



Physicochemical Equivalence and Validation of an HPLC Analytical Method for the Quantification of Glibenclamide and Its Sulfonamide Impurity in Prescribed Glibenclamide Tablets in Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors JOT and SOO designed the study, managed the data collection and analyses, performed the statistical analysis, wrote the protocol, managed the literature searches, and wrote the first and final draft of the manuscript. Author BKA participated in data collection and analyses. All authors read and approved the final manuscript.

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ABSTRACT

Objective: To investigate the physicochemical equivalence of four brands of commercially available glibenclamide tablets in Nigeria and to develop a validation method using HPLC for the quantitative determination of glibenclamide and its sulfonamide impurity present in these tablets.

Methods: Uniformity of weight, friability tests, hardness/crushing strength, dissolution, and disintegration tests were carried out on tablets/drug samples of each brand. Their functional groups were determined and compared with pure glibenclamide sample (reference standard) using Fourier

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Transform Infrared Spectroscopy (FTIR) between a range of 4000cm^{-1} to 400cm^{-1} . High-Performance Liquid Chromatography (HPLC) was used to determine the percentage of glibenclamide and its sulfonamide impurity present in each tablet brand.

Results: From the physicochemical evaluation of the four brands of glibenclamide tablets tested, the brands passed all the British Pharmacopeia specifications, but they all failed the hardness/crushing strength tests and one of the brands failed the assay test requirement for drug content. The developed HPLC method had a percentage recovery between the acceptable limit of 95-105% with percentage relative standard deviation (%RSD) of $< 3\%$ while the precision of the method was 0.102% and 0.383% for glibenclamide and its sulfonamide impurity, respectively. The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed analytical method for the four brands were $0.075\mu\text{g/ml}$ and $0.227\mu\text{g/ml}$ for glibenclamide while that of sulfonamide impurity were $0.114\mu\text{g/ml}$ and $0.345\mu\text{g/ml}$, respectively. In addition, the percentage impurity of sulfonamide in all the brands was less than the acceptable limit of 1%.

Conclusion: The results from the physicochemical evaluation of the glibenclamide brands justified the need for constant monitoring of marketed drug products. The results obtained from the HPLC quantification method developed for this study show that our data is reproducible based on the linearity, precision, and accuracy of data generated for glibenclamide and its sulfonamide impurity in the four brands of glibenclamide tablets prescribed to DM patients in Nigeria, which were judged to be satisfactory at the time of this study.

Keywords: Glibenclamide; Diabetes mellitus; HPLC; FTIR; Sulfonamide impurity.

1. INTRODUCTION

Diabetes Mellitus (DM) is a chronic non-infectious and metabolic disorder that comes with high blood glucose levels and impaired carbohydrates, proteins and lipid metabolism caused by either the inability of the human body to produce sufficient insulin or improper utilization of the insulin produced. Other causes of DM include excessive growth hormones, exocrine pancreatic defects, and chronic inflammation, among others [1]. DM has become a disease of great concern due to a global increase in its prevalence. In 2017, about 425 million people were diagnosed with diabetes globally and expected to rise by 48% to about 629 million people in 2045. In Africa, about 16 million people were diagnosed with disease in 2017 and expected to rise by 156% to about 41 million people in 2045 [2]. Symptoms associated with DM include increased thirst, blurry vision, weight loss, and polyuria. DM, when not well managed and treated on time can cause long-term debilitating conditions, such as retinopathy, autonomic dysfunctions, neuropathy, nephropathy, among others. People diagnosed with DM have a high risk of developing cardiovascular, cerebrovascular and peripheral diseases [3]. DM can be classified into type 1, type 2, gestational diabetes, and other specific types, which include latent autoimmune diabetes in adults (LADA), maturity-onset diabetes of the young (MODY), and secondary DM [4].

Type 2 DM (also known as non-insulin dependent or adult-onset diabetes) is the most common type of diabetes and accounts for about 90-95% of diabetic cases [5]. It results mainly from a combination of genetic (insulin resistance and impaired secretion of insulin) [6,7] and lifestyle factors [8,9] (such as obesity [10], sedentary lifestyle [11], lack of exercise [12], smoking [13], and alcohol consumption [14]). Other factors that could cause type 2 DM include stress and aging [15]. The early stage of type 2 DM is characterized by reduced insulin sensitivity, which can be reversed by using various medications and measures to enhance insulin sensitivity and decrease the production of glucose in the liver [16]. DM can be managed and treated with the aid of pharmacological agents, which can be administered through various routes of administration. Oral hypoglycemic/anti-hyperglycemic agents are commonly used for the treatment and management of type 2 DM [16]. These agents can lower the blood glucose levels by increasing the amount of insulin secreted in the pancreas, increasing the sensitivity of target organs to insulin, or decreasing the absorption rate of glucose from the gastrointestinal tract [17]. Classes of oral hypoglycemic/anti-hyperglycemic agents include sulfonylureas, metformin, thiazolidinediones, and alpha-glucosidase inhibitors [17].

Glibenclamide, 5-Chloro-N-(2-{4-[(cyclohexylcarbamoyl) sulfamoyl] phenyl} ethyl)

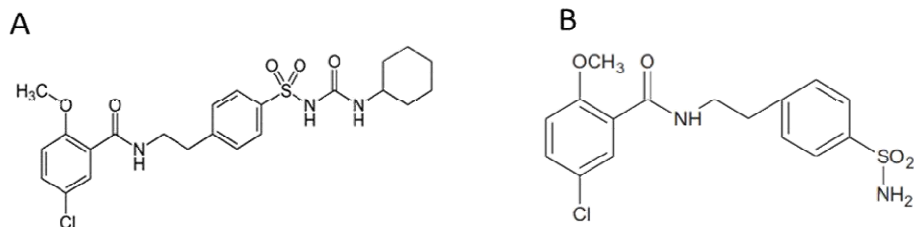


Fig. 1A. Chemical structure of Glibenclamide; Fig. 1B. Chemical structure of Sulfonamide

2-methoxybenzamide, belongs to the class of sulfonamides and it is commonly used in the management of type 2 DM [18]. Glibenclamide works by stimulating insulin secretion and increasing the response of β -cells to glucose and non-glucose secretagogues. This elevates the amount of insulin secreted by the pancreas. Glibenclamide also lowers blood glucose level concentrations by reducing serum glucagon levels [19,20]. For diseases, such as diabetes to be effectively treated and managed, drugs must have the mandatory quantity and quality of active pharmaceutical ingredients that conforms to the official requirements laid down by monographs are required [21]. In addition, the International Conference on Harmonization (ICH) mandates that all existent and probable impurities in drug substances and products should be identified, qualified and quantified by drug manufacturers in order to establish the biological safety of the impurities and their threshold limits [22]. The records from the British Pharmacopoeia and European Pharmacopoeia suggest that several impurities, such as sulfonamides may be formed during the synthesis of glibenclamide.

The present study was carried out to evaluate the physicochemical equivalence of four different brands of glibenclamide tablets prescribed to DM patients in Nigeria and also to develop and

validate an HPLC analytical method for the quantification of glibenclamide component and its sulfonamide impurity in these tablets.

2. MATERIALS AND METHODS

2.1 Sampling and Chemicals

Pure glibenclamide and sulfonamide powders were given as a gift from Swipha pharmaceutical limited, Lagos Nigeria. Four different brands of glibenclamide tablets were identified and purchased from various pharmacy stores across Southwest Nigeria. All tablets were within their shelf lives at the time of the investigation. All other reagents used were of analytical grade while the water used was distilled. HPLC grade of acetonitrile (Fischer scientific, U.K.) and analytical grade of sodium dihydrogen phosphate dehydrate (Merck, Darmstadt, Germany) were used in during HPLC analysis.

All brands of uncoated glibenclamide tablets (5mg) were separately assayed according to British Pharmacopoeia standards. Table 1 shows the brands of tablets studied with the name of manufacturers, batch numbers, NAFDAC (National Agency for Food and Drug Administration and Control) numbers, and manufacture and expiry dates.

Table 1. Brands of Glibenclamide (5 mg) tablets used for the study

Code	Tablets' brand name	Manufacturer	Batch number	NAFDAC number	Manufacture date	Expiry date
A	Daonil	Swiss Pharma, Nigeria	T202	04-0744	04/2015	04/2018
B	Clamide	Hovid Bhd, Malaysia	BGO 1744	04-4015	01/2016	01/2019
C	Diatab	May & Baker, Nigeria	A150149	04-7837	02/2015	01/2018
D	Glanil	Nigerian-German Chemical, Nigeria	E0701	04-2450	05/2015	05/2018

2.2 Uniformity of Weight Test

The uniformity of weight test for each brand of tablets was derived by selecting twenty tablets from each brand randomly. Selected tablets were weighed individually using an electronic weighing balance (Mettler Toledo, Switzerland). The uniform weight for each brand was determined by calculating the average weight of selected tablets in mean \pm SD.

2.3 Tablet Friability Test

Twenty tablets were weighed before placing them in a friabilator (Erweka GmbH, Germany), which was rotated for 4 mins at 25 rpm. After 4 mins, the tablets were removed from the tumbling chamber of the friabilator and were de-dusted and reweighed. The following formula was used to calculate the percentage weight loss of the tablets;

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

2.4 Tablet Hardness Test (Crushing Strength)

The crushing strength of each of the tablets from each brand was determined using a Monsanto hardness tester. Each tablet was placed in contact with the lower plunger of the hardness tester and zero readings were taken. The upper plunger of the hardness tester was used to crush the tablets and the force applied was documented as the crushing strength of the tablets and the mean \pm SD of the recorded values was evaluated.

2.5 Tablet Disintegration Time Test

The differences in disintegration time for six (6) tablets per brand were determined using a disintegration tester (Eagle Scientific Limited, Nottingham, UK) at (37.0 \pm 0.5) $^{\circ}$ C with distilled water. The disintegration tester which is an apparatus that consists of an assembly of tubes were covered at the lower end with a No. 10 mesh of 2mm diameter leaving the upper end opened. One Tablet was placed in each tube and the apparatus was immersed inside a beaker containing distilled water. The beaker was placed in a water bath that had a constant temperature of (37.0 \pm 0.5) $^{\circ}$ C and the tubes oscillated at a constant rate. The upward stroke made about 2.5ml of the tube to be immersed in the medium

and the downward stroke allowed the tube to be immersed deeply in the medium leaving about 2.5ml of the tube exposed. The disintegration time of the tablets was evaluated by recording the time taken for the tablets to disintegrate into granules and pass through the mesh. Three sets of readings were taken per brand and the average disintegration time calculated in mean \pm SD.

2.6 Tablet Dissolution Test

A dissolution apparatus (Erweka, GmbH, Germany) was used to evaluate the dissolution rates of the different brands of glibenclamide tablets according to pharmacopeia specifications using the paddle method. A dissolution medium of 900ml, 200mM phosphate buffer with a pH of 6.8 was prepared and kept at a temperature of 37.0 \pm 0.5 $^{\circ}$ C. Tablets of each brand of glibenclamide were placed in the vessel of the dissolution apparatus with a paddle rotation of 50 rpm for 30 mins. The samples were filtered and 5ml of the filtrate was withdrawn at intervals, subsequently replaced with an equivalent volume of the dissolution medium maintained at 37.0 \pm 0.5 $^{\circ}$ C. The samples that were withdrawn were diluted with an equivalent volume of phosphate buffer and their absorbance measured at a λ_{max} of 276nm using a UV spectrometer (Shimadzu, Japan). The concentration and the percentage of glibenclamide released was evaluated using the formula below;

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

Where Cs is the calculated concentration of glibenclamide in the sample in mg/ml.

2.7 High-Performance Liquid Chromatography Method

HPLC System: A High-Performance Liquid Chromatography system with a UV-Visible detector was used for this analysis and the data recorded using Empower 2 software.

Column: A C-18 stainless steel column was used to analyze the samples.

Mobile Phase: The mobile phase for the HPLC experiment was prepared according to BP 2009. A mixture of acetonitrile (ACN) and potassium dihydrogen orthophosphate (KH₂PO₄) in a ratio of 43:57 was used respectively with pH adjusted to 3 using orthophosphoric acid.

Chromatographic Conditions: All the analyses were performed at 30°C with a flow rate of 0.7 mL/min, a detection wavelength of 250nm and a C18 of 25cm length, 4.5nm diameter and 5µm particle size. Standards and test samples were filtered before analyzing using a 0.45µm filter and the injector volume used for both standards and test samples was 20µl.

2.7.1 Preparation of reference standard solutions

To prepare the different concentrations of reference standard solution for glibenclamide, 100mg of pure glibenclamide standard was weighed and transferred to a 100ml volumetric flask and dissolved with 50ml of methanol which was made up to the 100ml volume mark using the same solvent. A final concentration of 1mg/ml (1000µg/ml) stock solution was obtained. From the stock solution, serial dilutions of 50, 75, 100, 125, 150 and 175µg/ml were made for calibration concentrations used for the linearity study.

To prepare the different concentrations of reference standard solution for sulfonamide, 50mg of sulfonamide was dissolved in 50ml of methanol in a 100ml volumetric flask and was mixed well after making the volume to the 100ml mark using methanol. A final concentration of 0.5mg/ml (500µg/ml) stock solution was obtained. From the stock solution, serial dilutions of 25, 50, 75, 100 and 125µg/ml were made for calibration concentrations used for the linearity study.

2.7.2 Analysis of Glibenclamide content in each brand

To determine the content of glibenclamide in each brand, twenty tablets were randomly selected from each brand, they were weighed and pulverized. The weight of powder equivalent to the amount of 200mg of glibenclamide was transferred into a 100ml volumetric flask and dissolved in 50ml of methanol. The volume was made up to the 100ml mark of the volumetric flask using the same solvent. The mixture was sonicated until it was well dispersed and then filtered using a 0.45µm membrane filter. From the above stock solution, further dilutions were made to get a final concentration of 0.02mg/ml (20µg/ml). Each sample (per brand) was analyzed using HPLC using a 20µl volume injected six times using three separate preparations. In addition, six injections of a

standard solution of glibenclamide were likewise injected. The area of the glibenclamide peaks obtained for each sample was quantified with the area of the standard glibenclamide peaks in order to determine the percentage of glibenclamide present in each brand. Empower 2.0 software was used in integrating and analyzing the HPLC peak responses for quantitation of the peaks by area percent.

2.7.3 Analysis of Sulfonamide content in each brand

The sulfonamide content in each brand was determined using the method previously described. Twenty (20) tablets were randomly selected from each brand, they were weighed and pulverized. The weight of powder equivalent to the average weight of a powdered tablet of reference glibenclamide (5mg) was transferred into a 100ml volumetric flask and dissolved in 50ml of methanol. The volume was made up to the 100ml mark of the volumetric flask with methanol. The mixture was sonicated until it was well dispersed and then filtered using a 0.45µm membrane filter. A final concentration of 0.04mg/ml (40µg/ml) obtained from the above stock solution was analyzed using the developed HPLC method.

2.8 Functional Group Identification

Hypoglycemic sulfonylureas, such as glibenclamide have an aryl-sulfonyl-urea sequence in common that is responsible for their hypoglycemic properties. Their R and R¹ radicals regulate their pharmacological and pharmacokinetic profiles. They also possess a –SO₂-NH-CO-NH- moiety which is hydrophilic in nature. In addition, their aryl and R portions are lipophilic in nature and are responsible for the differences in their potencies i.e. sulfonylurea receptor binding properties, metabolism, and routes of elimination [23].

In this study, Fourier Infrared Spectroscopy (FTIR) was used to compare and ascertain whether chemical functional groups in the aryl-sulfonyl-urea sequence present in each brand of glibenclamide tablets are the same with the functional groups present in a pure sample of glibenclamide (reference standard). The samples were scanned using a Shimadzu FTIR spectrometer with wavelengths ranging from 4000 – 400cm⁻¹ and a resolution of 4cm⁻¹.

2.9 Validation Method for Glibenclamide and Its Sulfonamide Impurity

The developed HPLC method for the quantification of glibenclamide and its sulfonamide impurity was validated in terms of linearity, precision, accuracy, and sensitivity (LOD and LOQ) according to the ICH tripartite guidelines [24].

3. RESULTS AND DISCUSSION

The physicochemical parameters of all the drug samples evaluated are presented in Table 2.

3.1 Uniformity of Weight of Tablets

Uniformity of weight test is an important quality control parameter for solid dosage forms that corresponds to compendial requirements as it determines the uniformity of dosage units and ensures that all tablets contain an almost equal amount of drug substance intended. The uniformity of dosage units can be estimated through the evaluation of variations in the weight or drug content of tablets within a batch. In other words, it directly or indirectly estimates the variations in the composition or amount of active pharmaceutical ingredients and excipients present in a tablet. The variations in weight of active ingredients can affect the *in vivo* and *in vitro* performance of a drug and cause adverse side effects while variations in excipients can affect drug delivery, patient compliance, bioavailability and stability of the drug. The uniformity of weight for all the brands A, B, C, and D complied with the BP (2007) standard [25], as none of them deviated from the mean of more than 5% (Table 2).

3.2 Hardness or Crushing Strength of Tablets

Tablet hardness testing (in Kilopond (Kp)) is used to test the breaking point and structural integrity of a tablet. The hardness or crushing strength of a tablet can affect its rate of

disintegration. Tablets that are too hard may not disintegrate at the appropriate time and tablets that are too soft will not be able to withstand any further processes such as coating, packaging, and distribution. Tablets possessing high hardness or crushing strength could be as a result of the use of high concentration of binders and low concentration of disintegrants during their formulations. Other factors, such as the method of granulation employed, and high compressive force used during the compression of tablets are also important. The crushing strength requirement for a satisfactory tablet as recommended by BP is between 5-8Kp [26]. All the brands failed the hardness test. Diatab tablets had the highest crushing strength of all the four brands with a mean hardness or crushing strength of 15.4Kp (Table 2).

3.3 Friability of Tablets

Friability test for compressed uncoated tablets like glibenclamide tablets measures the tendency of compressed uncoated tablets to chip, break into smaller pieces or crumble when subjected to mechanical shock and attrition. It is another important quality control parameter that measures the loss in weight of compressed uncoated tablets which occurs as a result of the loss of fine particles from tablet surfaces [27]. This parameter plays a vital role in evaluating the ability of tablets to withstand hazards (such as mechanical, biological and chemical) that can be encountered during packaging, storage, and transportation [28]. The friability for all the brands was less than 1% of weight loss, which was within the BP (2007) specification limits (Table 2). Sample D had the highest percentage friability, which could be as a result of the amount and quality of binders used and hazards encountered during the packaging of the tablets.

3.4 Dissolution of Tablets

Dissolution test is a critical test for all oral solid dosage forms that measures the time taken for a certain amount of a drug substance to be released from a dosage form into a dissolution

Table 2. Physicochemical properties of Glibenclamide brands

Brand code	Mean weight \pm SD (mg)	Crushing strength (Kp)	Friability (%)	Disintegration time (mins)	Dissolution (%) at 30 mins
A	168.0 \pm 0.10	15.0	0.12	2.20	87.05 \pm 2.56
B	165.10 \pm 0.55	14.9	0.11	1.52	84.51 \pm 2.34
C	181.4 \pm 0.31	15.4	0.06	1.34	79.67 \pm 1.79
D	163.15 \pm 1.1	15.0	0.24	2.10	73.51 \pm 2.06

medium. The test provides *in vitro* drug release information of solid oral dosages and monitors its consistency in the drug's batches [29]. It can be used as a guide during the development of formulations, identification of critical manufacturing parameters, and evaluation of the bioavailability and bioequivalence of drugs [30]. The BP specifications require that more than 70% of the stated amount of active pharmaceutical ingredient should be released after 45 mins [31]. The dissolution test revealed that all the brands released more than 70% of the active pharmaceutical ingredient (glibenclamide) in 30 mins, which follows the dissolution order: D < C < B < A or A > B > C > D (Table 2).

3.5 Disintegration of Tablets

Disintegration involves the breakdown of tablets into smaller pieces or granules within a prescribed time in a liquid medium, such as gastric juice and intestinal fluid, which occurs before the dissolution of tablets in the body [32]. It is the rate determining step in drug absorption [33]. The disintegration test of tablets is an important test to evaluate the controlled and sustained release capacities of drug products. The test can be influenced by certain factors such as crushing strength, quality of disintegrants, and compactness. Also, the bioavailability of the active pharmaceutical ingredients present in the drugs may be influenced by the amount and the quality of excipients used for its formulation [34]. The

disintegration time for each sample met the BP 2009 requirements of ≤ 15 mins for uncoated tablets [26], which falls within 1.34 to 2.20 mins in this study.

3.6 Identification using IR Spectroscopy

Fourier Infrared Spectroscopy was used to identify the chemical functional groups present in the different glibenclamide tablet brands (sample A-D) and then compare to a reference standard. The IR spectra obtained from the pure glibenclamide and all the brands show peaks as a result of N-H stretch, N-H bend, O=S=O stretch, C=O stretch, C-H and =C-H bend signifying the presence of urea, sulfonamide, carbonyl, and aryl groups, respectively. This observation indicates that all the samples have aryl-sulfonyl-urea sequence of hypoglycemic sulfonylureas in them, which is common to glibenclamide. Table 3 below gives a summary of the peaks and their functional groups observed from the IR spectrum of pure glibenclamide (reference standard) and all the brands of glibenclamide tested. Figs. 2-6 show the image of the IR spectra obtained for them.

3.7 HPLC Validation Method

From the HPLC method developed, typical chromatographs for the standard and sample solutions of the different brands of glibenclamide tablets tested were obtained and shown in Figs. 7-12.

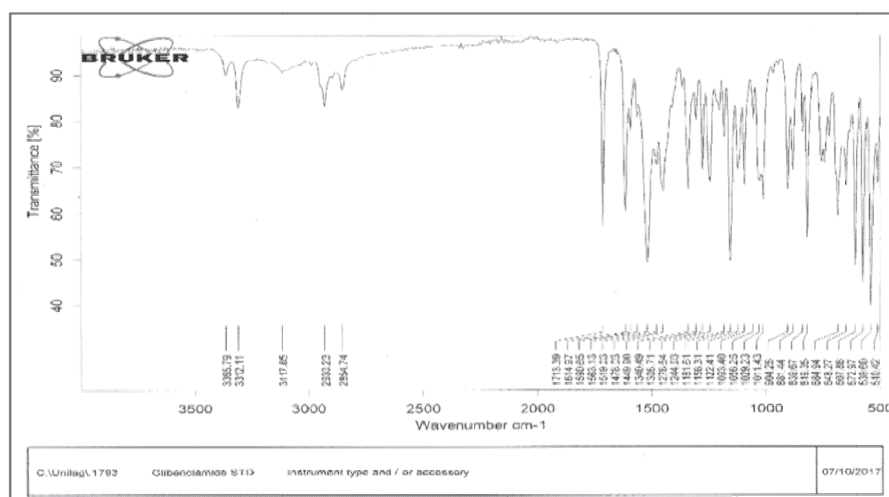
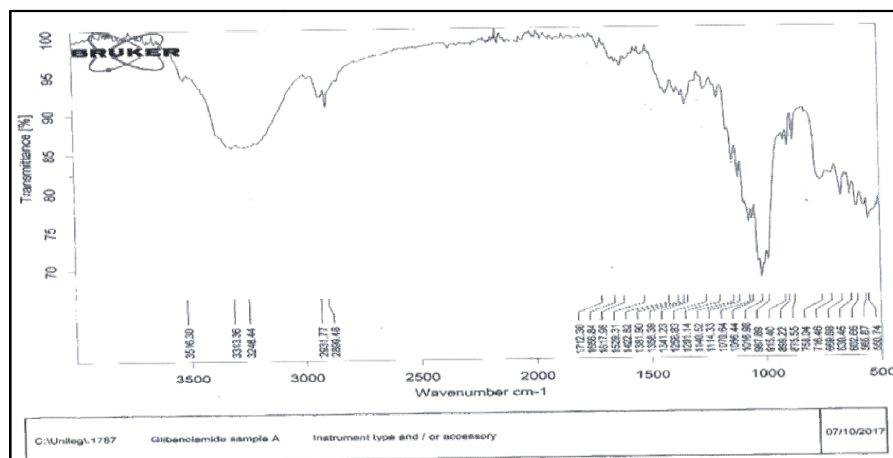


Fig. 2. FTIR spectra of pure Glibenclamide (reference standard)

Table 3. Summary of peaks and functional groups obtained from the IR spectrum obtained

Functional group	Standard	Brand A	Brand B	Brand C	Brand D
N-H stretch	3365.8	3516.3	3522.2,	3521.8	3521.8
	3312.9	3313.4	3315.7	3314.2	3315.2
	3117.9	3248.4			3266.2
N-H bend	1614.9	1656.8	1618.0	1617.3	1617.4
	1590.7	1617.9	1522.5	1520.9	
	1563.1	1529.3			
	1519.0				
C-H stretch	2930.3	2931.8	2932.5	2931.9	2931.3
	2854.7	2899.5	2899.9	2899.9	2899.4
C=O bend	1713.4	1712.3	1714.5	1714.5	1714.1
C-Cl bend	839.7	758.0	758.9	757.2	754.2
	819.4	716.5			
O=S=O stretch	1340.5	1341.2	1340.5	1340.4	1339.9
C-H bend	648.3	630.5	630.6	630.6	672.9
					630.1
Aromatic C-H in plane bend	1122.4	1140.5	1201.1	1141.5	1201.0
	1093.4	1114.3	1140.7	1115.0	1165.3
	1011.4	1070.7	1114.4	1071.3	1138.9
		1056.4	1070.7	1029.9	1115.1
		1016.9	1017.4		1091.4
		987.89			1057.9
Aromatic C-H out of plane bend	684.9	716.5	899.4	987.9	754.2

**Fig. 3. FTIR spectra of sample A revealing the functional groups present**

3.7.1 Linearity

Linearity involves the tendency of getting test results that are in direct proportion to the concentration of the analyte, which can be estimated by injecting a series of five to six injections of different concentrations of

glibenclamide (50-175 μ g/ml) and sulfonamide (25-125 μ g/ml) using a standard calibration curve. The calibration curves for both glibenclamide and sulfonamide show linearity (Figs. 13 and 14). For both, regression coefficients (R^2) of 0.9993 and 0.9991, as well as linear regression equations of

$Y=30338x-19975$ and $Y=35.268x+519$ were recorded, respectively. Table 4 gives a summary of the results obtained from the linearity and sensitivity analyses.

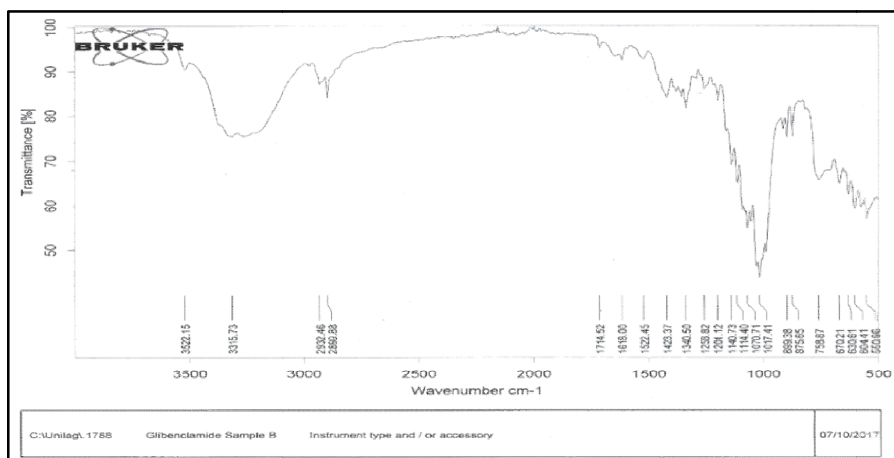


Fig. 4. FTIR spectra of sample B revealing the functional groups present

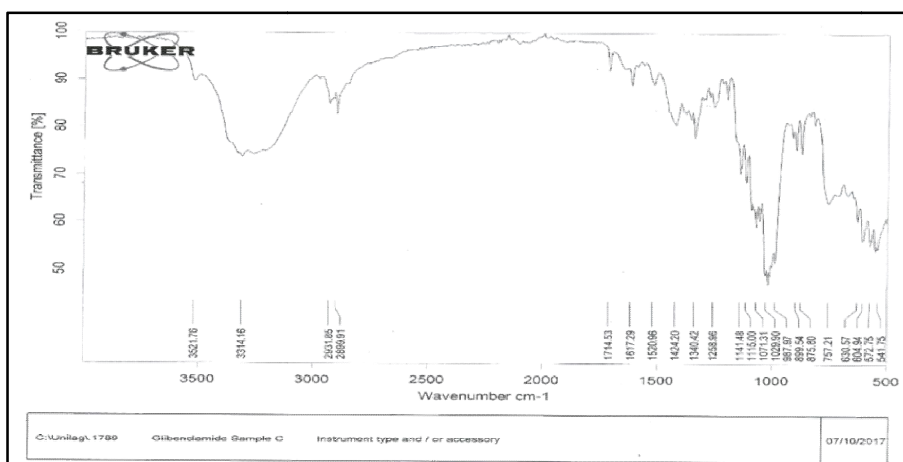


Fig. 5. FTIR spectra of sample C revealing the functional groups present

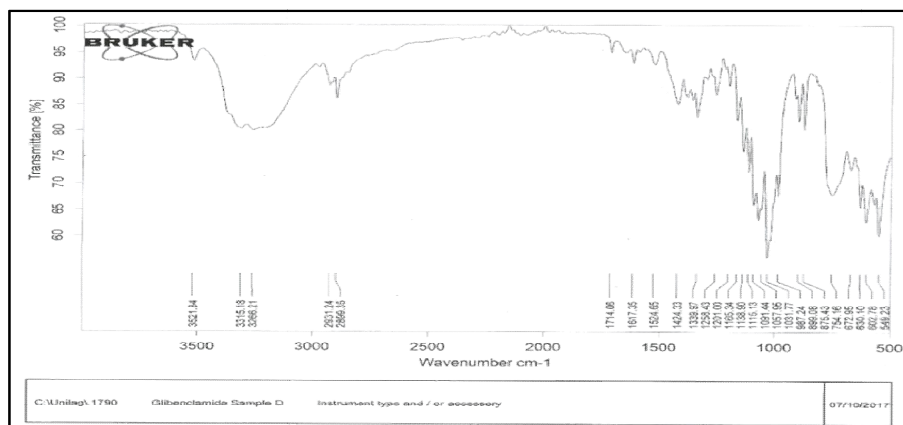


Fig. 6. FTIR spectra of sample D revealing the functional groups present

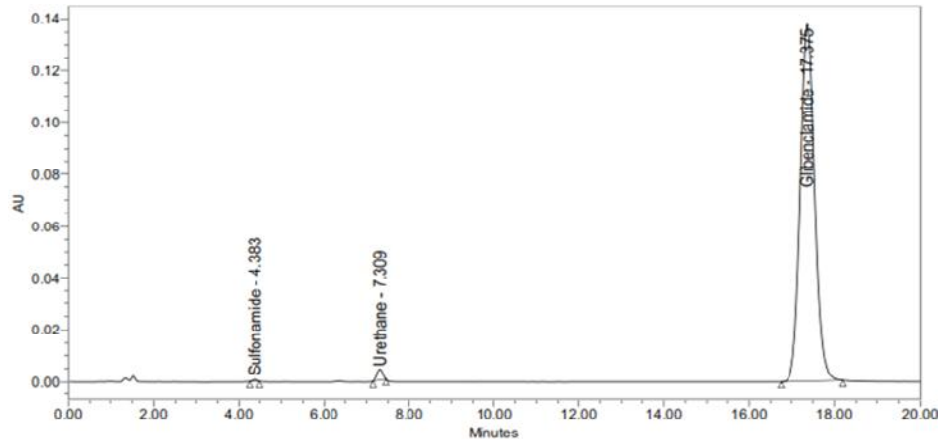


Fig. 7. HPLC chromatogram of Glibenclamide and related impurities

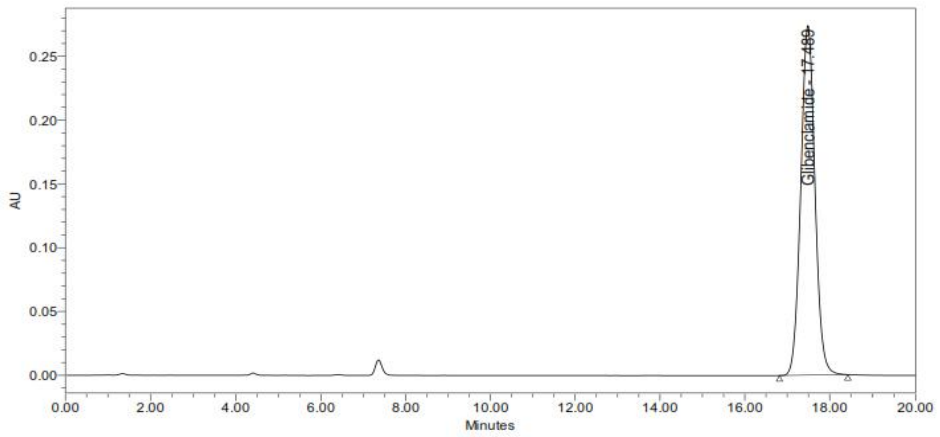


Fig. 8. HPLC chromatogram of Glibenclamide reference standard

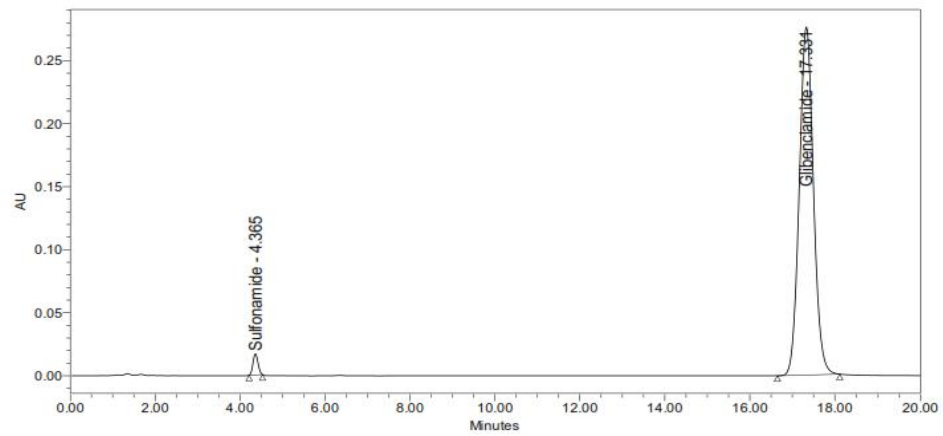


Fig. 9. HPLC chromatogram of sample A revealing the presence of Glibenclamide and its Sulfonamide impurity

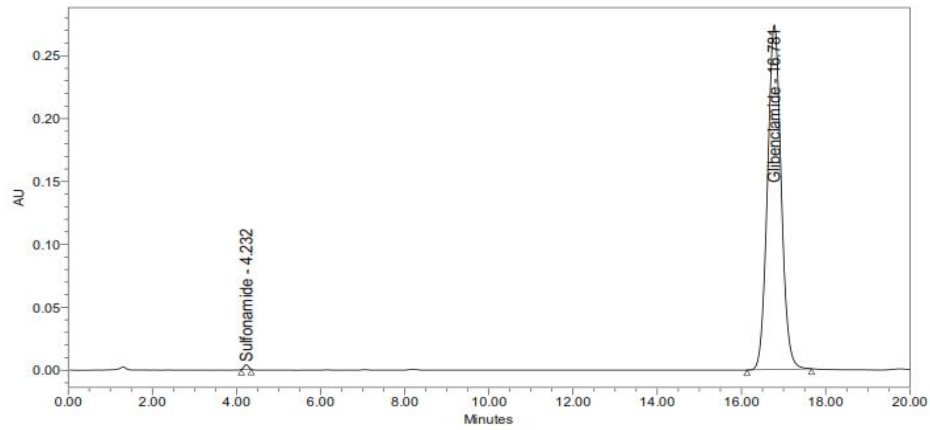


Fig. 10. HPLC chromatogram of sample B revealing the presence of Glibenclamide and its Sulfonamide impurity

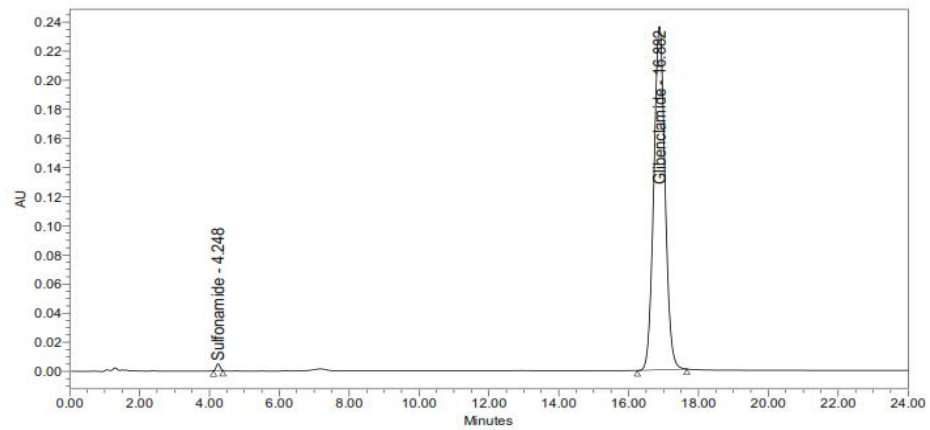


Fig. 11. HPLC chromatogram of sample C revealing the presence of Glibenclamide and its Sulfonamide impurity

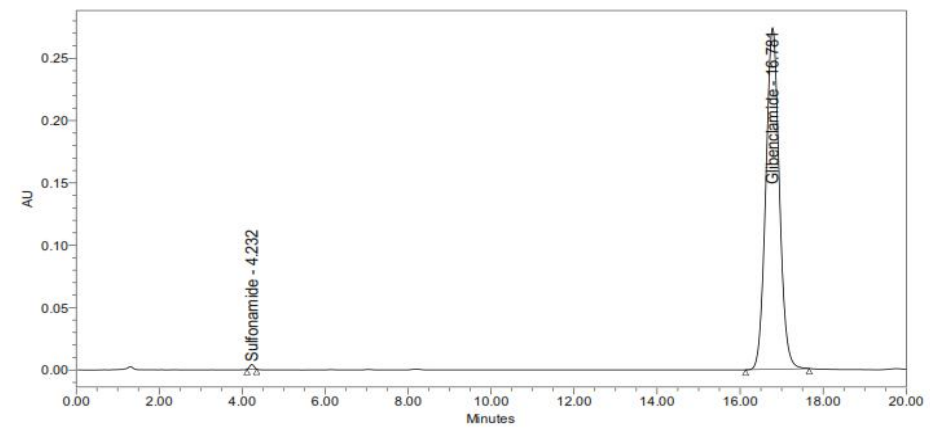


Fig. 12. HPLC chromatogram of sample D revealing the presence of Glibenclamide and its Sulfonamide impurity

3.7.2 Precision

The precision with respect to the degree of repeatability of the analytical method used was determined by calculating the %RSD of the peak areas of six individual injections (n=6) of pure

glibenclamide stock solution at a concentration of 100µg/ml and was discovered to be 0.102%. In addition, the %RSD of the peak areas of sulfonamide impurity was calculated using this method at a concentration of 75µg/ml and was found to be 0.383% (Table 5).

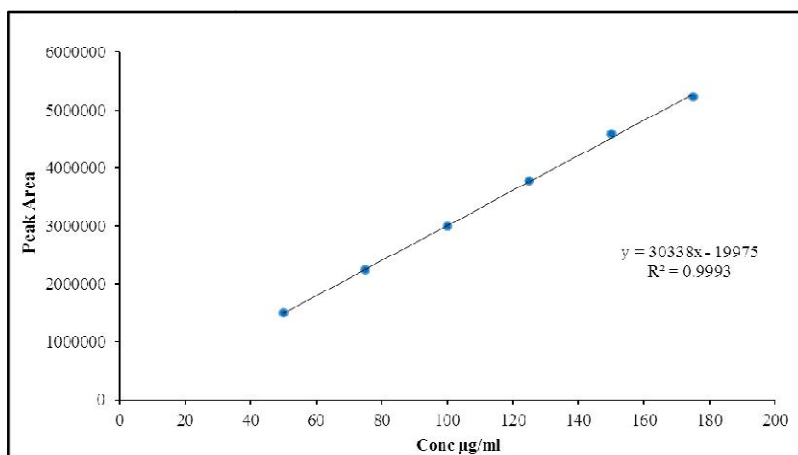


Fig. 13. Standard calibration curve for Glibenclamide

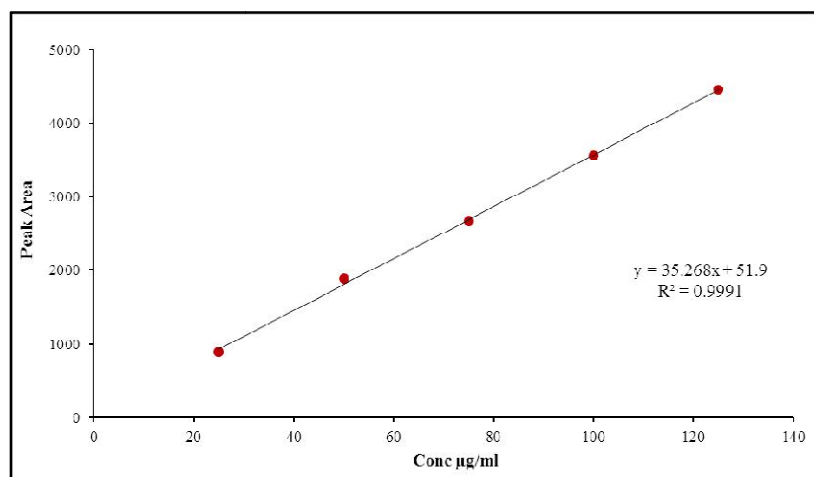


Fig. 14. Standard calibration curve for sulfonamide impurity

Table 4. Linearity and sensitivity data for Glibenclamide and its sulfonamide impurity

Parameters	Glibenclamide	Sulfonamide
Regression Equation	$Y=30338x-19975$	$Y=35.268x+51.9$
Regression Coefficient (R^2)	0.9993	0.9991
Slope	30338	35.268
Intercept	19975	51.9
LOD (µg/ml)	0.075	0.114
LOQ (µg/ml)	0.227	0.345

Table 5. Precision data for glibenclamide and its sulfonamide impurity

Determination of precision (repeatability)		
	Glibenclamide (100µg/ml)	Sulfonamide (75µg/ml)
n	Recovered (µg/ml)	Recovered (µg/ml)
1	98.88	74.40
2	98.77	74.77
3	98.76	74.77
4	98.69	74.18
5	98.64	74.80
6	98.61	74.91
Mean	98.88	74.40
SD	0.101	0.285
%RSD	0.102	0.383

Table 6. Percentage recovery studies for glibenclamide and its sulfonamide impurity analysis

Content of Glibenclamide and Sulfonamide in Daonil (Brand A)									
Glibenclamide					Sulfonamide				
Conc (µg/ml)	FC (µg/ml) Mean ±SD	% Recovery Mean	SEM	%RSD	Conc (µg/ml)	FC (µg/ml) Mean ±SD	% Recovery Mean	SEM	%RSD
50	48.72±0.020	97.44±0.042	0.024	0.040	25	24.02±0.028	96.08±0.113	0.065	0.118
100	98.10±0.015	98.10±0.017	0.133	0.228	50	51.95±0.113	103.89±0.227	0.131	0.219
150	150.72±0.01	100.48±0.010	0.005	0.009	75	74.40±0.115	99.21±0.153	0.088	0.154
Content of Glibenclamide and Sulfonamide in Clamide (Brand B)									
Glibenclamide					Sulfonamide				
Conc (µg/ml)	FC (µg/ml) Mean ±SD	% Recovery Mean	SEM	%RSD	Conc (µg/ml)	FC (µg/ml) Mean ±SD	% Recovery Mean	SEM	%RSD
50	49.44±0.003	98.87±0.007	0.004	0.010	25	24.61±0.085	98.46±0.340	0.196	0.346
100	101.52±0.001	101.52±0.003	0.001	0.001	50	50.64±0.150	101.29±0.300	0.173	0.296
150	150.99±0.024	100.66±0.020	0.009	0.016	75	74.80±0.128	99.74±0.170	0.098	0.179
Content of Glibenclamide and Sulfonamide in Diatab (Brand C)									
Glibenclamide					Sulfonamide				
Conc (µg/ml)	FC (µg/ml) Mean ±SD	% Recovery Mean	SEM	%RSD	Conc (µg/ml)	FC (µg/ml) Mean ±SD	% Recovery Mean	SEM	%RSD
50	49.52±0.010	99.04±0.020	0.011	0.019	25	24.02±0.085	96.08±0.340	0.196	0.354
100	101.89±0.03	101.89±0.027	0.015	0.026	50	51.69±0.087	103.39±0.173	0.100	0.168
150	151.59±0.141	101.06±0.094	0.054	0.093	75	76.22±0.102	101.63±0.136	0.078	0.134
Content of Glibenclamide and Sulfonamide in Glanil (Brand D)									
Glibenclamide					Sulfonamide				
Conc (µg/ml)	FC (µg/ml) Mean ±SD	% Recovery Mean	SEM	%RSD	Conc (µg/ml)	FC (µg/ml) Mean ±SD	% Recovery Mean	SEM	%RSD
50	49.67±0.090	99.34±0.181	0.104	0.180	25	25.04±0.071	100.16±0.285	0.165	0.285
100	101.89±0.063	101.89±0.063	0.036	0.070	50	49.14±0.113	98.28±0.227	0.131	0.231
150	151.23±0.212	100.82±0.141	0.081	0.144	75	74.77±0.199	99.70±0.266	0.153	0.287

Abbreviations: FC: Found/Recovered Concentration, SD: Standard Deviation, SEM: Standard Error Mean; RSD: Relative Standard Deviation.

Table 7. Analysis of the amount and percentage of drug content in Glibenclamide in the brands

Brand code	Label claimed (mg/tablet)	Amount detected (mg/tablet)	% Assay
A	5.0	5.36	107.2
B	5.0	5.12	102.4
C	5.0	4.85	97.0
D	5.0	4.88	97.6

Table 8. Analysis of the amount and percentage of drug content in Sulfonamide in the brands

Brand code	Amount of Sulfonamide per tablet (mg)	% of Sulfonamide
A	0.008	0.16
B	0.017	0.34
C	0.025	0.49
D	0.009	0.18

3.7.3 Sensitivity

The sensitivity of the method was determined using Limit of Detection (LOD) and Limit of Quantification (LOQ). LOD is the lowest quantity of an analyte in a sample that can be detected but not necessarily quantitated as an exact value while LOQ is the lowest quantity of analyte in a sample that can be quantitatively determined with appropriate precision and accuracy. The LOD and LOQ for glibenclamide and sulfonamide were determined using the formula $LOD=3.3(\bar{\delta}/s)$ and $LOQ = 10(\bar{\delta}/s)$ according to ICH guidelines [24]. Where $\bar{\delta}$ is the standard deviation of response (peak area) and (s) is the slope of the calibration curve. The LOD and LOQ were calculated from the linearity calibration curve and it was found to be 0.075 μ g/ml and 0.227 μ g/ml for glibenclamide, respectively, while 0.114 μ g/ml and 0.345 μ g/ml was obtained for sulfonamide impurity, respectively (Table 4).

3.7.4 Accuracy

The Accuracy (% Recovery) of the method refers to the nearness of agreement between an accepted reference value and the obtained value. This was determined by calculating the percentage recovery of the recovered analyte. This was done at three different concentrations of 50, 100 and 150 μ g/ml of the standard glibenclamide solution and 25, 50 and 75 μ g/ml of the standard solution of sulfonamide. The data obtained were statistically analyzed using the following formula;

$$\% \text{Recovery} = \left[\frac{\text{Found/Recovered concentration}}{\text{the injected concentration}} \right] * 100.$$

It was discovered that the % recovery of glibenclamide and sulfonamide for all the brands

were between 97.44-101.89% and 96.08-103.89%, respectively. The %RSD at all levels for both glibenclamide and its sulfonamide impurity was < 3%, which is within the acceptable limits (Table 6).

3.7.5 Analysis of Glibenclamide and its Sulfonamide impurity in each brand

The amount of glibenclamide present in each brand was found to be within the BP specification range of 95-105% for the active drug content [25]. The only exception was brand A (Daonil), which exceeded the specification by having 107% of the drug content (Table 7). This might be due to the interference with excipients used for its formulation. While test results for the amount of sulfonamide in each brand revealed that the brands had impurities between the ranges of 0.16-0.49% (Table 8). Sample C (Diatab) has the highest impurity content while sample A (Daonil) has the lowest impurity content. All brands have sulphonamide impurity within acceptable limits [22].

4. CONCLUSION

The physicochemical equivalence of the four brands of glibenclamide tablets was evaluated and all the brands were within the British Pharmacopeia specifications in terms of uniformity of weight, friability, dissolution and disintegration tests, but they all failed to meet the BP specifications for hardness/crushing strength. The FTIR spectra of all the brands, when compared with the reference standard, revealed that they have aryl-sulfonyl-urea sequence of hypoglycemic sulfonylureas. Tablets from one of the brands (Daonil) was found to have drug content above the limit set by the British Pharmacopeia (95-105%). All the brands

sampled have less than the 1% of sulfonamide impurities, which is within the acceptable limit for impurities. The physicochemical evaluation of the brands of glibenclamide tablets tested justifies the need for constant monitoring of the physicochemical equivalence of marketed drug products, which ensures their efficacy, quality, and safety.

Furthermore, the results obtained in this study showed that the developed analytical technique/method is simple, accurate, precise, sensitive and reproducible. This method can comparatively detect and quantify glibenclamide (active ingredient) and sulfonamide impurity in different brands of glibenclamide tablets. In addition, the developed analytical method can be modified and utilized economically as a quality control tool for the determination of active pharmaceutical ingredients of different drugs in final dosage forms and their related impurities, as well as for the identification and elimination of counterfeit or adulterated tablets.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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