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Analysis of Some Brands of Glibenclamide Marketed and Used In Maiduguri Metropolis, Using Ultra Violet Spectrophotometry and High Performance Liquid Chromatographic (HPLC) Methods.

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ABSRACTS

The study involves quantitative analysis of five (5) brands of Glibenclamide using UV Spectrophotometry and High Performance Liquid Chromatography. The UV analysis of Glibenclamide showed that Glanil, Daonil, Diatab and Clamide passed the test with 98%, 102%, 96% and 96% while Gliben J failed the test with 94% using the standard range of 95-105% as specified by BP, 2008. While the HPLC analysis revealed that Glanil, Daonil and Gliben J passed the test while Diatab and Clamide failed the test with 139.2% and 81.6% respectively using the standard of 95-105% as stated by BP, 2008.

Keywords: Glibenclamide, UV, HPLC.

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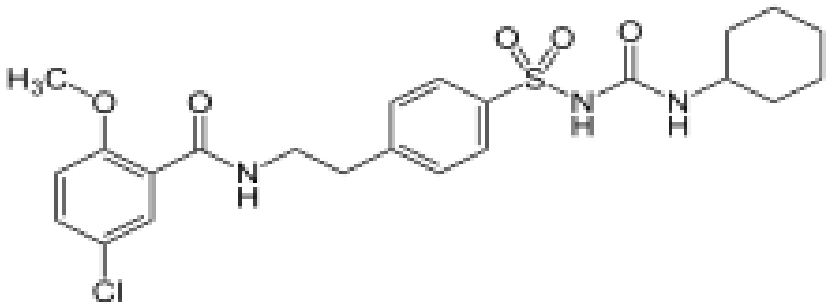
INTRODUCTION

The proliferation of substandard and adulterated pharmaceutical products is a global phenomenon which has been of great concern to many countries including Nigeria [1]. The resurgence of standard drug product especially in Nigeria is as a result of a number of factors, which include poor drug procurement and of drug distribution practices, low literacy level, inadequate information on the circulation of substandard products, lack of facilities for effective quality control analysis as an important element in quality surveillance and effectiveness of drug regulatory authority. The problems have been further compound in that Nigeria imports most drugs to meet its health care needs from Far East Asian countries such as China and India. [2] It was not long ago that the World Health Organization rose to the challenge of recommending that all importing countries should protect themselves from this menace by under taking sampling products within the distribution network as an important element in Quality surveillance [3].

Glibenclamide

Glibenclamide (INN), also known as **glyburide** (USAN), is an antidiabetic drug in a class of medications known as sulfonylureas, closely related to sulfa drugs. It was developed in 1966 [4].

Glibenclamide [5].



[6]. in the International Journal of Current Pharmaceutical Research carried out a research with the aim of preparing solid dispersion of Glibenclamide using different carriers such as PEG 6000, Polyvinyl pyrolidine (PVP) and Poloxamers in different ratios (1:1, 1:2, 1:3, 1:4 and 1:5) by Solvent Evaporation method. Drug carrier interactions were analysed by X-ray diffraction and Infra-Red Spectroscopy. Dissolution studies using the USP paddle method were performed for all solid dispersions. All solid dispersions showed increased dissolution rate as compared to pure Glibenclamide and PVP was found to be better than PEG and Poloxamer. The tablets were formulated using solid dispersion of Glibenclamide containing PVP as carrier. The tablets containing solid dispersion exhibited better dissolution profile than commercial tablets. Thus solid dispersion technique can be successfully used for improvement of dissolution of Glibenclamide.

In another study by (Alaa A.A. et *al.,*) in Sultan Qaboos University Medical journal, carried out a work on Comparative Effects of Glibenclamide and Metformin on C-Reactive Protein and Oxidant/Antioxidant Status in Patients with Type II Diabetes Mellitus,There were significant differences between patients prescribed metformin and glibenclamide and the controls with regard to serum hs-CRP, MDA and TAS. There was a significant reduction in the serum MDA and a significant raise in the serum TAS levels, with no significant effects on serum hs-CRP levels after metformin therapy, but no significant effects on these parameters after glibenclamide therapy. The percentage of variation in these parameters after both drugs, showed a significant raise in serum TAS levels with the metformin therapy with no significant effects in serum MDA and hs-CRP. [7].

Mechanism of action

The drug works by inhibiting the sulfonylurea receptor 1 (SUR1), the regulatory subunit of the ATP-sensitive potassium channels (K_{ATP}) in pancreatic beta cells. This inhibition causes cell membrane depolarization opening voltage-dependent calcium channel. This results in an increase in intracellular calcium in the beta cell and subsequent stimulation of insulin release. After a cerebral ischemic insult the blood brain barrier is broken and glibenclamide can reach the central nervous system. Glibenclamide has been shown to bind more efficiently to the ischemic hemisphere. Moreover, under ischemic conditions SUR1, the regulatory

subunit of the K_{ATP-} and the NC_{Ca-ATP}-channels, is expressed in neurons, astrocytes, oligodendrocytes, endothelial cells and by reactive microglia [8].

Medical uses

It is used in the treatment of type 2 diabetes. As of 2011, it is one of only two oral antidiabetics in the World Health Organization Model List of Essential Medicines (the other being metformin). As of 2003, in the United States, it was the most popular sulfonylurea [9].

Additionally, recent research shows that glibenclamide improves outcome in animal stroke models by preventing brain swelling and enhancing neuroprotection. A retrospective study showed that in type 2 diabetic patients already taking glyburide, NIH stroke scale scores were improved on discharge compared to diabetic patients not taking glyburide. [10].

Side effects and Contraindications

This drug is a major cause of drug induced hypoglycemia. Cholestatic jaundice is noted. Glibenclamide may be contraindicated in for those with G6PD deficiency, as it may cause acute haemolysis. Recently published data suggest glibenclamide is associated with significantly higher annual mortality when combined with metformin than other insulin-secreting medications, after correcting for other potentially confounding patient characteristics. The safety of this combination has been questioned. Glibenclamide causes cholestasis as the major side effect [11].

Synthesis

The N-acetyl derivative of β-phenethylamine is reacted with chlorosulfonic acid to form the para sulfonyl chloride derivative. This is then subjected to ammonolysis, followed by base-catalyzed removal of the acetamide. This is then acylated with 2-methoxy-5-chlorobenzoic acid chloride to give the amide intermediate. This is then reacted with cyclohexyl isocyanate to yield the sulfonylurea glibenclamide [12].

METHODOLOGY

Materials

- Five (5) Glibenclamide were used for the study
- Pure sample of the drugs were obtained from NAFDAC which serve as standard
- Writing and labeling materials

Measuring cylinder, Beakers, 1000ml volumetric flask, 100ml volumetric flask, 50ml volumetric flask, Sonicator, Filter paper, Spatula, High performance liquid chromatography set up, UV Visible spectrophotometer (Beckman), Analytical weighing balance, Pestle and mortar, Distilled water

All reagents used were obtained from NAFDAC office, Maiduguri.

Practical Method

The methods employed for the purpose of this study are the UV visible spectrophotometer and high performance liquid chromatographic methods. [13].

Practical Procedure

UV Procedure for Glibenclamide

Preparation of Reagent

The reagent is prepared using dilution formular $C_1V_1 = C_2V_2$

C_1 the molar concentration of HCl = 11.5M
V_1 is the volume of the concentration of HCl measured so as to dilute with the methanol
C_2 is the new concentration to the prepared i.e. 0.01N
V_2 is the volume required for the analysis, it can be 250, 500, 1000 e.t.c
$C_1 = 11.5$ $V_1 = ?$
$C_2 = 0.01$ $V_2 = 1000$
$C_1V_1/ C_1 = C_2V_2/ C_1$
$V_1 = 0.01 \times 1000/11.5 = 0.86 = 0.9ml$

Therefore 0.9ml of the conc HCl is measured and transferred into a 1000ml volumetric flask and make up with Methanol up to the mark and stirred for 3minutes using ultrasound machine.

Method

Equivalent of 5mg of each of the sample were weighed accurately using the electronic weighing balance and then transferred into 1000ml volumetric flask carefully. The remaining particles of the sample were then washed into the 100ml volumetric flask using the reagent prepared above. The sample is then shaken mechanically so as to mix tissue sample. Finally using the same solvent it was made up to 100ml.

The UV spectrophotometer is then calibrated and blanked using the same reagent and limiting the absorbance, each of the samples were taken using at a wavelength of 300nm. The results are then recorded.

HPLC Procedure for Glibenclamide [14].

Assay

Weigh and powder 10 tablets and carry out the method for liquid chromatography using the following solution

- Mix with the aid of ultrasound, a quantity of the powder tablets containing 5mg of Glibenclamide with a mixture of 2ml of water and 20ml of methanol until fully dispersed and filter through a 0.2µm membrane filter
- Dissolve 50mg of glibenclamide BPC in 10ml of methanol with the aid of ultrasound for 20mins, add sufficient methanol to produce 50ml and dilute 1 volume of this solution to 4 volumes with methanol. To 20ml of this solution add 2ml of water and mix.

Chromatographic conditions

a. Stainless steel column (10cm x 4.6mm) packed with Octadecylsilyl silica gel for chromatography (5µm).
b. Isocratic system using the mobile phase below
c. Flow rate of 1.5ml per minute
d. Ambient column temperature
e. Detection wavelength of 300nm
f. Injection volume of 20µm for each solution

Mobile phase

A mixture of 47 volumes of acetonitrile and 53volumes of a 1.3% w/v solution of potassium dihydrogen orthophosphate previously adjusted to Ph 3.0 with orhtophosphoric acid. Calculate the content of Glibenclamide

RESULTS

Table 1

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Brand Name	Brand Code
Glanil	Q
Daonil	R
Gliben J	S
Diatab	T
Clamide	U

The tables below show the result of UV spectrophotometer which is used to calculate the percentage and milligram content of the following drugs.

The results are as follows:

UV For Glibenclamide

Q

C = A/ab
C = 31.023/63 x 1
C = 0.49g/100ml
C = 4.9mg/ml
% content = actual/expected x 100
= 4.9/5 x100 = 98%
Mg content = % content/100 x manufacturers claim
= 98/100 x 5 = 4.9mg

R

C = A/ab
C = 31.949/63 x 1
C = 0.51g/100ml
C = 5.1mg/ml
% content = actual/expected x 100
= 5.1/5 x100 = 102%
Mg content = % content/100 x manufacturers claim
= 102/100 x 5 = 5.1mg

S

C = A/ab
C = 29.442/63 x 1
C = 0.47g/100ml
C = 4.7mg/ml
% content = actual/expected x 100
= 4.7/5 x100 = 94%
Mg content = % content/100 x manufacturers claim
= 94/100 x 5 = 4.7mg

T

C = A/ab
C = 30.088/63 x 1
C = 0.48g/100ml
C = 4.8mg/ml
% content = actual/expected x 100
= 4.8/5 x100 = 96%

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Mg content = % content/100 x manufacturers claim
= 96/100 x 5 = 4.8mg

U

C = A/ab
C = 30.312/63 x 1
C = 0.48g/100ml
C = 4.8mg/ml
% content = actual/expected x 100
= 4.8/5 x100 = 96%
Mg content = % content/100 x manufacturers claim
= 96/100 x 5 = 4.8mg

Table 2: Percentage Content and Mg Content of Glibenclamide (5mg) USING UV

Sample	%content	mg content
Q	98	4.9
R	102	5.1
S	94	4.7
T	96	4.8
U	96	4.8

HPLC for Glibenclamide

Analyst: manager

Sample ID: T 130613 **Vial: 200** **Injection Volume: 20**

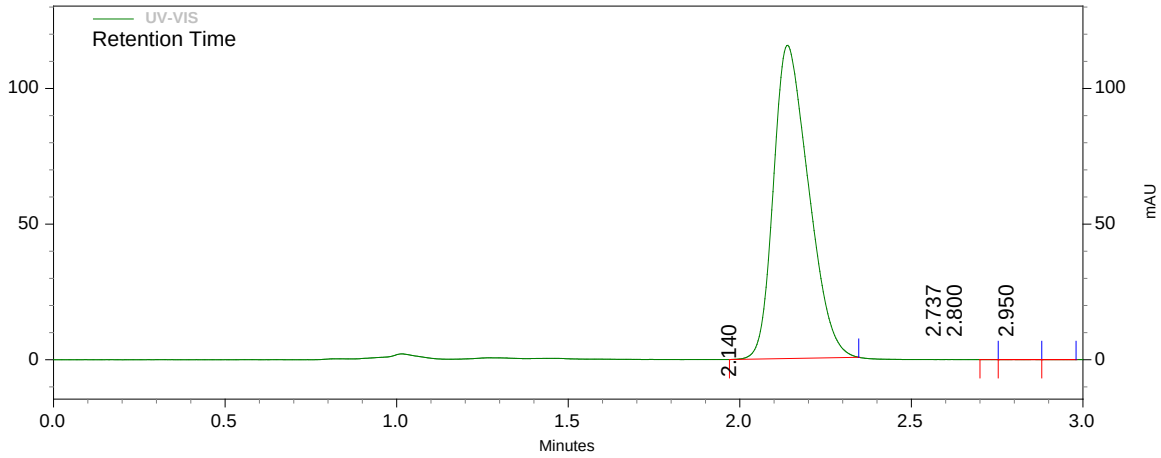

TABLE 3

UV-VIS Results				
Name	**Retention Time**	**Area**	**Area Percent**	**Integration Codes**
	2.140	3279697	99.977	MM
	2.737	124	0.004	BB
	2.800	426	0.013	BV
	2.950	219	0.007	VE
Totals				
		3280466	100.000	

% content = 3279697 x 100 = 139.2%
2356599

Mg content = 139.2 x 400 = 6.96mg

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100

Analyst: manager

Sample ID: U 130613 **Vial: 199** **Injection Volume: 20**

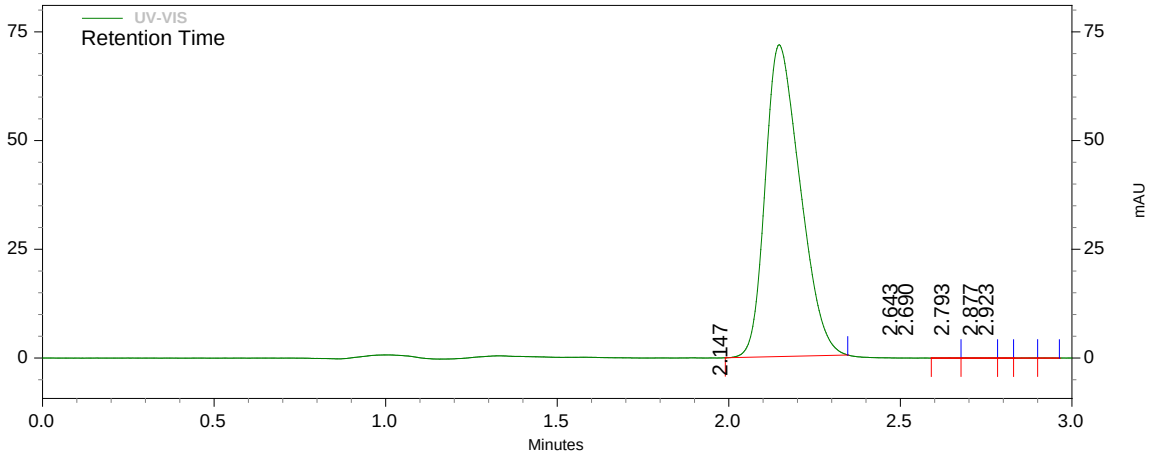

TABLE 4

UV-VIS Results				
Name	**Retention Time**	**Area**	**Area Percent**	**Integration Codes**
	2.147	2028100	99.952	MM
	2.643	279	0.014	BV
	2.690	371	0.018	VV
	2.793	73	0.004	VB
	2.877	142	0.007	BV
	2.923	118	0.006	VE
Totals				
		2029083	100.000	

% content = 2028110 x 100 = 86.1%
2356599

Mg content = 86.1 x 5 = 4.3mg
100

Analyst: manager

Sample ID: S 130613 **Vial: 198** **Injection Volume: 20**

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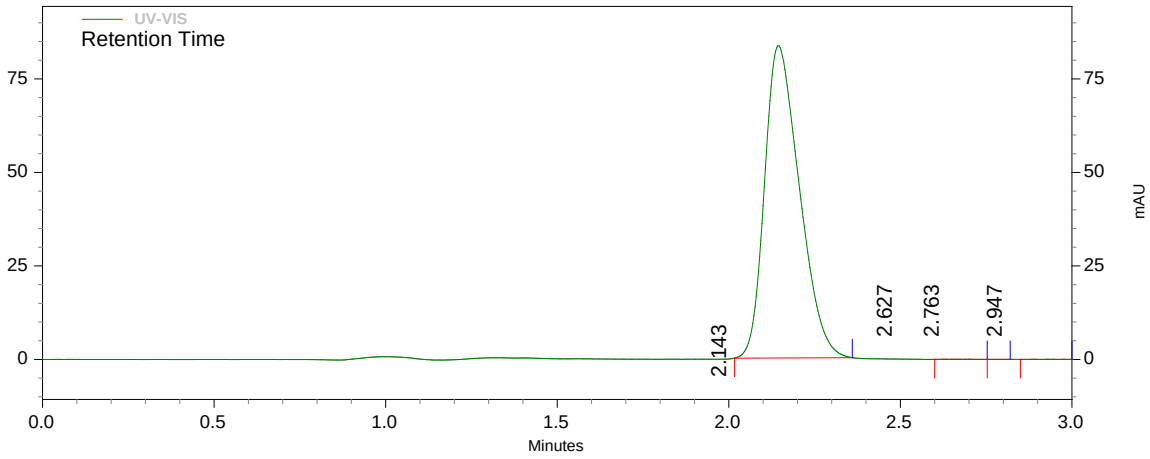

TABLE 5

UV-VIS Results				
Name	**Retention Time**	**Area**	**Area Percent**	**Integration Codes**
	2.143	2356599	99.967	MM
	2.627	361	0.015	BV
	2.763	92	0.004	VB
	2.947	328	0.014	BE
Totals				
		2357380	100.000	

% content = $\frac{2356599}{2356599} \times 100 = 100\%$

Mg content = $\frac{100}{100} \times 5 = 5mg$

DISCUSSION

According to British Pharmacopoeia volume III, 2008 [15].a glibenclamide contain not less than 95% and not more than 105% of glibenclamide tablet. E 1% 1cm at 63 molar absorptivity was used in calculating the standard at a wavelength of 300nm percentage content of Q 98%, R 102%, S 94%, T 96%, and U 96%. For Glibenclamide, all the samples passed within the range except for S which is slightly lower and may be due to experimental error.

In the HPLC, analysis carried out on the same samples using sample using the same limit set by the B.P and USP.

For Analysis of glibenclamide using HPLC S was used as the secondary standard. % content of T is 139.2%, U 81.6%,, both samples failed the test because T was above while U was below the specified limit.

CONCLUSION

For the analysis of glibenclamide using UV, the entire sample passed according to specified limit laid down by the British Pharmacopoeia except one, for analysis of Glibenclamide using HPLC all the samples passed except two that failed the test.

RECOMMENDATION

When a drug taken does not contain the specified amount of active principle, and due to the great association between dose and response, the response may not be obtained which may require increase or decrease in dose.

It should be recommended that each batch of the tablet or capsules produced by every company undergoes quantitative assay, to ensure that they contain the right amount of the active principle as specified by the official books.

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