

Pharmaceutical Evaluation of Type II Oral Antidiabetic Agent

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ABSTRACT

In this study, the pharmaceutical evaluation including physical and chemical evaluation of commercial Glibenclamide tablets (Gliboral®, Glynase® and Glib-5®) that are available in the Libyan market was performed according to the British Pharmacopoeia (BP) monograph (2009). The obtained results from this work indicated that, significant differences in the dissolution behavior were observed between the different tested commercial products. Gliboral® exhibited the lowest dissolution profile while the other products showed dissolution profiles that were almost twice that of Gliboral®. However, no significant differences were observed for the percentage (%) of weight loss (friability) and disintegration time for the tested products with % RSD value of less than 2 %. Additionally, all products were found satisfactory in terms of identification using infrared (IR) spectroscopy in comparison to reference spectra. Moreover, the assay results for the % content of glibenclamide using high performance liquid chromatography (HPLC) showed that all tested products were found to pass and satisfied with the BP specifications which required glibenclamide content to be within the range of 95 to 105 % of the labeled content. The precision of the analysis was also evaluated and calculated and the results showed good precision (interday and intraday) with % RSD value of less than 1.9 % (based on 6 injections).

Keywords: Dissolution Testing, glibenclamide tablet, high performance liquid chromatography, infrared spectroscopy, quality control

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INTRODUCTION

Quality control of pharmaceutical products is essential and to a larger extent, the quality control consider an integral to all modern industrial process and become very important and has driven the development of analytical techniques particularly chromatographic techniques. Testing a pharmaceutical product involved chemical, physical and sometimes microbiological analysis [1]. Quality control is therefore important for monitoring the effect of many drugs especially those drugs used for treatment of chronic and fatal diseases to insure the safety and efficacy. The absence of quality control measures in many countries led to the production and prevalence of drugs which do not meet

quality specifications like substandard, fake or counterfeit drugs since such drugs may contain for instance low or high concentration of active ingredients, poor quality ingredients, poor stability and packaging [1, 2]. Such drugs may not have only inadequate physicochemical behavior, but can also influence the clinical outcome of the use of that preparation [3]. Many studies have shown the presences of substandard drugs available in the markets of several countries and significant difference in the dissolution and disintegration of tablets were revealed [4-9].

In this study, the quality of the type II oral anti-diabetic drug, glibenclamide products (5 mg) that are widely available in the Libyan market was evaluated through direct purchase of the products from local private pharmacies and subjecting them to analysis according to the British Pharmacopoeia (BP 2009) [10]. Glibenclamide (**figure 1**) which also known

as Glyburide in USA, is a second generation belonging to class of a sulfonylurea oral hypoglycemic drug which has long been in clinical use [11]. It chemically known as 5-chloro (4-[N(cyclohexylcabonyl) sulfonyl [phenylethyl)]-2-methoxy benzamide.

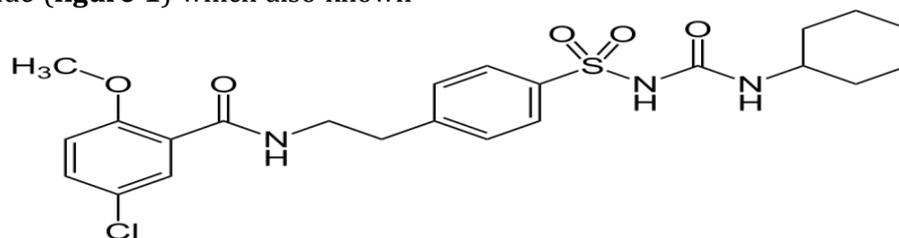


Figure 1: Chemical structure of Glibenclamide product

This class of drug works by binding to and activates the sulfonylurea receptor 1 and cause inhibition of ATP sensitive potassium channel in pancreatic beta cells. This inhibition cause cell membrane depolarization that lead to opening voltage dependent calcium channels, thus triggering to increase intracellular calcium Beta cells that stimulate insulin release [11, 12]. Glibenclamide may also be used parallel with metformin when diet and glibenclamide or diet and metformin alone do not result in adequate glycemic control [11].

The quality control tests used for pharmaceutical products can be physical test like (hardness, weight variation, content uniformity, disintegration, dissolution or friability) and chemical test using different instrumental and detection techniques like (HPLC, CE, TLC, GC, MS, NMR, IR) in addition to Microbiological test [10, 13-20]. Accordingly, in this work, the pharmaceutical quality of the three commercial available glibenclamide products in Libya was evaluated and assessed by conducting friability, disintegration and dissolution tests followed by IR identification and HPLC assay for the percentage content. Thus the aim of this project was directed toward pharmaceutical evaluations of three different imported commercial brands of drugs widely available and commonly used for treatment of type II diabetic patient in Libya.

MATERIALS AND METHODS

Chemicals and Reagents

All reagents were of pro analysis grade and all solutions were prepared in distilled water. Potassium dihydrogen orthophosphate (KH_2PO_4), dipotassium phosphate (K_2HPO_4) and phosphoric acid (H_3PO_4) were used for buffer preparation and was obtained from SD Fine-Chem Limited (Mumbai). High performance liquid chromatography (HPLC) grade acetonitrile (ACN) and methanol (MeOH) were obtained from TEDIA Company (INC, USA). Hydrochloric acid (HCl), chloroform (CH_2Cl), potassium bromide (KBr) was obtained from (Merck KGaA, Damstadt, Germany). Working standard of glibenclamide was obtained from the Food and Drug Control Center (Tripoli, Libya). The examined tablets of the three commercially available glibenclamide products A, B and C (Gliboral[®], Glynase[®] and Glib-5[®]) respectively were purchased from local private pharmacies in Tripoli. A list of the tested products with their details is shown in (Table 1).

Instrumentation and Operation Conditions

Dissolution test was performed to measure the release of drug in specified time under specific condition to be ready for absorption [15]. The test was conducted by placing one tablet of each product containing 5 mg of glibenclamide in the dissolution apparatus (one in each vessel or basket) (G.F.L NO-10688101, Germany) and

immersed in beaker containing the medium 900 mL 0.1N HCl. Rotation was set at 50 rpm for 30 min and temperature was set at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. All samples were then filtered and 10mL of the filtrate was taken and diluted to 50 mL using 0.1N HCl and the UV

absorption was recorded at 276nm. The percentage release of glibenclamide was determined by using the following equation:
 $\% \text{ Released} = [\text{Cs} \times (0.9) / 5] \times 100$
 Where Cs is the calculated concentration of glibenclamide in the sample ($\mu\text{g}/\text{mL}$).

Table 1: List of the commercial evaluated products containing Glibenclamide

Brand name	Manufacturer	Batch No.	Manufacture date	Expiry date
Gliboral®	Italy (Menarini international)	502	04/2013	04/2018
Glynase®	U.A.E (Julphar)	470	02/2009	02/2014
Glib-5®	India (micro-LABS limited)	1107	05/2009	02/2014

Friability test was performed to measure the resistance of the tablet to abrasion during packaging or handling. This property related to hardness, weight variation and content uniformity problem. Ten tablets were accurately weighed then and placed in a Friabilitor (ERWEKA, Germany) and rotated (tumbled) at 25 rpm 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 (% friability) was recorded as percentage weight loss according to the following formula:

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

Disintegration test was also performed to estimate the time required for a dosage form to break down in to granules of specified size under carefully specified conditions [6]. The test was conducted by taking six tablets of each products and immersed in the basket rack (ERWEKA, Germany, MODEL ZT321) containing 0.1N HCl for 15 min and the result was recorded.

Identification using IR spectroscopy: the IR spectroscopy was used to determine the chemical functional groups in the tested products since different functional groups absorb characteristic frequencies of IR radiation [21]. In this study, Fourier transform IR instrument was used (IR Prestige-21, SHIMADZU, Japan). The advantages of this instrument is that a full spectral scan be acquired in about one second compared with the 2-3 min required for a dispersive instrument which is another type of IR instrument. Also, several spectral scans can be taken and averaged in

order to improve the noise ratio for the spectrum. The procedure of extraction before IR identification was carried out as following: five tablets of each product were taken and 25 mL of distilled H_2O was added, shake and sonicate for 5 min. Then 25mL of CH_3Cl was added and shaken further for another 5min. This was followed by centrifugation at 2500 rpm for 5 min and the CH_3Cl layer was separated and evaporated using rotovapour and then the extract was dried at 60°C under vacuum. 2 mg was then taken from the dry extract with 200 mg KBr and the mixture was grained with care under vacuum at pressure 80 Mpa to obtain transparent KBr pressed disk for IR identification. The IR identification was achieved by comparing/matching the obtained IR spectra of the commercial glibenclamide products tested to the standard fingerprint spectra.

Assay of glibenclamide content: the HPLC experiments in this study were carried out using the HPLC system (Hitachi, model L-7400, Tokyo-Japan). The system was equipped with a UV detector and the HPLC-UV measurements were carried out at 300 nm for the tested products. The recommended mobile phase by the BP (2009) consisted of a mixture of potassium dihydrogen orthophosphate (KH_2PO_4) buffer at pH 3 and acetonitrile (ACN) in a ratio of 53:47, respectively. The pH was adjusted using microprocessor pH meter, HANNA Instruments (Romania) and the mobile phase was vacuum filtered through $0.2 \mu\text{m}$ cellulose acetate membrane and then degassed using ultrasonic water bath.

The mobile phase was delivered by a Jasco pump (Jasco, Tokyo, Japan) operating at a flow rate of 1.5 mL/min. The HPLC column used was stainless steel column packed with octadecylsilyl silica gel for chromatography (C18, 150 mm × 4.6 mm i.d) (Thermo Scientific, USA), Isocratic elution was used and the injected volume was 20 μ L (n=6). The column was set at room temperature and equilibrated to a stable base line before start of injections.

Preparation of sample solutions according to BP (2009)

The British pharmacopoeia BP monograph (2009) was used to test all collected commercial glibenclamide products. For each product, ten tablets were accurately weighed, powdered and a quantity equivalent of the average weight of one powdered tablet (5 mg) of glibenclamide was transferred to a 20 mL volumetric flask and mixed with 2 mL of water and the volume was completed to mark with methanol. The mixture was then sonicated until fully dispersed and then was filtered through a 0.2 μ m membrane filter [10]. Two

separate preparations were made for each product and each preparation was injected six times along with six injections of a standard solution of glibenclamide.

RESULTS

Physical quality control including dissolution, friability and disintegration test were carried out and the obtained results were reported. (Figure 2A) showed the results obtained from dissolution test of all tested commercial Glibenclamide samples using 0.1 N HCl as dissolution media.

Friability test and disintegration test were also conducted for all Glibenclamide products and obtained results are represented in (figure 2B and 2C) respectively.

Additionally, IR of the commercial Glibenclamide products (A, B, C) as well as reference standard was investigated and the reference results spectrum was used for comparison and identification. All samples and standard IR spectrum are given in (Figure 3).

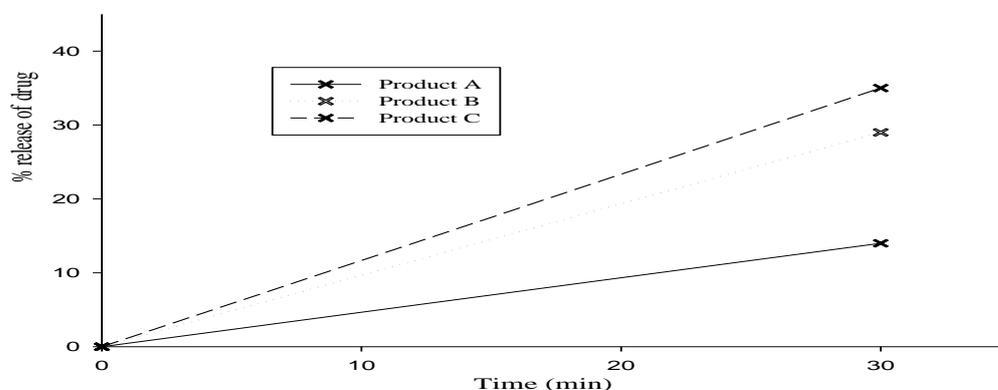


Figure 2A: Dissolution profile for the commercial Glibenclamide product tested using 0.1 N HCl as dissolution media

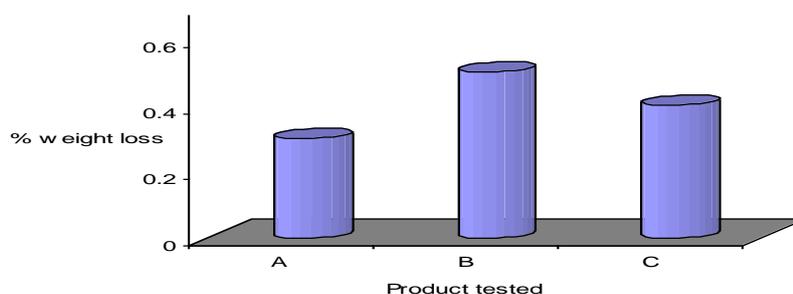


Figure 2B: The obtained percentage of friability for the tested Glibenclamide products

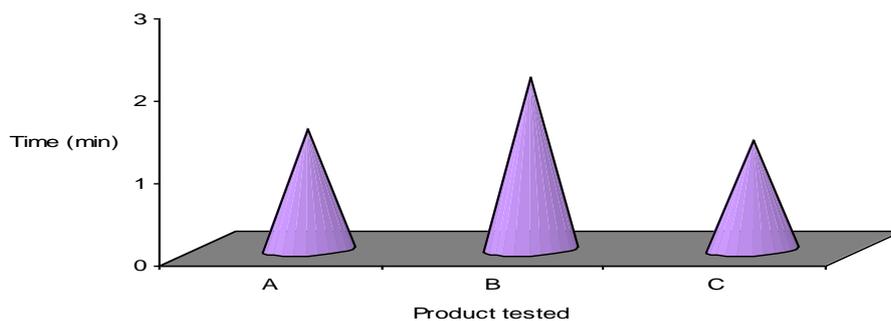
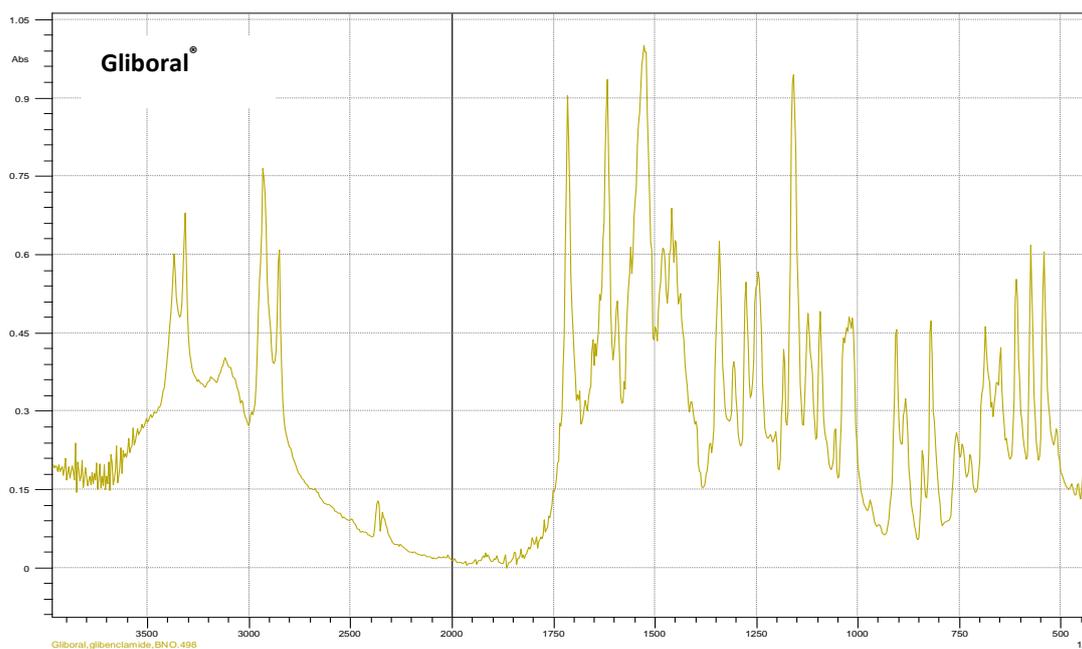
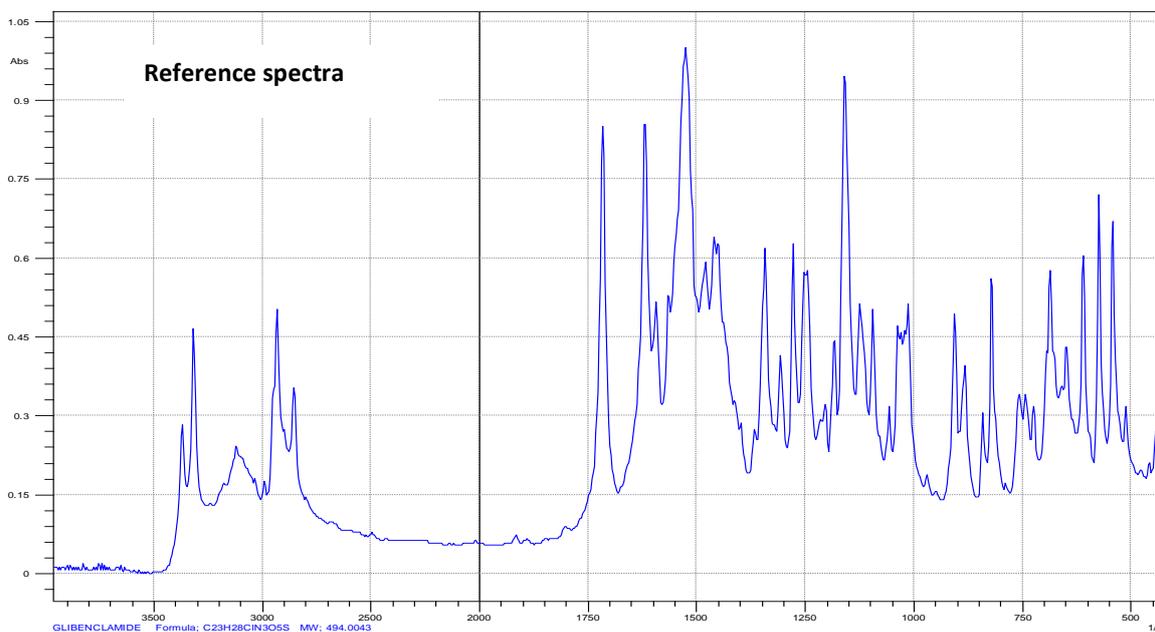


Figure 2C: The obtained disintegration time for the tested Glibenclamide products



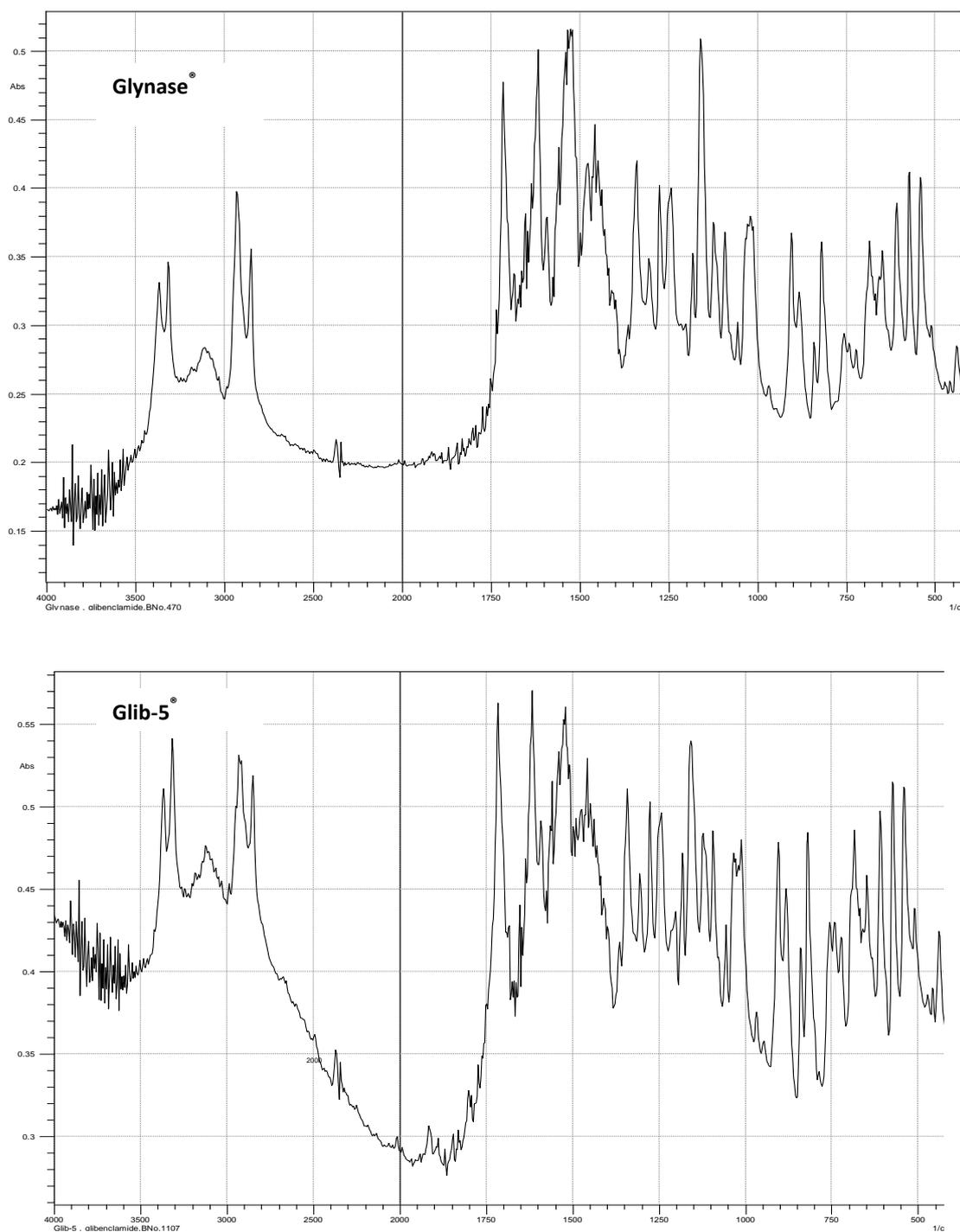


Figure 3: IR spectra of the tested commercial Glibenclamide products (A, B and C) compared to the reference spectra

Moreover, HPLC was used for the assay of all samples under investigation; the obtained chromatograms for the samples are shown in (Figure 4). The AUP from

$$\% \text{ content} = \frac{\text{AUP STD}}{\text{concentration STD}} \times \frac{\text{concentration sample}}{\text{AUP sample}} \times 100$$

The % content was 102.7 %, 99.5 % and 97.2 % (n=3) for Gliboral, Glynase and Glib-5 respectively.

chromatogram was used for estimation of % content of Glibenclamide in each sample by applied the following formula:

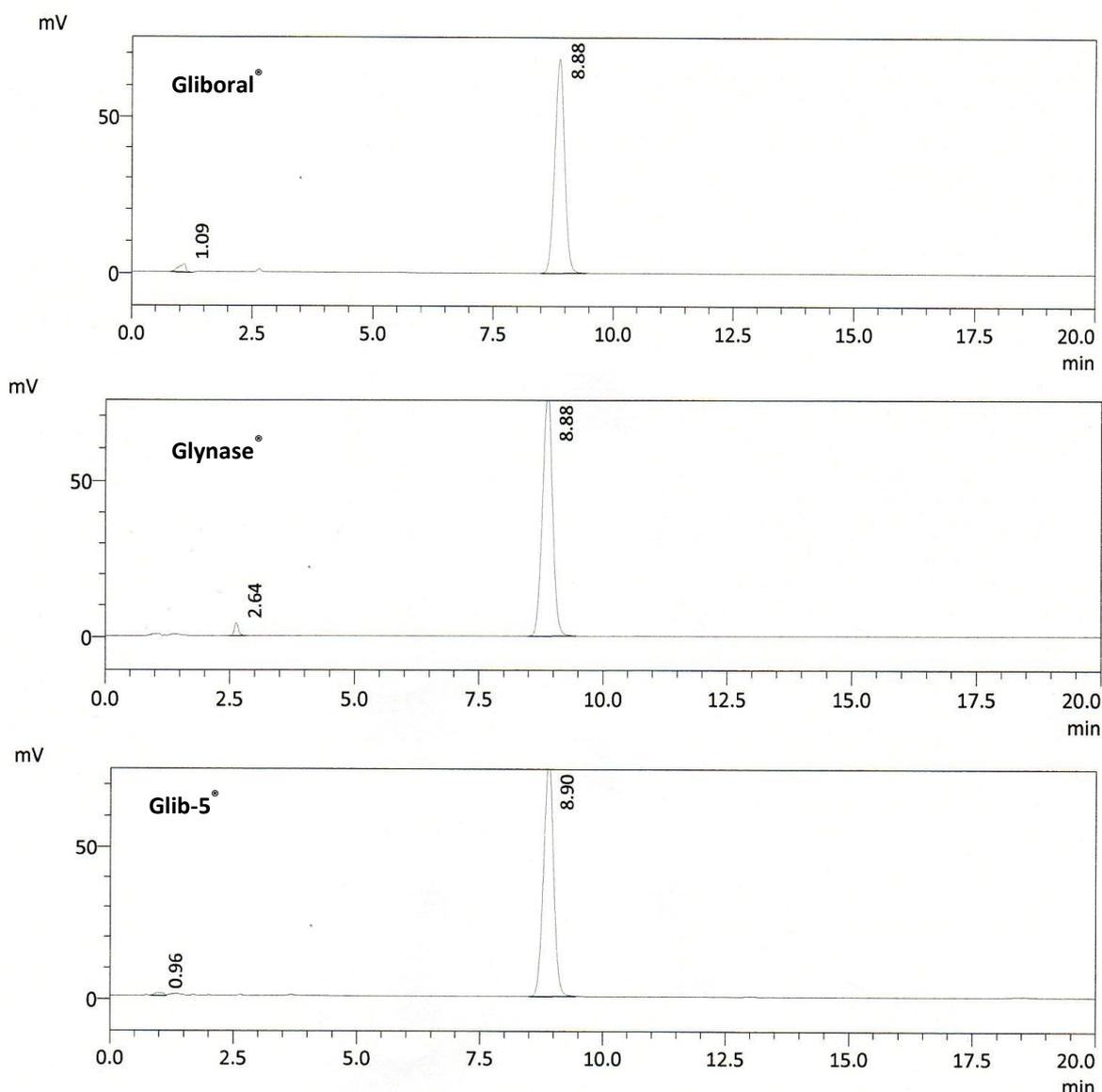


Figure 4: Chromatograms of Glibenclamide solutions prepared from commercial tablets

DISCUSSION

The obtained dissolution profile as it can be seen in (figure 2A) indicated that there are significant differences between the tested products. All tested samples did not release a significant percentage, since (80 %) of the drug should be dissolved within 30 min according to BP. Product A (Gliboral®) showed the lowest percentages released (14 %) over the entire dissolution curve, while product B (Glynase®) and product C (Glib-5®) showed the highest percentage and twice that of product A (35 %). In this study, 0.1 N HCl was used as dissolution media with UV recording since the pharmacopeia did not specify a specific dissolution media for glibenclamide. Some research studies indicated the use of borate

buffer as dissolution media whereas others using phosphate buffer [9,15]. The observed differences between the tested commercial brands of glibenclamide may related to manufacturing process or other factors that affects on dissolution rate can be a reason such as: particle size, additives used, the force of tablet compression of tablet or the tablet shape. A study by El-Sabawi et al. showed a significant difference in the dissolution behavior of different brands of glibenclamide products available in the Jordanian market using phosphate buffer as a dissolution media [9].

The obtained results for the % friability indicated that all tested products have good % friability (not more than 1 %) as it can be seen in (figure 2B). Although, product (A)

has the lowest weight loss followed by product C and B, however, this difference is not significant which could indicate that all tested products of glibenclamide have good resistance to abrasion during storage or handling.

Disintegration test is important since for the drug to be absorbed from a solid dosage form after oral administration it must be in solution form and this is the first important step toward this condition which involves the breakup of the tablet. According to the BP, the uncoated tablet required to be disintegrated within 15 min. The obtained disintegration profile indicated that there are a slight difference in the disintegration time between the different products, where product (C) has the shortest disintegration time followed by product (A), whereas product (B) has the longest disintegration time as it can be seen in **(figure 2C)**. However, all products complied with the limit of BP (disintegrate in less than 15 min). The obtained slight difference in the disintegration time between the products can be related to the manufacturing process like for instance hardness of tablets or type of disintegrator used.

IR identification was carried out by comparing the obtained IR spectra of the tested commercial products (A, B, C) with the reference spectra. As it can be seen in **(figure 3)**, the obtained IR spectra for product A (Gliboral®) are very close to reference spectra following with product B (Glynase®) and C (Glib-5®).

For the assay of samples, the HPLC analysis was performed for the commercial products using the same condition. As it can be seen in the obtained chromatograms in **(figure 4)**, the HPLC analysis was obtained in less than 10 min with good repeatability of the Rt with % RSD value less than 1.5 %. The peak were symmetrical with low tailing factor (less than 1.04) and with % RSD value for the peak area of less than 1.7 %.

The content limit according to BP for the amount of glibenclamide should be (95 % - 105 %) and the obtained results satisfied with the BP requirement for the content of glibenclamide. The precision of the measurements (intra-day and inter-day) was also evaluated by injecting the sample solution in the first day and then injecting

the same sample solution in third day. The obtained % RSD of peak area was less than 1.9 % which could indicate a good precision for the obtained measurements. Moreover, stability of the sample solutions during the analysis was also studied, since the chemical stability of drug substance in solution form is the most common interpretation and that will distinguish active ingredient from its degradation products, so that active pharmaceutical ingredient content can accurately be measured. Also, to evaluate how the drug stability can change during the analysis due to the environmental factor such as: temperature, light, moisture. The test was conducted by injecting the same drug solutions for six consecutive days under the same experimental condition and the same chromatographic condition of HPLC assay. The average % RSD value of the peak area was less than 2.3 % which indicated that the products were stable during the analysis.

CONCLUSION

The obtained results indicated that, all tested commercial glibenclamide tablets from the Libyan market were within the British Pharmacopeia (BP) specifications in terms of dissolution, disintegration and friability test, identification using IR spectroscopy and HPLC assay. All tested products pass the BP requirements of percentage per label and IR identification. The tested products differed mostly in their dissolution behavior when tested. Gliboral® showed the lowest dissolution profile of all the products tested whereas, the other products showed a percentage release of the drug, almost twice that of Gliboral®. The obtained differences in the dissolution profiles of tested products are highly expected to reflect possible differences in their therapeutic outcome. Consequently, additional investigation for the reasons behind such differences and bio-equivalence studies for the tested commercial glibenclamide products are required and highly recommended.

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