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Pharmaceutical evaluation of glibenclamide products available in the Jordanian market

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The pharmaceutical quality of five generics of glibenclamide that are available in the Jordanian market was assessed according to the British Pharmacopoeia (BP) monograph (2009). Similarly, the originator glibenclamide (Daonil®) which was obtained from the Saudi market was subjected to analysis and used as a reference product. All products were found satisfactory in terms of identification and related substances as per the BP requirements. However, the assay results showed that only two products, in addition to the reference (Daonil®) satisfied the BP specifications which required glibenclamide content to be within the range: 95 to 105% of the labeled content. All products, in spite of marginal deviations for two of them, were found to pass the United States Pharmacopoeia (USP) assay specifications (90 to 110%). Significant differences in dissolution behavior were observed between the different generics and the originator (Daonil®). Daonil® exhibited the lowest dissolution profile while some products showed dissolution profiles that were almost twice that of Daonil®.

Key words: Glibenclamide tablets, pharmaceutical equivalency, dissolution testing, quality control.

INTRODUCTION

The absence of quality control measures or effective drug regulatory agencies in many countries led to the production and prevalence of substandard, fake, and counterfeit drugs (World Health Organization (WHO), 1999). Substandard drugs have been defined as those which do not meet quality specifications set for them, as to contain under or over concentration of ingredients, contamination, poor quality ingredients, poor stability and inadequate packaging (Green et al., 2000; Newton et al., 2001). Conventional generics for an orally administered drug are considered to be therapeutically equivalent to a reference, once pharmaceutical equivalence and bioequivalence have been established (Schellekens et al., 2011). In practice, despite the presence of legislations for bioequivalence, generic products can differ significantly from the reference drug and amongst themselves (Genazzani and Pattarino, 2008). Many studies worldwide have shown significant percentages of substandard medicines available in the markets of several countries (Ehianeta et al., 2012; Eichie et al., 2009; Smith et al., 2006; Vial et al., 2008). For example, one of two marketed amoxicillin generics from Italian market was not bioequivalent to the brand leader product (Del Tacca et al., 2009). In another study comparing 13 copies of alendronate, significant differences in dissolution and disintegration of tablets were revealed (Epstein et al., 2003).

Interestingly, some studies have shown that the effect of substandard preparations might not be limited to inadequate physicochemical behavior, but can also be extended to influence the clinical outcome of the use of that preparation (Margolese et al., 2010). According to the current regulations issued by the Jordan Food and Drug Administration (JFDA), only random batches of each product are tested after establishing a record for successful testing results for a particular product. JFDA also requires every generic product to have a valid

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bioequivalence study that demonstrates its equivalence to the originator brand. However, questions are probably still being raised by professionals and the public pertaining to the quality of generics available in the Jordanian market. The result reported by a recent study (Schellekens et al., 2011) is particularly alarming in this regard. According to Schellekens et al. (2011), around 56% of amoxicillin preparations available in regional markets, including the Jordanian market, were out of pharmacopoeial specifications.

In this study, the quality of the oral antidiabetic drug, glibenclamide preparations was assessed through direct purchase of the relevant preparations from local community pharmacies and subjecting them to analysis according to the British Pharmacopoeia (BP). Glibenclamide (Figure 1), also known as glyburide (in the United States), is a sulfonylurea oral hypoglycemic drug which has long been in clinical use (Luzi and Pozza, 1997). In Jordan, five generics are officially registered by the JFDA and are available in the local market. The originator (Daonil®) however, is currently not available in the Jordanian market. In this study, the pharmaceutical qualities of the five generics available locally were compared to that of the originator (Daonil®) which was obtained from the Saudi market.

MATERIALS AND METHODS

Chemicals

Working standards of glibenclamide were obtained from medicine testing laboratories (Amman, Jordan). High-performance liquid chromatography (HPLC) grade acetonitrile and methanol were obtained from TEDIA Company (INC, USA). Potassium dihydrogen orthophosphate was obtained from SD Fine-Chem Limited (Mumbai). Tablets of the five commercially available glibenclamide products were obtained from the local market. The examined tablets were purchased from local community pharmacies in just the same way that the patient might have bought them from such pharmacies. A list of the tested products with their details is shown in Table 1.

Apparatus

The HPLC system employed in this study consisted of a Ultra violet (UV) detector (Merck-Hitachi, model L-7400, Tokyo-Japan), a pump (Merck-Hitachi, model L-7400, Tokyo-Japan) and an integrator unit (Merck-Hitachi, model D-7500, Tokyo-Japan). The employed HPLC column was C18, 5 µm, 200 × 4.6 mm i.d. (Thermo Scientific, USA). Dissolution experiments were carried out using a Copley scientific dissolution apparatus, DIS6000 (UK). Friability testing was carried out using an Erweka TAR Roche Friabilator (Germany). Measurements of pH were made using microprocessor pH meter, HANNA Instruments (Romania).

Tests performed according to BP (2009)

In general, the British pharmacopoeial monograph (British Pharmacopoeia, 2009) was adopted for testing all collected commercial products. Identification test, related substances and assay were performed. The details for each test are described.

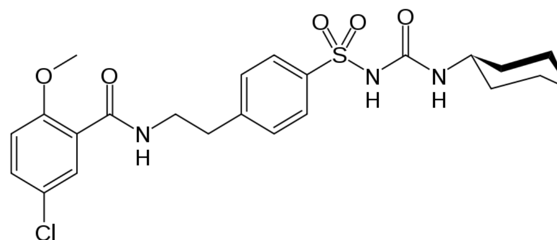

Figure 1. Chemical structure of glibenclamide.

Identification test

According to BP monograph, identification of glibenclamide was performed via thin layer chromatography (TLC). This was achieved by comparing the retention index (R_f values) for the chromatogram of a standard glibenclamide solution to that obtained for the extracted tablets. Further identification was performed by comparing the HPLC retention times of the major peaks for the standard and samples during the conductance of the assay (according to the same pharmacopoeial monograph). The conditions for TLC were those described for the related substances tests.

Related substances

The test sets limits for two specified impurities which are 4-[2-(5-chloro-2-methoxybenzamido)ethyl] benzenesulphonamide (CEBSA) and methyl N-4-[2-(5-chloro-2-methoxybenzamido)ethyl] benzenesulphonyl carbamate (MCEBSC). The test was performed by applying solution 1 representing the extract of commercial tablets containing the equivalent of 0.5% glibenclamide, and solution 4 representing the standard solution of glibenclamide at 0.5% as specified in BP. Solutions 2 and 3 were supposed to be prepared using the two potential glibenclamide impurities. As these impurities were not available, two solutions of standard glibenclamide were prepared at concentrations similar to the concentrations specified for impurities solutions. These glibenclamide standard concentrations were 0.012 and 0.002%, corresponding to CEBSA and MCEBSC, respectively.

Assay

The recommended mobile phase by BP was employed. It comprised a mixture of potassium dihydrogen orthophosphate buffer (pH 3) and acetonitrile in a ratio of 53:47, respectively. The overall chromatographic run time was less than 13 min. The buffer was vacuum filtered through 0.2 µm cellulose acetate membrane and then mixed with acetonitrile. The mobile phase was degassed in an ultrasonic bath. The column was set at room temperature and equilibrated to a stable base line before start of injections. The flow rate was set at 1.5 ml/min.

Preparation of sample solutions according to BP

For each product, 4 tablets were accurately weighed, crushed and the equivalent of the average weight of one tablet was transferred to a 20 ml volumetric flask. The volume was completed to mark with methanol, and additional 2 ml of water was added. The mixture was then sonicated and filtered through a 0.45 µm syringe filter. Three separate preparations were made for each product, and each preparation was injected three times along with three injections of a properly prepared standard solution of glibenclamide.

Dissolution test

The tests were performed according to pharmacopoeial specifications using Apparatus 2 (paddle method). The medium employed was 900 ml of 200 mM phosphate buffer (pH 6.8). Paddle rotation was set at 75 revolutions per minute. Medium temperature was set at 37°C ± 0.5°C. Six tablets of each product were placed in the dissolution apparatus (one in each vessel). Samples (5 ml) were withdrawn at pre-determined time points (10, 20, 30, 45, 60 and 120 min) and the withdrawn samples were replaced with buffer solution. All samples were then filtered before being injected into the HPLC column. The amount of dissolved glibenclamide was determined using the chromatographic conditions recommended in the BP. However, the wavelength was adjusted to 250 nm instead of 300 nm in order to maximize the sensitivity of the method and to enable accurate measurement of the lowest concentration possibly obtained during dissolution testing. The linearity of the method was ensured by injecting standard solutions of glibenclamide in the concentration range 1.135 to 5.675 µg/ml which covers 20 to 115% of the anticipated concentration resulting from the dissolution of the 5 mg tablet in 900 ml of buffer. A good correlation coefficient for the average calibration equation was obtained (0.9972). The percentage release of glibenclamide was determined by using the following equation:

$$\text{Released (\%)} = [Cs \times (0.9) / 5] \times 100$$

Where Cs is the calculated concentration of glibenclamide in the sample (µg/ml). Dissolution profile for each generic product was obtained by plotting the percentage released against time of sampling.

Friability

Twenty tablets were accurately weighed then tumbled at 25 rounds per minute for a period of 4 min. The tablets were then removed from the tumbling chamber, de-dusted on a sieve and re-weighed. The loss in weight due to the tumbling action was recorded as percentage weight loss according to:

$$\text{Friability (\%)} = [\text{Initial weight - Final weight / Initial weight}] \times 100$$

RESULTS AND DISCUSSION

Identification

According to BP monograph, identification of glibenclamide can be achieved using two chromatographic tests. The first chromatographic test employs the same HPLC conditions that are recommended for the assay of glibenclamide in this monograph. The test is based on comparing the retention time (t_r) of the analyte (glibenclamide) from commercial tablets to that of a standard preparation of glibenclamide. The second identification test is based on TLC which utilizes the same chromatographic conditions that are used for related substances test in the same monograph. According to the test, the substance is positively identified if it exhibits a similar $R_{f\ value}$ of a standard solution. All commercial preparations tested exhibited practically similar R_f and t_r values when compared to standard glibenclamide. Thus, all the tested preparations can be said to contain the correct active ingredient as per the pharmacopoeial specifications.

Related substances

The BP test for related substances in glibenclamide tablets is a semi-quantitative test that is based on TLC. The test states that the spots corresponding to the mentioned impurities in sample should not exceed in intensity that for a standard preparation for each of them, which means that the maximum allowed limit for CEBSA and MCEBSC were 0.012 and 0.002%, respectively. The results indicated that, in all of the tested preparations, only one major spot could be seen in the extracted preparations (solution 1) which corresponds to glibenclamide as confirmed by the spot in the standard solution (solution 4). This suggests that all of the tested preparations met the pharmacopoeial specifications pertaining related substances.

Assay

All of the obtained generic tablets of glibenclamide were assayed as recommended by BP. The peak of the analyte in the chromatogram of the solution prepared from tablets was confirmed by comparing the retention time (11.3 min) with that of a standard solution of glibenclamide. A reasonable peak shape was obtained for the analyte (Figure 2). Before the start of analysis, standard solutions of glibencalmide were injected every day in triplicate and relative standard deviation (RSD) values for peak areas were calculated. Three injections were applied to HPLC from each of the three preparations prepared for each product. RSD for triplicate injections of either standard glibenclamide solution or solutions prepared from tablets were always less than 2%. Summary of the obtained assay results expressed as percentage per label for all generics tested, together with RSD values, are presented in Table 2.

In general, the precision of analysis was satisfactory as judged by the obtained RSD values of less than 2%. Strictly speaking, only two preparations can be said to pass the BP assay requirements which are Glucomid® (97.2%) and Glunil® (105%). This is because the BP (British Pharmacopoeia, 2009) requires glibenclamide tablets to contain not less than 95% and not more than 105% of the claimed amount. However, if the USP (United States Pharmacopoeia, 2005) specifications were to be considered, which allows percentage per label to be in the range 90 to 110%, then all of the tested preparations would pass the assay requirements with two preparations being on the borderline (Melix®,110.3% and Glibemide®, 111.5%).

Since the manufacturers of the assayed products might have adopted the USP specifications rather than the BP ones, it could be concluded that the assayed products

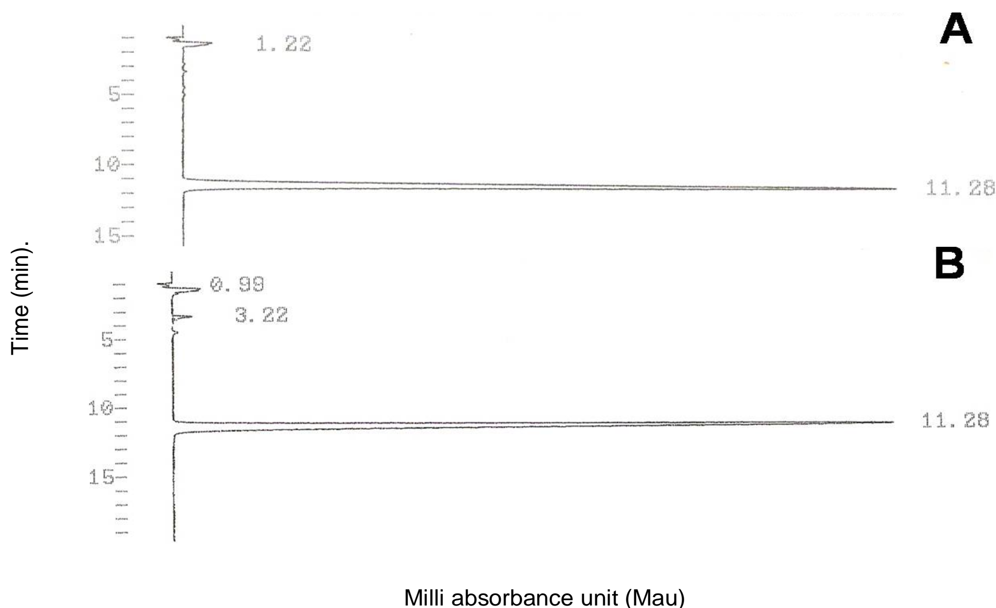

Figure 2. (A) Sample chromatograms for standard glibenclamide and (B) a solution prepared from commercial tablets of Daonil®.

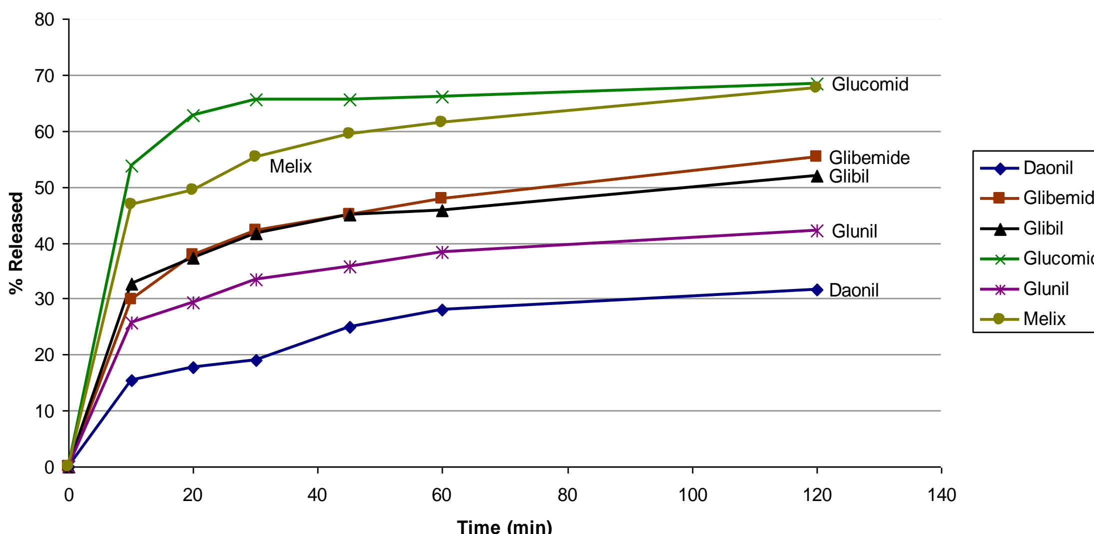

Figure 3. Average dissolution profile for the various commercial products tested in phosphate buffer pH 6.8.

demonstrated generally satisfactory assay results.

Dissolution test

A simple medium of phosphate buffer at pH 6.8 was adopted to carry out the dissolution studies. A complete dissolution profile for each product was obtained. Dissolution profiles are believed to better reflect (than single point determination) the *in vivo* bioavailability of drugs, particularly for those drugs which are classified as class II in the bioclassification system (Dressman et al., 1998). Such drugs are generally known as low solubility high permeability drugs (Dressman et al., 2001). Glibenclamide is known to be classified as a class II drug (Dressman et al., 2001), therefore its *in vitro* dissolution profile could be expected to reflect the *in vivo* performance of the formulation.

The average dissolution profile obtained for each of the tested commercial preparations is shown in Figure 3. From

Table 1. List of the tested generics and reference tablets containing glibenclamide.

Brand name	Manufacturer	Batch No.	Manufacture date	Expiry date
Daonil®	Sanofi-Aventis France	0CH9A	04/2010	04/2012
Melix®	Bio-Strata Pharmaceuticals	6074	08/2006	08/2011
Glunil®	Ram Pharmaceuticals, Jordan	L09	09/2009	09/2011
Glibil®	Hikma Pharmaceuticals, Jordan	6222	10/2010	10/2013
Glibemide®	United Pharmaceuticals, Jordan	7720	08/2010	08/2013
Glucomid®	APM, Jordan	756076	02/2007	02/2012

Table 2. Average percentage per label obtained for each of the tested products.

Product	Percentage per label	RSD% (n = 3)
Daonil®	104.2	0.56
Melix®	110.3	0.93
Glunil®	105	1
Glibil®	110	0.75
Glibemide®	111.5	1.1
Glucomid®	97.2	0.86

RSD: relative standard deviation.

the figure, it can be seen that all products, including the originator (Daonil®) did not release significant percentage of the drug within the first 30 min. In fact, Daonil® exhibited the lowest percentage release within the first 30 min (20%) while other products varied in the range of 35 to 65%. This might be unexpected but indeed explainable. The pharmacopoeia did not specify a dissolution medium for glibenclamide tablets and left the choice of the medium to the manufacturer, so if the medium was different (for example, simulated gastric or intestinal fluid), a significant increase in the dissolution of glibenclamide may be anticipated. In fact previous reports on the dissolution of Daonil® in simple phosphate buffer (pH 6.8) obtained almost identical results to those obtained in this study that is, no more than 40% of glibenclamide in Daonil® was released within 120 min (Lee et al., 1999). Moreover, the dissolution of Daonil® was shown to significantly increase (from ~40 to ~90%) by changing the pH by one unit only (from 6.8 to 7.8), which indicates the high sensitivity of glibenclamide solubility to pH of the medium.

A previous study on the originator Euglucon N® which is the trade name of glibenclamide in Britain (made by Boehringer Mannheim/Hoechst, Germany), showed that no more than ~20% of the drug was released over 30 min in simple phosphate buffer (pH 6). However, significantly higher dissolution rates were obtained when dissolution was studied in simulated fluids (Löbenberg et al., 2000). Yet another confirmation came from a study that compared the performance of Daonil® (Hoechst) to other two suggested formulations of glibenclamide. The results showed that the percentage release of glibenclamide from Daonil® in phosphate buffer (pH 7.4) was just below 40% over 1 h (Tashtoush et al., 2004).

More importantly, the obtained dissolution profiles in this study for the tested commercial products were obviously different, with Daonil® showing the least percentages released over the entire dissolution curve. For some products, the percentages dissolved over the time range 60 to 120 min were almost twice that of Daonil®. For better evaluation of similarity of dissolution profiles, the similarity factor (f2) value was calculated for each dissolution curve in comparison to Daonil® and they were 34.5, 35.3, 19.3, 47 and 23.5 for Glibemide®, Glibil®, Glucomid®, Glunil®, and Melix®, respectively.

According to the estimated f2 values, none of the profiles could be considered similar to that of Daonil® which is quite alarming. In principle, it should not be taken that the products with higher percentage dissolution are better performing than Daonil® because Daonil® is the originator on which relevant clinical, pharmacokinetics and toxicity studies were performed before authorization. Therefore, if a generic of glibenclamide released double the percentages of Daonil®, and that was highly correlated with *in vivo* performance, then that generic would provide almost double the dose of Daonil® which essentially should not be acceptable. However, the question here is whether the obtained dissolution profiles correlate directly with *in vivo* bioavailability profiles for the products. An answer to this question could be found in relevant literature.

A study (Löbenberg et al., 2000) has shown very strong *in vitro*/*in vivo* correlation for glibenclamide preparations when investigated in fasted simulated intestinal fluid, and

some weak correlations in other dissolution media. Yet another study has shown that some *in vitro/in vivo* correlation exist for different glibenclamide preparations even in simple dissolution media such as phosphate buffer at pH 7.4 (Dressman et al., 1998). Therefore, it is quite likely that the observed differences in the dissolution profiles of glibenclamide generics might be reflected on *in vivo* bioavailability performance with potential ramifications in their clinical effects. This is particularly true for those products whose dissolution profiles were significantly different from that of Daonil® (Melix® and Glucomid®). Other commercial products of glibenclamide in different countries with dissolution profiles that are not similar to that of the originator (Daonil®) have been previously reported (Lee et al., 1999).

Friability of tablets is a criterion that measures the tendency of tablets to shed powder, so that more friable tablets could be anticipated to be more easily crushable and disintegrated, which in turn may influence their dissolution. Due to the observation that some of the tested generics appeared to have higher levels (than the originator) of shed fine powder; while in their blister packs and in attempts to explain the difference in dissolution behavior of the generics studied, friability tests were performed according to the pharmacopoeial requirements. The obtained percentage friability [weight loss (%)] were: 0.094, 0.079, 0.228, 0.075, 0.125 and 0.262 for Daonil®, Melix®, Glunil®, Glibil®, Glibemide® and Glucomid®, respectively. Although all of the tested commercial products were of satisfactory limits for friability, different generics exhibited slightly different values of percentage weight loss. However, no obvious correlation was obtained between the percentage loss and percentage release of the drug. Together with the observation that all preparations reached their maximum percentage release within 50 min or less (no further release occurred up to 120 min), the observed differences in dissolution behavior of the tested generics can not be attributed to factors such as friability and disintegration rate.

Overall, the study demonstrated that generics of glibenclamide available in Jordan market were of satisfactory quality attributes pertaining to identification, related substances and content of the active substance. However, the studied generics exhibited dissolution profiles that are significantly different from each other and from that of the originator Daonil®. In light of the relevant literature, it could be anticipated that the observed *in vitro* dissolution inequivalence of the different products, is likely to be reflected as *in vivo* bio-inequivalance. Therefore, further *in vivo* bio-equivalence studies are essential to confirm or refute these *in vitro* findings.

Conclusion

In general, all tested commercial glibenclamide generic tablets from the Jordanian market were within the BP specifications in terms of identification and related substances. All tested products can be said to pass the USP requirements of percentage per label. The tested generics differed mostly in their dissolution behavior when tested in phosphate buffer (pH 6.8). Daonil® showed the lowest dissolution profile of all the products tested. Some generics showed a percentage release of the drug, almost twice that of Daonil®. The differences in dissolution profiles are likely to reflect potential differences in clinical performance of the tested generics. Properly controlled bio-equivalence studies are strongly recommended to further investigate the potential inequivalence of the tested products.

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REFERENCES

British Pharmacopoeia (2009). Glibenclamide (and tablet) monograph Volume 1 and 2. London: The stationary office.

Del Tacca M, Pasqualetti G, Di Paolo A, Virdis A, Massimetti G, Gori G, Versari D, Taddei S, Blandizzi C (2009). Lack of pharmacokinetic bioequivalence between generic and branded amoxicillin formulations. A post-marketing clinical study on healthy volunteers. Br. J. Clin. Pharmacol. 68:34-42.

Dressman JB, Amidon GL, Reppas C, Shah VP (1998). Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. Pharm. Res. 15:11-22.

Dressman J, Butler J, Hempenstall J, Reppas C (2001). The BCS: where do we go from here? Pharm. Tech. 25:68-76.

Ehianeta T, Williams B, Surakat J, Mohammed N, Anyakora C (2012). Quality survey of some brands of artesunate-amodiaquine in Lagos drug market. Afr. J. Pharm. Pharmacol. 6:636-642.

Eichie FE, Arhewoh IM, Ezeobi OC (2009). *In-vitro* evaluation of the pharmaceutical quality of some ibuprofen tablets dispensed in Nigeria. Afr. J. Pharm. Pharmacol. 3:491-495.

Epstein S, Cryer B, Ragi S, Zanchetta JR, Walliser J, Chow J, Johnson MA, Leyes AE (2003). Disintegration / dissolution profiles of copies of Fosamax (alendronate). Curr. Med. Res. Opin. 19:781-789.

Genazzani AA, Pattarino F (2008). Difficulties in the production of identical drug products from a pharmaceutical technology viewpoint. Drugs R&D 9:65-72.

Green MD, Mount DL, Wirtz RA, White NJ (2000). A Colorimetric field method to assess the authenticity of drugs sold as antimalarial artesunate. J. Pharm. Biomed. Anal. 24:65-70.

Lee MS, Chang YC, Shih CC, Chen CJ, Chang BL (1999). Study on dissolution profile of commercial glyburide tablets. J. Food Drug Anal. 7:82-93.

Löbenberg R, Krämer J, Shah VP, Amidon GL, Dressman JB (2000). Dissolution testing as a prognostic tool for oral drug absorption: dissolution behavior of glibenclamide. Pharm. Res. 17:439-444.

Luzi L, Pozza G (1997). Glibenclamide: an old drug with a novel mechanism of action? Acta. Diabetol. 34:239-244.

Margolese HC, Wolf Y, Desmarais JE, Beauclair L (2010). Loss of response after switching from brand name to generic formulations: three cases and a discussion of key clinical considerations when switching. Int. Clin. Psychopharmacol. 25:180-182.

Newton P, Proux S, Green M, Smithuis F, Rozendaal J, Prakongpan S, Chotivanich K, Mayxay M, Looareesuwan S, Farrar J, Nosten F, White NJ (2001). Fake artesunate in South-east Asia. Lancet

357:1948-1950.
Schellekens H, Klinger E, Mühlebach S, Brin JF, Storm G, Crommelin DJ (2011). The therapeutic equivalence of complex drugs. Regul. Toxicol. Pharmacol. 59:176-183.
Smith JC, Tarocco G, Merazzi F, Salzmann U (2006). Are generic formulations of carvedilol of inferior pharmaceutical quality compared with the branded formulation? Curr. Med. Res. Opin. 22:709-720.
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.
United States Pharmacopoeia (2005). The official compendia of standards. United States Pharmacopoeia Convention, INC. 12601, Twinbrook parkway, Rockville, MD 20852.
Vial J, Cohen M, Sassiat P, Thiébaut D (2008). Pharmaceutical quality of docetaxel generics versus originator drug product: a comparative analysis. Curr. Med. Res. Opin. 24:2019-2033.
WHO (1999). Counterfeit drugs: guidelines for the development of measures to combat counterfeit drugs, department of essential drugs and other medicines. Geneva: WHO.