. The concentration of glimepiride was determined by a standard linear calibration curve in the concentration range of 25–5000 ng/mL.

Pharmacokinetic data analysis Noncompartmental pharmacokinetic analysis was conducted to calculate the area under the curve from 0 to 36 h (AUC_{0-36}). The peak plasma concentration (C_{max}) and the time to reach peak plasma concentration (T_{max}) of the different dosage forms were determined by a visual inspection of the experimental data. The AUC was estimated according to the linear trapezoidal rule. The threshold for differences to be considered significant was set at P 0.05. The ≤ relative bioavailability (F) of Formulation 1 and Formulation 2 to the commercial tablets (reference) was calculated using the following equation:

$$\text{Relative bioavailability (F\%)} = \frac{AUC_{0-36}\text{test}}{AUC_{0-36}\text{reference}} \times 100$$

In vitro–in vivo correlations analysis

An *in vitro–in vivo* correlations (IVIVC) for glimepiride was evaluated by plotting the mean T_{max} of the three formulations versus the mean *in vitro* accumulative dissolution at 10 min (D_{10}) and AUC of 0–20 min (AUC_{0-20}) in various media, respectively. Linear regression analysis was applied to fit the data and R^2 was calculated to evaluate the robustness of IVIVC.

Results and discussion

Phase solubility study of glimepiride PMs and SDs

The solubility phase diagram of glimepiride as a function of PVPk30 proportion in phosphate buffer solution of pH 7.8 at 37°C is shown in Figure 1. For all the PMs and SDs tested at different weight ratios, an increase in glimepiride solubility was found linear with respect to the weight fraction of polymer in the binary system. The increase of the solubility with increasing PVPk30 concentration indicates the solvent and wetting properties of PVP for drug. For example, at the highest PVP/drug ratio (12:1 w/w), the solubility of glimepiride was ~2-fold compared with pure drug powder.

PMs exhibited an improvement in solubility over pure glimepiride coarse powder, maybe due to increased wettability of the drug and the inhibition of drug particle aggregation by the polymer carrier. However, all SDs enhanced solubility greater than their corresponding PMs. The enhancement of glimepiride solubility by the SD technique over PMs was attributed to the wettability effect of the carrier and dispersed state of the drug. Furthermore, size reduction and molecularly/

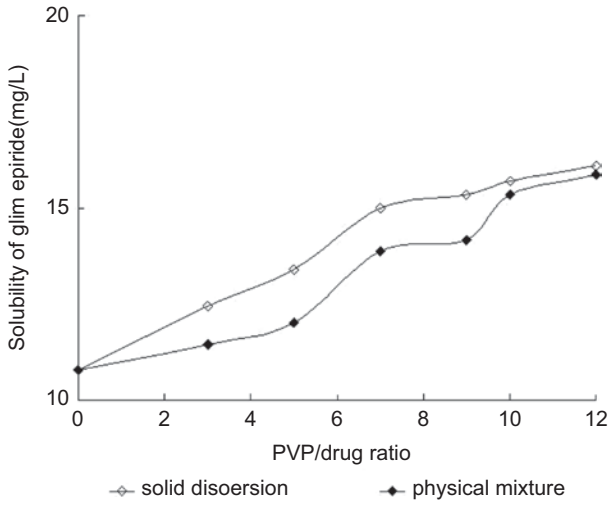

Figure 1. Effects of the weight fraction of PVPk30 on solubility from glimepiride–PVPk30 systems in phosphate buffer solution of pH 7.8 at 37°C: physical mixtures (◆) and solid dispersions (◊). Each point represents the means ± SD of six experiments.

RIGHTSLINK

nanosized dispersed state of drug molecules in the SD also accounted for the enhanced solubility. In addition, these results are in accordance with the well-established formation of soluble complexes between water-soluble polymeric carriers and poorly soluble drugs[18].

Thermal analysis by DSC

DSC curves obtained for glimepiride, PVPk30, the PMs, and the SDs prepared with PVP are shown in Figure 2. Powdered glimepiride showed a melting endothermic peak at 215.3°C, and PVP showed an endotherm at 202.7°C. While these features are easily identified in the PM, the glimepiride endothermic peak disappeared totally. This suggests that the glimepiride was amorphous in SD.

X-ray powder diffraction

XRPD is the standard technique for studying the crystalline or amorphous nature of drugs in solid state. The

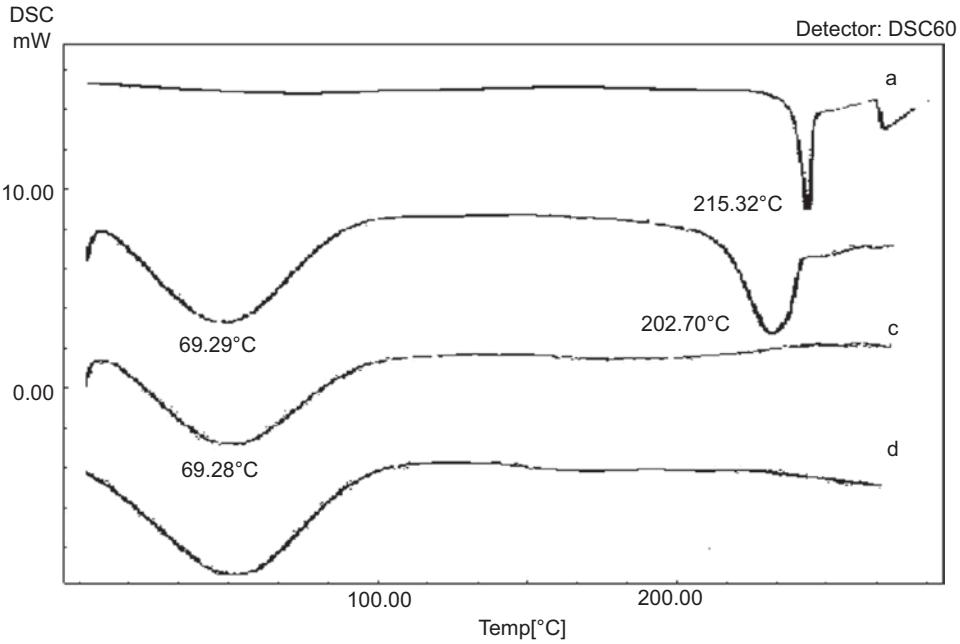

Figure 2. DSC curves of glimepiride (a), physical mixtures (1:9, w/w) (b), PVP (c), and glimepiride–PVP solid dispersions (1:9, w/w) (d).

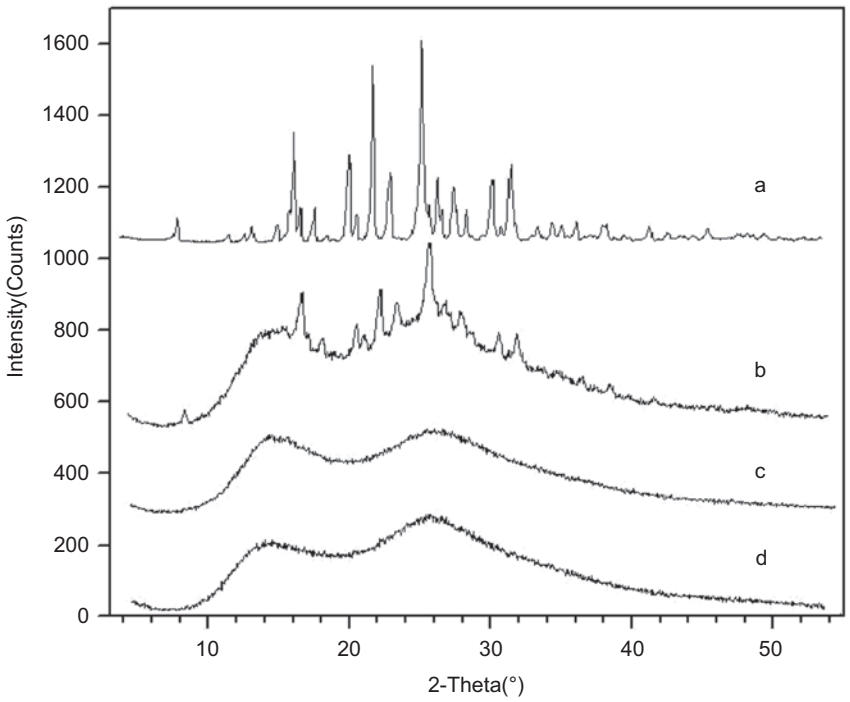

Figure 3. X-ray diffraction patterns of glimepiride (a), physical mixtures (1:9, w/w) (b), PVP (c), and glimepiride–PVP solid dispersions (1:9, w/w) (d).

© 2011 Informa Healthcare USA, Inc.

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/30/12
For personal use only.

RIGHTSLINK

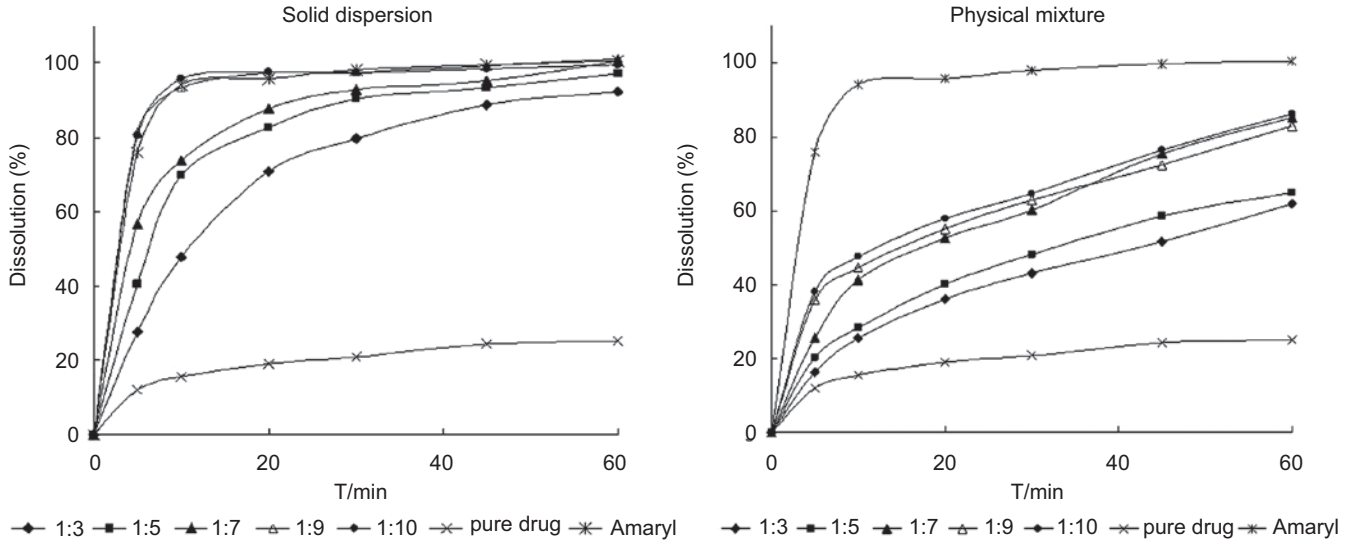

Figure 4. Dissolution profiles of glimepiride from solid dispersions or physical mixtures with various weight fractions of PVPk30 as a function of time (min) in 0.02% Tris solution: (a) 1:3 w/w (◆); (b) 1:5 w/w (■); (c) 1:7 w/w (▲); (d) 1:9 w/w (Δ); and (e) 1:10 w/w (•) are the drug–carrier ratio. Each value represents the mean ± SD of six experiments. For comparison: dissolution profiles of marketed Amaryl® 1 mg tablets (–×–) and pure glimepiride (–✳–).

XRPD patterns for glimepiride, PVP, glimepiride–PVP SD (1:9, weight ratio), and the corresponding PMs are shown in Figure 3. In the X-ray diffraction spectrum of glimepiride powder (Figure 3), sharp and intense peaks at a diffraction angle of 2θ 13.36°, 16.62°, 18.10°, 19.12,° 22.00°, 25.21°, 26.32° and a series of smaller peaks at 2θ angles of 6.36°, 14.60°, 17.16°, 22.97°, and 23.64° were present and suggested that the drug was present as a crystalline material. Some glimepiride crystalline peaks were still detected in the binary mixtures. In contrast, there were no sharp peaks attributable to the crystalline form of glimepiride in the SDs, suggesting that glimepiride in this SD system was in amorphous state.

In vitro dissolution testing

Investigation of dissolution in Tris solution

The *in vitro* dissolution profiles of the SD-containing preparations and corresponding PMs are shown in Figure 4. Tris solution (pH 10.4) was selected as a dissolution medium since it has been reported to have sufficient discriminating capability to evaluate the relationship between *in vitro* dissolution and *in vivo* absorption of glimepiride products[19]. The Formulation 1 prepared by drug–PVPk30 SD at weight ratio of 1:9 and 1:10 (drug:carrier) exhibited rapid drug dissolutions, with >75% drug released within 5 min, whereas pure glimepiride coarse powder only released 30% of the drug after 1 h.

The dissolution rates of glimepiride at 5 min from PM and SD with increasing proportion of PVPk30 are investigated in Figure 5. The established relationship revealed a nonlinear pattern of enhanced solubilization and dissolution behavior. Indeed, there is a progressive increase in the dissolution rates of glimepiride up to 90% weight ratio of PVPk30 for PM and SD. These data indicated that PVP is effective in improving the dissolution

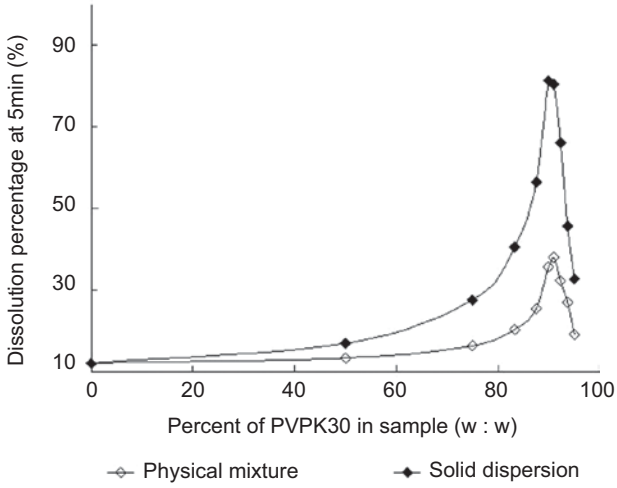

Figure 5. Effects of weight fraction of PVPk30 on the dissolution percentage at 5 min of glimepiride released from physical mixtures (◊) and solid dispersions (◆).

of poor water-soluble drugs regardless of whether the drug is present as a PM or SD[20]. However, all SDs showed faster dissolution rate than their corresponding PMs. The enhanced dissolution rate of SDs may be attributed to many factors such as decreased particle size of drug, specific dispersed state of drug in these SDs. In addition, PVP shows excellent water solubility. This polymer could improve the wettability of the glimepiride particles and the drug solubility at the diffusion layer surrounding the particles[18]. Furthermore, PVP would inhibit the crystallized process of the drug and then glimepiride was in the molecular dispersion in the SD as described earlier. It was well-known that amorphous drug demonstrates the faster dissolution than crystalline drug[21].

The dissolution rates of glimepiride attain the maximal at weight fractions of about 0.9 of the polymer,

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/30/12
For personal use only.

RIGHTSLINK

evidencing that these ratios are crucial to achieve optimal solubilization of glimepiride. For SDs, 81.4 ± 1.6% and 80.4 ± 2.3% of glimepiride dissolved within the first 5 min in comparison with 35.8 ± 1.3% and 36.9 ± 2.4% for the corresponding PMs of the same compositions. In contrast, the dissolution rate of glimepiride declines above the drug to carrier ratio of 1:9. This feature may reflect the delayed dissolution process of the drug induced by the PVP in excess probably due to the relatively elevated viscosity in the microenvironment around the drug particle[22]. Moreover, the overall viscosity of the material increased as the amount of PVP increases. The hardness of the tablets was high so that disintegration delayed, and ultimately affected the drug dissolution rate and extent.

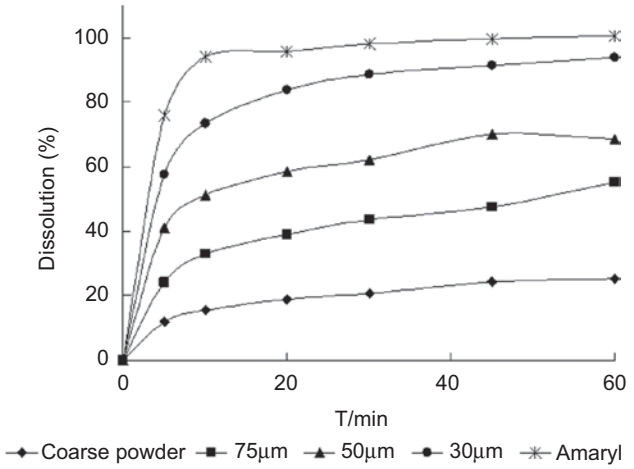

Figure 6. Effect of particle sizes of glimepiride on dissolution in 0.02% Tris solution: 10 μm (•), 50 μm (▲), 75 μm (■), 145 μm (◆) are the particle sizes of glimepiride. Each value represents the mean ± SD of six experiments. For comparison: dissolution profiles of marketed 1 mg tablets (–✳–).

The results of this study demonstrated that 1:9 might be the most appropriate ratio to prepare the SD in glimepiride–PVP binary system and was employed in the optimal Formulation 1.

The *in vitro* dissolution profiles of the micronized powder tablets (Formulation 2) were shown in Figure 6. The enhancement of the dissolution rate from crude to micronized glimepiride was in accordance with its pronounced reduction in particle size. The accumulative dissolution at 1 h was ~5-fold with particle size 10 μm of glimepiride compared with coarse powder. This was due to increased specific surface area of the drug as a consequence of reduced particle size. When particle size reduced to 30 μm, both *in vitro* dissolution rate and amount of glimepiride from tablets achieved ideal effect

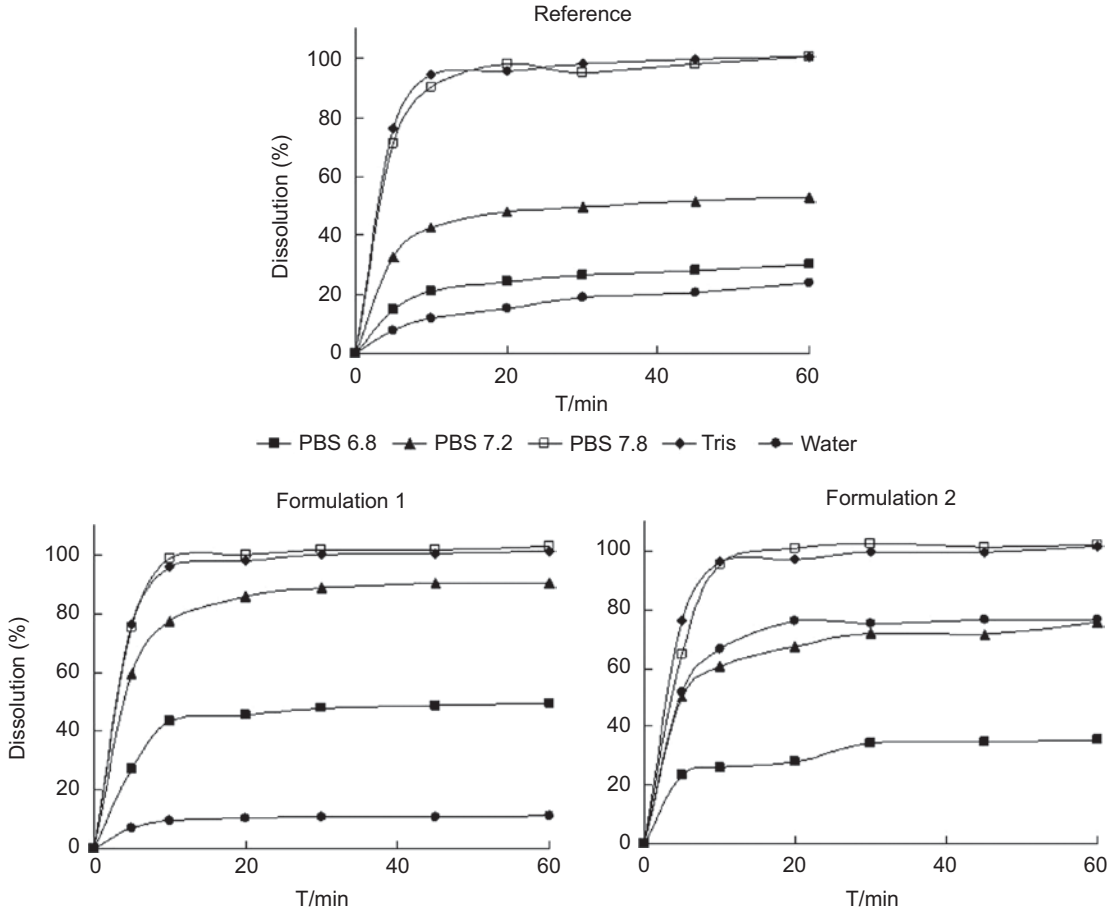

Figure 7. The dissolution profiles of glimepiride from optimal Formulation 1, Formulation 2, and reference products in phosphate buffer solution at pH 6.8 (■), 7.2 (▲), 7.8(□), water (◆), and Tris (•) solution, respectively. Each data point represents the mean ± SD of six measurements.

© 2011 Informa Healthcare USA, Inc.

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/30/12
For personal use only.

RIGHTSLINK

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/30/12
For personal use only.

in Tris solution and this condition was employed as the optimal Formulation 2.

It was worth mentioning that among the carriers used in the formation of SDs, PVP is the most commonly used. This polymer shows excellent water solubility. When preparing the SD-based tablets, the difference between povidone as the SD carrier and crospovidone as the disintegrant for tableting purposes is the swelling capability. For example, PVPP is a highly cross-linked version of PVP, exhibits excellent water-absorbing action, and swells very rapidly to about 4–6 times their dry volume and sets up localized stresses that spread throughout the tablet and break up the tablet from within. In contrast, PVP shows excellent water solubility, improves the wettability of the poorly soluble drugs, but has poor swelling capability.

Testing of solubility and dissolution in various media

To compare the dissolution behavior of optimal Formulation 1, Formulation 2, and marketed tablets, six different dissolution media were investigated covering the gastrointestinal physiological pH range of 1.2–7.8. The dissolution profiles for the three products are shown in Figure 7. The dissolution of the drug was strongly affected by the pH, significantly greater dissolution at pH 7.8 than at pH 6.8. The dissolution at pH 1.2 was less than the limit of quantitation and cannot be detected. This observation was supported by the equilibrium solubility data where a moderate increase was observed above pH 1.2 followed by a marked increase above pH 6.8 (Figure 8). Glimepiride is a weak monoacid. The solubility of drug to the pK_a was calculated using the following equation:

$$\mathrm{pH} = \mathrm{p}K_a + \lg \frac{S - S_0}{S_0}$$

where S and S_0 are the equilibrium solubility and intrinsic solubility of drug, respectively, and pK_a is the dissociation constant of drug. The pK_a value of glimepiride has been reported is ~6.5. Experimental results showed that there was a poor solubility value of glimepiride under the pH <6.5, and increased solubility with pH >6.5. For example, the solubility was 0.1 μg/mL in pH 5.0 and 10.79 μg/mL in pH 7.8.

Table 1. Similarity factors (f_2) of the three formulations in different media.

Media	Formulation 1	Formulation 2	Marketed tablet
Water	55.54	13.31	100
PBS6.8	55.16	59.70	100
PBS7.2	22.10	54.82	100
PBS7.8	63.90	65.27	100
Tris	88.47	90.64	100

The similarity factors (f_2) that were calculated between the reference and Formulation 1/Formulation 2 are presented in Table 1. The results of f_2 showed that the profiles of Formulation 1 are not significantly different from the reference except in phosphate buffer pH 7.2 where the dissolution rate and extent of Formulation 1 were both superior to the reference. Formulation 2 and the commercial tablets are mostly similar except in water where Formulation 2 demonstrated substantial rapider dissolution. The results confirmed that both SD and micronization techniques are capable of improving dissolution of glimepiride to a similar extent as the marketed product[23,24]. Based on the *in vitro* dissolution performances, it was expected that the test and reference products may be bioequivalent, but still needed further bioequivalence verification.

Pharmacokinetic studies

Pharmacokinetics of the two optimal prepared tablets of glimepiride (SD and micronized powder tablets) compared with the commercial available tablets were investigated following oral administration of 3 mg to each of six healthy beagle dogs. The profiles of the mean plasma concentrations of glimepiride versus time are shown in Figure 9 and the main pharmacokinetic parameters are given in Table 2, respectively. Following oral administration of the two test formulations, absorption were both rapid with no statistically significant difference in mean T_{max} when compared with the reference. The results suggested that SD and micronization techniques could both improve drug release and absorption in GIT. This observation was supported by the data as shown in Table 3. Additionally, the results revealed that Formulation 1 gave the highest C_{max} value (2091.93 ng/mL, range

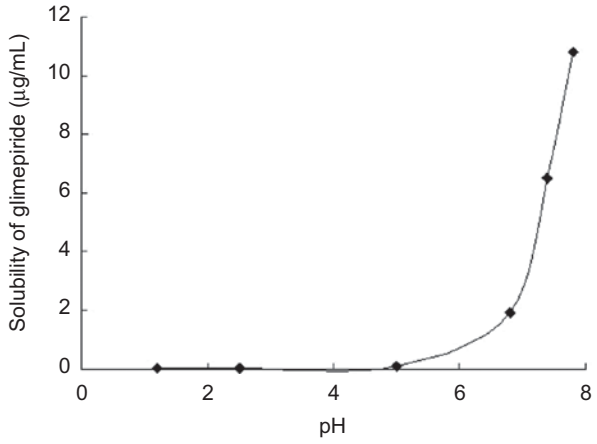

Figure 8. Equilibrium solubility of glimepiride in different media of pH values at 37°C.

Table 2. Pharmacokinetic parameters (±SD) of glimepiride after oral administration of the three different formulations in beagle dogs, including Formulation 1, Formulation 2, and marketed tablet.

Parameters	Ke (h^{-1})	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (ng h/mL)	F (%)
Marketed tablet	0.08 ± 0.03	9.45 ± 2.66	4.50 ± 1.02	1924.27 ± 776.76	26668.44 ± 7607.54	100.00 ± 0.00
Formulation 1	0.08 ± 0.02	8.64 ± 2.22	3.50 ± 1.97	2091.93 ± 875.47	28461.98 ± 9993.21	106.17 ± 16.66
Formulation 2	0.09 ± 0.02	8.63 ± 2.43	2.17 ± 0.68	1927.65 ± 752.75	26838.64 ± 8719.67	101.33 ± 16.26

RIGHTSLINK

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/30/12
For personal use only.

1254.80–3643.50 ng/mL), followed by Formulation 2 (1927.65 ng/mL, range 1345.73–3085.40 ng/mL) and the reference (1924.27 ng/mL, range 1269.91–3364.05 ng/mL), yet the differences are not regarded as substantial. The mean AUC value for Formulation 1 was 28461.98 ng·h/mL (range 17454.98–43666.89 ng·h/mL) and Formulation 2 was 26838.64 ng·h/mL (range 19492.24–41911.11 ng·h/mL), whereas Amaryl® produced a mean of 26668.44 ng·h/mL (range 17138.68–36065.39 ng·h/mL). The relative bioavailability of Formulation 1 and Formulation 2 against the reference calculated from AUC_{0-36} was 106.17% and 101.33%. Again, no substantial differences between the test samples and reference were observed. Similarly, no differences in half-life or elimination constant were found. It was noted that the inter-subject variability is relatively high, and so another test including more subjects is necessary to evaluate the present results or clinic study to judge whether it can be extrapolated to humans.

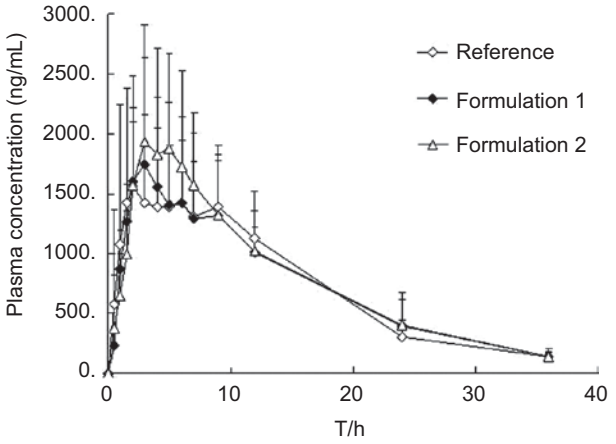

Figure 9. Average plasma concentration versus time profiles of glimepiride after oral administration (3 mg glimepiride doses) of marketed tablet (◊); Formulation 1 (◆) and Formulation 2 (Δ) in beagle dogs ($n = 6$). Each data point represents the mean ± SD.

Table 3. Wilconxon signed test results of T_{max} between test A/B and reference tablets for glimepiride.

	Reference	Test A	Test B	*P*	Conclusion
Mean ± SD	4.50 ± 3.02	2.17 ± 0.68	3.50 ± 1.97	>0.05	Not substantial
Max–min	9.00–1.00	3.00–1.50	6.00–2.00		
Median	4.5	2	2.5		

IVIVC analysis

Since the level A of IVIVC in which the entire *in vivo* time course is correlated with the *in vitro* data is difficult to acquire for the immediate-release dosage forms, the level C of IVIVC that establishes a single-point relationship between dissolution and a pharmacokinetic parameter was developed in this study. Two dissolution rate parameters were selected, including D_{10} (cumulative release at 10 min) and AUC_{0-20} (area under the curve of the cumulative release from 0 to 20 min). T_{max} was chosen to describe the *in vivo* absorption rate.

The regression analysis results are summarized in Table 4. The R^2 values between D_{10} in Tris buffer and pH 6.8, 7.2, and 7.8, with *in vivo* T_{max} data, were 0.79, 0.02, 0.21, and 0.26, respectively. Furthermore, R^2 values between AUC_{0-20} in Tris buffer and pH 6.8, 7.2, and 7.8, with *in vivo* T_{max} data, were 0.88, 0.02, 0.21, and 0.18, respectively. Both data sets demonstrated that the dissolution performance of the three glimepiride formulations in Tris buffer correlated best with the *in vivo* T_{max} data and can reflect the *in vivo* dissolution behavior of the glimepiride tablets. On the basis of IVIVC analysis, it was concluded that Tris solution was the good *in vitro* dissolution medium to screen and discriminate the different glimepiride formulations, and also the Tris solution was the optimum dissolution screening medium in the *in vitro* dissolution testing analysis, such as Figures 5–7. Additionally, the results were in a good agreement with the results of the previous report[19].

Conclusion

Compared with traditional glimepiride tablets prepared with pure drug coarse powder, the two formulation tablets by SD and micronized powder showed significant improvement in dissolution. Furthermore, the dissolution profiles of the two new formulations are comparable with the commercial product Amaryl. The pharmacokinetic values for C_{max}, T_{max}, and AUC were similar for all formulations with no statistical differences among them. These data suggest that the investigated three formulations are comparable based on *in vitro* characterization and are bioequivalent in terms of pharmacokinetic variables. Therefore, both the SDs and the micronization techniques are able to improve the *in vitro* dissolution and *in vivo* bioavailability of glimepiride following oral administration, to a similar extent as the marketed product Amaryl.

Table 4. Regression analysis for *in vitro* and *in vivo* relationship.

	Regression equation and correlation coefficient			
Media	D_{10}[a] versus T_{max}		AUC_{0-20}[b] versus T_{max}	
PBS 6.8	$Y = -0.0125X + 3.7666$	$R^2 = 0.0156$	$Y = -0.0009X + 3.8663$	$R^2 = 0.0223$
PBS 7.2	$Y = -0.0301X + 5.206$	$R^2 = 0.2051$	$Y = -0.0018X + 5.2537$	$R^2 = 0.2122$
PBS 7.8	$Y = -0.1346X + 16.137$	$R^2 = 0.2587$	$Y = -0.0083X + 16.757$	$R^2 = 0.1808$
Tris	$Y = -0.9536X + 94.503$	$R^2 = 0.7893$	$Y = -0.0584X + 98.489$	$R^2 = 0.8820$
Water	$Y = -0.0322X + 4.3365$	$R^2 = 0.7873$	$Y = -0.0018X + 4.3341$	$R^2 = 0.7825$

[a]D_{10} is the cumulative release of the three studied formulations at 10 min.
[b]AUC_{0-20} is the area under the curve of the cumulative release from 0 to 20 min.

© 2011 Informa Healthcare USA, Inc.

RIGHTSLINK

Declaration of interest

We are grateful for financial support from the National Basic Research Program of China (973 Program) No. 2009CB930300 and Key Project for Drug Innovation (No. 2010ZX09401-304, 2009ZX09301-012, 2008ZX09401-004) from the Ministry of Science and Technology of China. The authors report no declarations of interest.

References

1. Sheen PC, Khetarpal VK, Cariola CM, Rowlings CE. (1995). Formulation studies of a poorly water-soluble drug in solid dispersions to improve bioavailability. Int J Pharm 118: 221–227.
2. Betageri GV, Makarla KR. (1995). Enhancement of dissolution of glyburide by solid dispersion and lyophilization techniques. Int J Pharm 126:155–160.
3. Arias MJ, Gines JM, Moyano JR, Perez-Martinez JI, Rabasco AM. (1995). Influence of the preparation method of solid dispersions on their dissolution rate: study of triameterene–D-mannitol system. Int J Pharm 123:25–31.
4. Leuner C, Dressman J. (2000). Improving drug solubility for oral delivery using solid dispersions. Eur J Pharm Biopharm 50: 47–60.
5. Verheyen S, Blaton N, Kinget R, Van den Mooter G. (2002). Mechanism of increased dissolution of diazepam and temazepam from polyethylene glycol 6000 solid dispersions. Int J Pharm 249: 45–58.
6. Okonogi S, Oguchi T, Yonemochi E, Puttipipatkhachorn S, Yamamoto K. (1997). Improved dissolution of ofloxacin via solid dispersion. Int J Pharm 156:175–180.
7. Tashtoush BM, Al-Qashi ZS, Najib NM. (2004). *In vitro* and *in vivo* evaluation of glibenclamide in solid dispersion systems. Drug Dev Ind Pharm 30:601–607.
8. Sethia S, Squillante E. (2004). Solid dispersion of carbamazepine in PVP K30 by conventional solvent evaporation and supercritical methods. Int J Pharm 272:1–10.
9. Vogt M, Kunath K, Dressman JB. (2008). Dissolution enhancement of fenofibrate by micronization, cogrinding and spray-drying: comparison with commercial preparations. Eur J Pharm Biopharm 68:283–288.
10. Perrut M, Jung J, Leboeuf F. (2005). Enhancement of dissolution rate of poorly-soluble active ingredients by supercritical fluid processes. Part I: Micronization of neat particles. Int J Pharm 288: 3–10.
11. Douroumis D, Fahr A. (2006). Nano- and micro-particulate formulations of poorly water-soluble drugs by using a novel optimized technique. Eur J Pharm Biopharm 63:173–175.
12. Löbenberg R, Amidon GL. (2000). Modern bioavailability, bioequivalence and biopharmaceutics classification system. New scientific approaches to international regulatory standards. Eur J Pharm Biopharm 50:3–12.
13. Grunenberg A, Keil B, Henck JO. (1995). Polymorphism in binary mixtures as exemplified by nimodipine. Int J Pharm 118: 11–21.
14. Amidon GL, Lennernäs H, Shah VP, Crison JR. (1995). A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. Pharm Res 12:413–420.
15. Xiao D, Lin B, Mei L. (2010). Comparison of dissolution of glimepiride tablets from different manufactures. China Pharm 19:52–55.
16. Moore JW, Flanner HH. (1996). Mathematical Comparison of curves with an emphasis on *in vitro* dissolution profiles. Pharm Tech 20:64–74.
17. Costa P, Lobo JMS. (2001). Modeling and comparison of dissolution profiles. Eur J Pharm Sci 13:123–133.
18. Mura P, Manderioli A, Bramanti G, Ceccarelli L. (1996). Properties of solid dispersions of naproxen in various polyethylene glycols. Drug Dev Ind Pharm 22:909–916.
19. He Z, Zhong D, Chen X, Liu X, Tang X, Zhao L. (2004). Development of a dissolution medium for nimodipine tablets based on bioavailability evaluation. Eur J Pharm Sci 21:487–491.
20. Lin CW, Cham TM. (1996). Effect of particle size on the available surface area of nifedipine from nifedipine polyethylene glycol 6000 solid dispersion. Drug Dev Ind Pharm 29:813–819.
21. Hancock BC, Zografi G. (1997). Characteristics and significance of the amorphous state in pharmaceutical systems. J Pharm Sci 86:1–12.
22. Naima Z, Chantal C, Philippe A, Siro T, Jerome D. (2001). *In vitro* and *in vivo* evaluation of carbamazepine–PEG 6000 solid dispersions. Int J Pharm 225:49–62.
23. Jung JY, Yoo SD, Lee SH, Kim KH, Yoon DS, Lee KH. (1999). Enhanced solubility and dissolution rate of itraconazole by a solid dispersion technique. Int J Pharm 187:209–218.
24. Zmeili S, Saket M, Qaisi A, Gharaibeh M, Amro B, Tutunji M, Razzak MA, Muti H. (1995). A comparative single-dose bioequivalence study of two brands of glipizide. Curr Ther Res 56: 38–46.

Drug Development and Industrial Pharmacy Downloaded from informahealthcare.com by Biblioteca Alberto Malliani on 01/30/12
For personal use only.

RIGHTSLINK