World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

IN-VITRO COMPARATIVE STUDY OF METFORMIN/GLYBURIDE COMBINATION GENERIC BRAND TO THE ORIGINATOR

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Article Received on 02/09/2020 **Article Revised on 23/09/2020** **Article Accepted on 13/10/2020**

ABSTRACT

The Sudan Pharmaceutical industry was slowly growing. Many generic brands are produced, the quality of which should be verified. To achieve this verification and guarantee the quality, glyburide/Metformin combination brand, GA, was tested for similarity with the originator; Glucovance and testing its uniformity in content. The physical properties were found to be within the specified limits. The API contents in both product s were within the pharmacopoeial limits. On studying the dissolution profile the similarity factor f_2 is 75 for the glyburide component and 80 for metformin component while the difference factor f_1 is 11 for glyburide component and 7 for the metformin components, indicating close similarity. The uniformity of content was within the USP limits. It had been concluded that Sudan pharmaceutical industry is promising and can produce acceptable quality to satisfy the population needs.

KEYWORDS: Metformin/Glyburide combination, Similarity with Originator, Quality of Generics, Sudan Pharmaceutical Industry.

INTRODUCTION

Glucovance was originated by Merck Sante as a first line in the treatment of diabetes type 2 together with the diet control and exercise. It is a combination of sulphonylurea and biguanide, Glyburide and Metformin. The Sudan pharmaceutical industry is slowly growing and the quality of the products should be verified and the generic GA analogue was produced.

Glyburide

$C_{23}H_{28}ClN_3O_5S$, molecular mass: 494.0

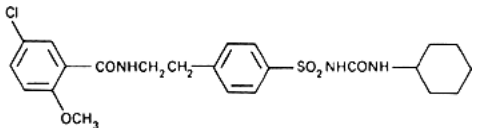

Figure 1: The structural formula of Glyburide.

Physical properties of Glyburide

Metformin Hydrochloride

$C_4H_{11}N_5$.HCl, molecular mass 165.62,

Physical properties of metformin:

It is a white or almost white crystals4 Molecular weight: 129.16 g/mol, Melting Point: 250º C4 Boiling Point: 224.1° C, slightly soluble in alcohol, practically insoluble in ether, chloroform, acetone, methylene chloride. In water, 2g is soluble in 10ml. 4

BCS Classification

The BCS is a scientific framework for classifying a drug substance based on its aqueous solubility and intestinal permeability. It allows the prediction of in vivo pharmacokinetics of oral immediate-release (IR) drug products by classifying drug compounds into four classes based on their solubility related to dose and intestinal permeability in combination with the dissolution properties of the dosage form. 14. Glyburide is a BCS class-II, has a less solubility outline, whereas Glyburide is connected with stability and solubility troubles. (Table 1)

Table 1: The Bio pharmaceutics classification system.

Class	Solubility	Permeability
I	high	High
II	Low	high
III	high	low
IV	low	low

Rahman Gul and, Syed Umer developed validated method for concurrent analysis of dissolution study of glyburide and metformin from fixed dose tablets. Dissolution medium was chosen on the establishment of

solubility, sink conditions for drugs. A variety of investigational conditions (kind of equipment, stir rate and volume of dissolution medium) were optimized, and the new method was validated according to ICH and USP guide lines. Investigation of dissolution samples was assessed by HPLC column as stationary phase. The composition of mobile phase was acetonitrile, phosphate buffer (pH 5.3, 50:50, v/v). The flow rate of the mobile phase was 1 ml/min at 40o C and the wavelength was set at 230 nm. The most excellent dissolution profiles were achieved with 900 ml dissolution medium of Phosphate buffer pH 7.4 at 370± 2C, stirred at 75 rpm utilizing apparatus -II. Total dissolution completed within 45 minutes. The Validation criteria revealed accuracy, precession and robustness of the novel method with no any intrusion through sample analysis.[1]

Fazlay Aimed to evaluate and compare dissolution pattern of locally branded drug products of Metformin Hydrochloride available in Bangladesh with each other. Glucophage is the patent drug of Metformin Hydrochloride. Three different brands of Metformin Hydrochloride tablets, Six tablets from each of the brands were used for the in- vitro dissolution study. Cumulative drug release was measured up to 50 minutes for all brands.[2]

Ganesh, *et al* used ultraviolet spectroscophotometry for the determination of metformin and glibenclamide simultaneously in a combined dosage form. The method utilized simultaneous equation method for analysis using 0.1 N HCL as a solvent. The two wavelengths 300 nm and 233 nm were selected for determination of Glibenclamide and Metformin Hydrochloride respectively. Beer's law was obeyed in concentration range of 10-60µg/ml and 2-12µg/ml for Glibenclamide and Metformin Hydrochloride respectively. The recovery studies confirms the accuracy of the projected method and the results were validated as per ICH guideline.[3]

Mohammed Motaher, *et al* formulated a combination product of Glyburide and Metformin Hydrochloride Tablets 2.5mg/500mg and to evaluate their physiochemical properties. Wet granulation method was adopted for preparation of tablet using different excipients for six different formulations. The granules for tableting were evaluated for angle of repose, bulk density, tapped density, compressibility index and drug content etc. The tablets were subjected to thickness, hardness, friability and disintegration. Granules for three formulas showed satisfactory flow properties, compressibility index and the physical parameters of tablets from these three formulations gave optimum result in comparison to innovator's brand. Disintegration time of these three formulations (7-8 min) was found similar with innovator's brand (6.30-7.30 min). Assay of formula of glyburide (97.97%) and Metformin HCl (100.2%) met the USP specification (90%-110%). It was also found that dissolution profile of Glyburide depends on particle size of Glyburide powder.[4]

G. Maggi Stella, *et al* Concluded that Metformin/ glyburide (Glucovance) fixed-dose combination tablets are a second-line drug treatment for type 2 diabetes mellitus when glycaemic control does not improve with metformin or sulfonylurea monotherapy. It is developed and validated by different instruments like UV-Visible spectroscopy, RP-HPLC and HPTLC. The developed methods were accurate, precise and linear.[5] Shweta and Sunil, Aimed for determination of glyburide, metformin hydrochloride and rosiglitazone maleate by reversed phase liquid chromatographic technique in tablet dosage form. The method was found to be precise and reproducible. The proposed method was successfully applied for the analysis of metformin hydrochloride, rosiglitazone maleate, glyburide as a bulk drug and in pharmaceutical formulation without any interference from the excipients.[6]

Villarroel and Clement, *et al* Compared the Dissolution Profiles of Seven Metformin Formulations in Simulated Intestinal Fluid. The study compared the dissolution profiles of seven immediate-release metformin 500-mg formulations, including two products with the trade name Glucophage, in simulated intestinal fluid (SIF) at pH 6.8 buffer without enzymes. Other characteristics investigated were absolute weight and drug content. The weights were significantly different (p "0.05), and each of the formulations demonstrated drug content between 99% and 103%. Only one of the formulations showed <85% drug release in 15 min.[7] Elhamili, and Bergquist, et al Performed physical and chemical evaluation of commercial Glibenclamide tablets (Gliboral®, Glynase® and Glib-5®). 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.[7]

Haile Kassahun, Kaleab Asres *et al* assessed quality as well as physicochemical bioequivalence of five brands of glibenclamide tablets marketed in Addis Ababa using *in vitro* and *in vivo* methods. Friability, disintegration, dissolution, and assay for the content of active ingredients were evaluated using the methods described in the British Pharmacopeia (2009) and United States Pharmacopeia (2007). All the brands of glibenclamide tablets complied with the official specification for hardness, friability, disintegration, and assay.[8]

As Glyburide Metformin combination is widely used, the local pharmaceutical industry tends to satisfy the population needs, although the quality should be

guaranteed. This made the evaluation of the quality of locally manufactured brand of high priority and so, physiochemical properties should be compared to the originator and the uniformity of content should be investigated.

MATERIALS AND METHODS

Materials

Glyburide, metformin reference standards, Blue Nile Research Centre, Potassium chloride extra pure.(Spain), Boric acid.(techno pharmachem, bahadurgarh,) Sodium chloride(Blue Nile, Sudan). 1-heptane sulfonic acid, sodium salt monohydrate, HPLC grade. Acetonitrile (methyl cyanide, India, Mumbai, Sodium hydroxide. (BDM chemicals ltd, England) Ortho-phosphoric acid, 85% Distilled water.

Instruments

Dissolution test apparatus, Electrolab tester,12vessels. PHmeter detector, HANNA. Disintegration test apparatus , Electrolab Tablet Hardness tester [NE4_Cop,(Copley, Scientific, serial NO:13010)]. Ultraviolet spectrophotometer, UV-1700 pharmaspec Shimadzu HPLC instrument [Shimadzu HPLC, prominence-ı,LC2030C 3D,Detector:PDA]. Electronic balance, Shimadzu AY220 [0.1mg-220g].

Methods

Tablet weight variation[9]

Using a sensitive balance, the weight of 20 tablets was determined and the total weight of tablets was calculated. According to the total weight of 20 tablets there is specified percent error as stated in the USP. Using this percent error range of weight variation is determined, upper and lower limit of weight were established and the weight produced in the same batch must hence fall within these limits (±7.5%)

Hardness test

The slide scale of hardness tester was made zero. One tablet was placed vertically between the two jaws of the tester. Force was applied with a screw thread and spring until tablet fractured. 16. The hardness of 20 tablets was measured and the average should not be less than 5 Kg/F.

Disintegration[9]

The disintegration time for 6 tablets was determined using disintegration apparatus, the test was carried out by agitating the tablets in an aqueous medium, the temperature was set to 37o C and the tablets were dropped into the six chambers (tubes), the time taken for the 6 tablets to disintegrate was recorded. According to the USP when the medium being used is water and the tablets were coated, the disintegration time should not exceed 15 minutes.

Glyburide Dissolution test

Medium : 0.05 M boric acid and 0.05M potassium chloride solution , prepared by dissolving 3.09g of boric acid and 3.73g of potassium chloride in approximately 250ml of water , adjusting 1N NaOH to a pH of 9.5 , and diluting with water to 1L;500L. Apparatus: II:75rpm. The amount of Glyburide (C23H28ClN3O5S) dissolved was determined by employing the following method.[9]

Glyburide component of the tablet dissolution test

Phosphate buffer: 28.7 g of monobasic ammonium phosphate in water was dissolved, and diluted with water to 1 L. Mobile phase: a filtered and degassed mixture of Phosphate buffer and acetonitrile (1:1) was prepared, and adjusted with 1 N sodium hydroxide to a pH of 5.3. Standard solution: about 10 mg of Glyburide WS was transferred, accurately weighed, to a 100-mL volumetric flask, dissolved in 20ml of acetonitrile, Diluted further with medium to obtain a solution having a Glyburide conc. In mg per ml of L/500, where L is the label claim. **Procedure:** HPLC equipped with a 230-nm detector and 4.6-mmx15-cm column that contains 5µm packing L7 column maintained 30o C, column efficiency not less 5000 theoretical plates , tailing factor is between 0.8 and 2.0 ; RSD for replicate injections is not more than 2%.[9]

Metformin HCl Dissolution Test

Medium **:** 0.05 phosphate buffer , pH6.8 , prepared by dissolving 6.8 of monobasic potassium phosphate in 1000ml of water and adjusting with 0.2 N NaOH to a pH 6.8± 0.1;1000ml Apparatus : II:50rpm.

Metformin HCl component of the tablet dissolution test

The amount of Metformin hydrochloride (C4H11N5 ·HCl) dissolved was determined by employing the following method. Standard solution: an accurately weighed quantity of Metformin Hydrochloride WS in Medium was dissolved. Diluted with Medium, if necessary, to obtain a solution having a concentration, in mg per mL, of Metformin hydrochloride of about L/1000, where L is the label claim, in mg, of Metformin hydrochloride. Test solution: a portion of the solution was passed under test through a 0.45-µm polypropylene filter or a 1-µm glass fiber filter. The percentage of C4H11N5·HCl dissolved was calculated. Procedure **:** UV absorbtion at the wave lengh of maximum absorbance 232nm on portions of the test solution in comparison of the Standard solution.[9]

Uniformity of content

The test for uniformity of content of single-dose preparation is based on the assay of the individual content of active substance of a number of single-dose units to determine whether the individual content are within limits set with the reference to the average content of the sample.

Using a suitable analytical method is determined the individual content of active substance of 10 dosage units taken at random. Apply the criteria of test A, test B, or test C as specified in the monograph.[9]

Identification test[9]
Retention time is the primary means for chromatographic peak identification.

Glyburide: The retention time of the glyburide peak of the Sample solution corresponds to that of the major peak of the Standard solution, as obtained in the Assay for Glyburide.

Metformin Hydrochloride: The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay for Metformin Hydrochloride.

Assay for Glyburide content, HPLC method
Solution A: 28.8 g/L of monobasic ammonium phosphate.
Mobile phase: acetonitrile and solution A (40:60), were adjusted with 1 N sodium hydroxide to pH 5.3.
Diluents: acetonitrile and water (50:50).
Standard stock solution: 0.25 mg/mL of USP glyburide WS was prepared as follows: was dissolved first in the acetonitrile using 50% of the final volume and then was diluted with water to volume.
Standard solution: 0.025 mg/mL of USP glyburide WS in diluents, from the standard stock. System

Suitability solution 1: a solution containing 0.025 mg/mL of USP glyburide related compound A WS was prepared in diluents. 50µL of this solution was transferred to a 50 ml volumetric flask, and diluted with standard solution to volume.

System suitability solution 2: 5.0ml of USP Metformin hydrochloride WS was dissolved in system suitability solution 1.

Sample solution: not less than 5 tablets were dissolved in diluents by stirring with a magnetic stirring bar for at least 1 hour, and then diluted to obtain a solution containing 0.025 mg/mL of glyburide based on the label claim. A portion of this solution was centrifuged at 3000 rpm for 10 minutes.[9]

Assay for Metformin hydrochloride content, HPLC method
Solution A: 1.0 g of each sodium heptanesulphonate and sodium chloride were transferred to 2000 ml volumetric flask. 1800 ml of water was added and adjusted with 0.06 M phosphoric acid to a pH of 3.85. It was then diluted to volume with water.

Mobile phase: acetonitrile and solution A (10:90)

Diluents: acetonitrile and water (1:40)

Standard solution: 0.025 mg/mL of USP Metformin hydrochloride WS in diluents.

System suitability stock solution: 25µg/mL of each USP Metformin related compound B WS and USP Metformin related compound C WS in diluents.

System suitability solution: 0.5 mL of the system suitability stock solution was transferred to a 50 ml volumetric flask and was diluted to volume with standard solution.

Sample solution: a portion of the retained sample solution was diluted with water from the assay to obtain 0.25mg/mL of Metformin hydrochloride based on the label claim.

RESULTS AND DISCUSSION

Physical tests
Table 2: Weight Variation.

Brands	Average weight	% Lowest	% Highest	RSD%
GA	0.6407	98	104.2	0.66
Glucovance5	0.6161	97.1	100.8	0.987

Table 3: Thickness

Brands	Average	% Lowest	% Highest
GA	5.833	98.6	101.3
Glucovance5	5.577	99.15	100.77

Table 4: Hardness.

Brands	Average Hardness
GA	13.89
Glucovance5	17.06

Table 5: Disintegration time.

Brands	Disintegration Time (minute)
GA	8.5 min
Glucovance5	4.5 min

Weight variation, hardness and disintegration tests complies the USP specifications.

DISSOLUTION

The dissolution test was performed according to USP for the two the brands.

Table 6: Dissolution test for Metformin component in GA 5mg tablets

Metformin in GA						
time min	0	5	10	20	30	45
release%	0	63.14	91.39	95.59	97.37	97.73

Table 7: Dissolution test for Metformin component in Glucovance 5mg tablets

Metformin in Glucovance						
time min	0	5	10	20	30	45
release %	0	41.85	92.61	87.99	98.36	97.56

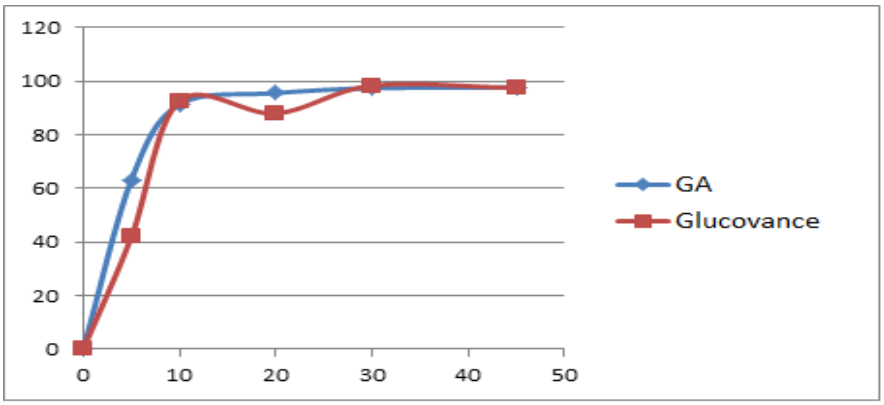

Figure 2: Dissolution Profile of Metformin in GA Generic and the Originator Brands.

Table 8: Glyburide.

Glyburide in GA						
time min	0	5	10	20	30	45
release %	0	73.1	92.5	98.1	95.7	94.6

Glyburide in Glucovance						
time min	0	5	10	20	30	45
release %	0	48.7	75.6	97.8	98.1	99

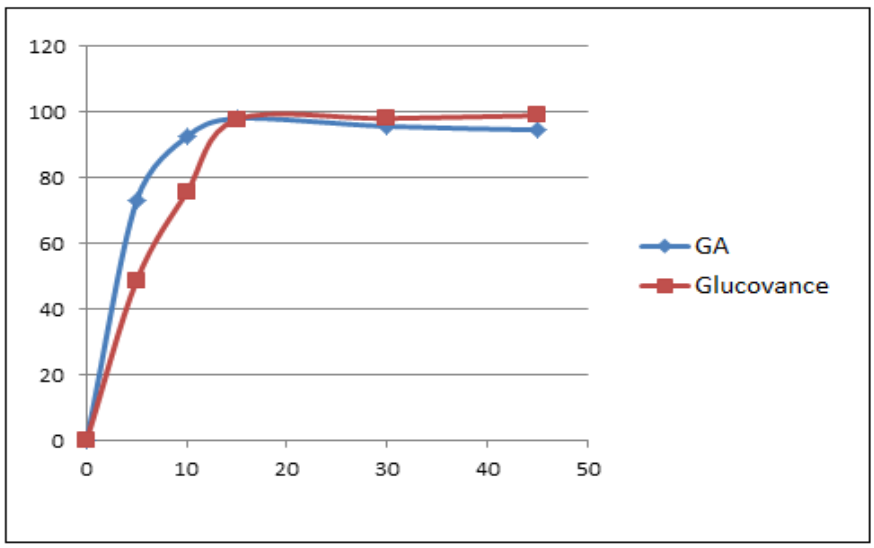

Figure 3: Dissolution Profile of Glyburide in GA Generic and the Originator Brands.

Similarity of GA with the Glucovance[10]

Table 9: Calculation of f_2 and f_1.

Time	Rt	Tt	{Rt-Tt}	$(Rt-Tt)^2$
5	73.10	48.70	24.4	595.36
10	92.50	75.60	16.9	285.61
15	98.10	97.80	0.3	0.09
30	95.70	98.10	2.4	5.76
45	94.60	99.00	4.4	19.36
		sum (Rt-Tt)		48.4
Glyburide		sum $(Rt-Tt)^2$		906.18
Number of time points, n = 5		sum Rt		454
		Similarity factor f2		75
		Difference factor f1		11
	Time	Rt	Tt	{Rt-Tt}	$(Rt-Tt)^2$
	5	41.85	63.14	21.29	453.26
	10	92.61	91.39	1.22	1.4884
	15	87.99	95.59	7.6	57.76
	30	98.36	97.37	0.99	0.9801
	45	97.56	97.73	0.17	0.0289
			sum (Rt-Tt)		31.27
	Metformin		sum $(Rt-Tt)^2$		513.52
			sum Rt		418.37
			Similarity factor f2		80
			Difference factor f1		7

The two products showed in-vitro similarity to a grater extend.

Component	f_2	Lower acceptable Limits	f_1	Upper acceptable Limits
Glyburide	75	50	11	15
Metformin	80	50	7	15

Chemical Assay, Table 10: Content Percentage of the two component in the two Brands.

Sample	% Metformin	Meformin Content in mg	%Glyburide	Glyburide content in mg	Comment
GA	100.07%	500.35 mg	101.44%	5.07 mg	Complies USP
Glucovance	100.14%	500.7 mg	105.28%	5.26 mg	CompliesUSP
The USP Tolerance = 90.0% to 110.0% 0f the stated amount.					

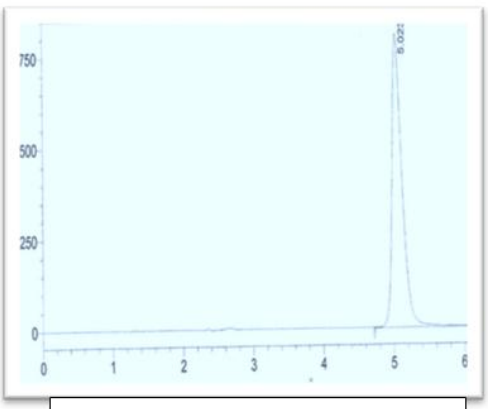

Figure 4: HPLC Chromatogram for Metformin Reference Standard

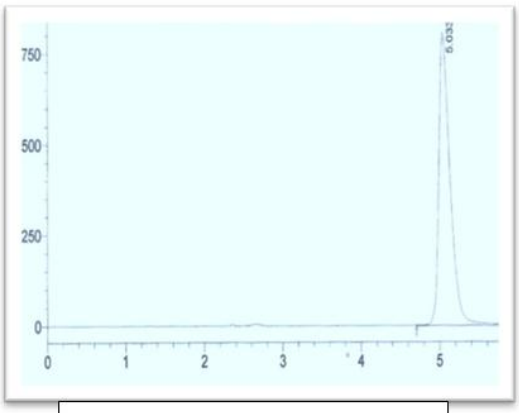

Figure 5: HPLC Chromatogram for Metformin in GA

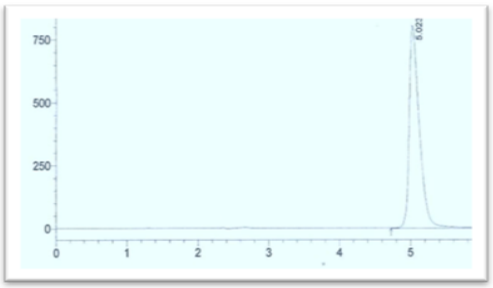

Figure 6: HPLC Chromatogram for Metformin in Glucovance

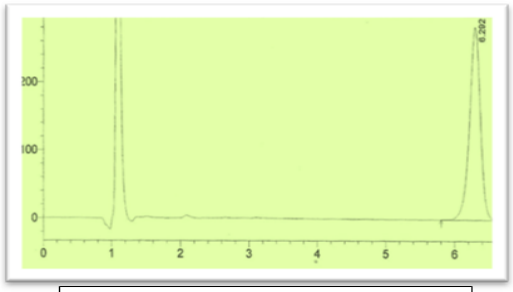

Figure 7: HPLC Chromatogram of Glyburide Reference Standard

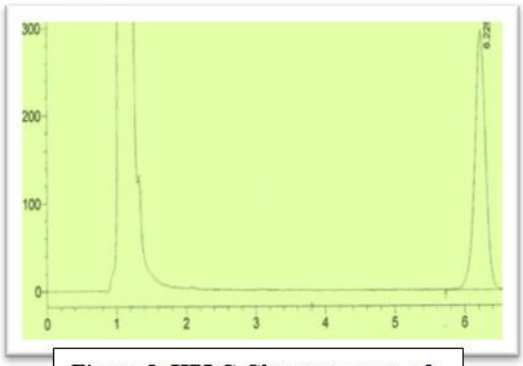

Figure 8: HPLC Chromatogram of Glyburide in GA

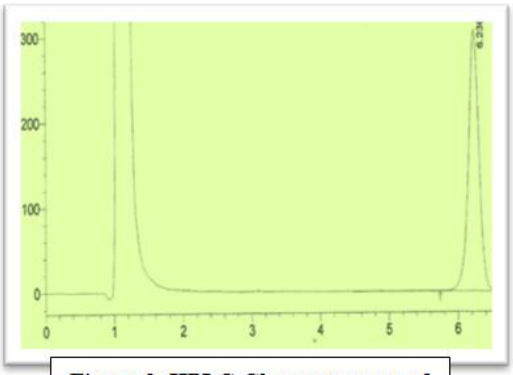

Figure 9: HPLC Chromatogram of Glyburide in Glucovance

Content Uniformity

Uniformity of the dosage unit is the degree of uniformity in the amount of drug substance among dosage units. It can be demonstrated by two methods, content uniformity or weight variation. Weight variation is applicable for tablets containing 25 mg or more of the drug substance or comprising 25% or more by weight so, this applicable for metformin component. Uniformity of content for less than 25mg or less than 25% of the drug substance, so, it is applicable the Glyburide component.

Weight Variation for Metformin Component

The average metformin content in 10 tablets is 100%, the standard deviation is 0.8.

A.V=K.S(when X value lies 98.5 to 101.5 where, X is the Mean value of 10 units, K is constant of using 10 units and is 2.4n and S is the Standard Deviation value. Thus, AV = 2.4×0.76 = 1.824.

Content Uniformity for Glyburide component

The average content of 10 tablets is 103.9 and standard deviation 2.74 so, $\bar{X}$is more than 101.5.

AV= $\bar{X}$ - 101.5 + KS, where, AV is the acceptable value, $\bar{X}$ is the average; K is 2.4 the 10 units constant and S is the standard deviation.

AV = 103.9 – 101.5 + 2.4 × 2.74 = 8.17

Since USP specifies the maximum acceptable value L_1= 15, thus all components are within the allowable acceptable value.[9]

CONCLUSION

The physical tests including weight variation, hardness, friability, disintegration and dissolution for both products were within the specifications. The dissolution test for the Glucovance in 45 min was 100.7% for Glyburide and 99.99% for Metformin. It is 95.9% for Glyburide and 98.61% for Metformin in GA. Both Complying the pharmacopoeial specifications for dissolution test. .The chemical assay for content using HPLC method for both component for each of the two brands are within the pharmacopeial specifications having values of (101.44%/100.14%), (105.28%/100.07%). The similarity factor f_2 is 75 for the Glyburide component and 80 for metformin indicating good similarity dissolution profile. The difference factor f_1 is 11 for Glyburide component and 7 for metformin indicating no significant difference. The uniformity of content in brand GA is within the acceptable USP limits L_1= 15; A.V for Metformin content is 1.824 and 8.17 for Glyburide indicating consistent production practice. This ppoints that the local pharmaceutical industry for generic s highly promising.

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