Original Research Article

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

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 Sulfonamide impurity present in the tablets.

Methods: Uniformity of weight, friability tests, hardness/crushing strength and dissolution, disintegration time tests were carried out on drug samples of each brand and their functional groups were determined and compared with that of pure Glibenclamide sample (reference standard) using Fourier Transform Infrared Spectroscopy (FTIR) between a range of $4000cm^{-1}$ to $400cm^{-1}$. High-Performance Liquid Chromatography (HPLC) was used to determine the percentage Glibenclamide content and Sulfonamide impurity present in each 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 Sulfonamide impurity, respectively. The LOD and LOQ of the developed method for the four brands were 0.075µg/ml and 0.227µg/ml for Glibenclamide while that of Sulfonamide impurity was 0.114µg/ml and 0.345 µg/ml respectively. In addition, the percentage impurity of Sulfonamide in all the brands was less than the acceptable limit of 1%.

Conclusion: The physicochemical evaluation of the brands of Glibenclamide tablets indicated the need for constant monitoring of marketed drug products while the results obtained from the validation of the developed method revealed that the linearity, precision, and accuracy for the quantification of Glibenclamide and Sulfonamide impurity of the four brands of Glibenclamide tablets were satisfactory.

Keywords: Glibenclamide; Diabetes Mellitus; HPLC; FTIR; Sulfonamide Impurity

1.0 Introduction

Diabetes Mellitus is a chronic non-infectious disease 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 diabetes include, excessive growth hormones, exocrine pancreatic defects, infections, among others [1]. Diabetes mellitus, a metabolic disorder has become a disease of great concern as there has been a global increase in its prevalence. In 2011, 366 million people globally had diabetes and by 2030, it is expected that 552 million people would be diagnosed with the

ailment. 425 million people around the world were diagnosed with diabetes in 2017 and by 2045 it is estimated to increase by 48% to 629 million people. In the same year, 16 million people in Africa had diabetes and it is also expected to increase by 156% in 2045 affecting 41 million people [2]. Symptoms associated with diabetes mellitus include increased thirst, blurry vision, weight loss, and polyuria. Diabetes mellitus if not managed and treated on time can cause long term effects such as retinopathy, autonomic dysfunctions, neuropathy, nephropathy etc. People diagnosed with diabetes have a high risk of developing cardiovascular, cerebrovascular and peripheral diseases [3]. Diabetes Mellitus can be classified into Type 1 diabetes mellitus, Type 2 diabetes mellitus, Gestational diabetes and other specific types which includes Latent Autoimmune Diabetes in Adults (LADA), Maturity Onset Diabetes of the Young (MODY) and Secondary Diabetes Mellitus [4].

Type 2 diabetes mellitus which is also known as non-insulin dependent or adult onset diabetes is the most common type of diabetes that 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] (obesity [10], sedentary lifestyle [11], lack of exercise [12], smoking [13], alcohol consumption [14]). Other factors that have been found to cause type 2 diabetes include stress and aging [15]. The early stage of Type 2 diabetes mellitus is characterized by reduced insulin sensitivity which can be reversed by using various medications and measures to enhance insulin sensitivity or decrease the production of glucose in the liver [16]. Diabetes Mellitus is managed and treated with the aid of pharmacological agents which can be administered through various routes of administration. Oral hypoglycemic/anti-hyperglycemic agents are used for the treatment and management of type 2 diabetes. They are agents that lower blood glucose levels by increasing the amount of insulin secreted by 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.

Glibenclamide, 5-Chloro-N-(2-{4-[(cyclohexylcarbamoyl) sulfamoyl] phenyl} ethyl) 2methoxybenzamide, belongs to the class of sulfonylureas and it is commonly used in the management of Type 2 diabetes [18]. Glibenclamide works by stimulating insulin secretion and increasing the response of β-cells to glucose and non-glucose secretagogues. This elevates the amounts of insulin secreted at different blood glucose level concentrations. 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 with the mandatory quantity and quality of active pharmaceutical ingredients and that also conforms to the official requirements laid down by monographs are required [21]. In addition, the International Conference on Harmonization (ICH) mandates that 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 British Pharmacopeia and European Pharmacopeia suggest that there are various types of Glibenclamide related impurities which include Sulfonamide (4-[2-(5chloro-2-methoxybenzamido) ethyl] benzene Sulfonamide) that is formed during the synthesis of Glibenclamide.

This present study was carried out to evaluate the physicochemical equivalence of four different brands of Glibenclamide tablets (5mg) sold in the south-western region of Nigeria and also to develop and validate an HPLC method for the quantification of Glibenclamide and Sulfonamide impurity in the tablets.

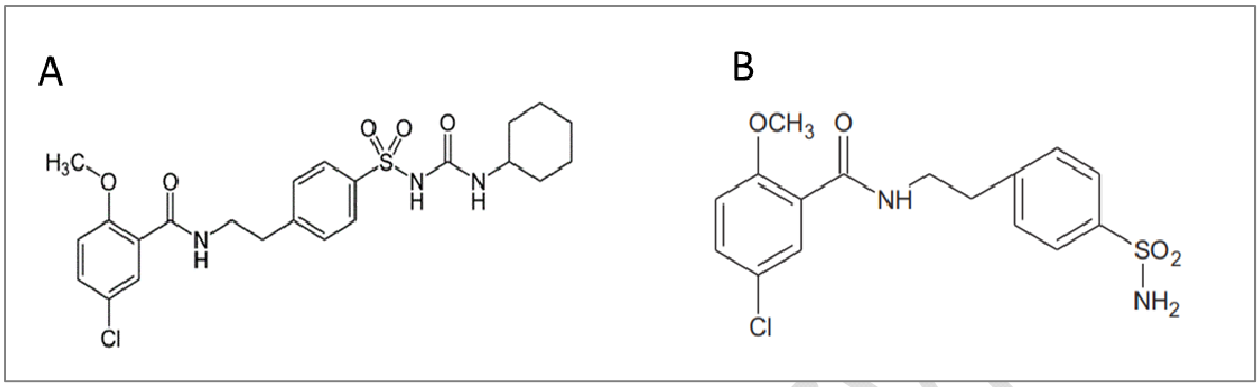

Figure 1A. Chemical Structure of Glibenclamide. Figure 1B. Chemical structure of Sulfonamide.

2.0 Materials and Methods

2.1 Sampling/ 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 and they were all within their shelf lives at the time of the investigation. All other reagents used were of analytical grade and the water used was distilled. HPLC grade of acetonitrile (Fischer scientific, U.K.) and analytical grade of sodium dihydrogen phosphate dehydrate (Merck, Darmstadt, Germany) was used.

All the different brands of uncoated Glibenclamide tablets with a strength of 5mg were assayed according to British Pharmacopeia standards. Table 1 shows the brands of tablets studied, manufacturers, batch number, NAFDAC (National Agency for Food and Drug Administration and Control) numbers, manufacture, and expiry dates.

Table 1: Brands of Glibenclamide (5mg) 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

The uniform weight of each brand of tablets was gotten by selecting twenty tablets from each brand randomly. They were weighed individually using an electronic weighing balance (Mettler Toledo, Switzerland) and their uniform weight was determined by calculating their average weight in mean ± SD.

2.3. Friability test

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

Friability (%) = [Initial weight – Final weight] / [Initial weight] * 100%

2.4. Hardness test (Crushing strength)

The crushing strength of each of the tablets from each brand was determined by a Monsanto, UK 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 ± SD of the recorded values was evaluated.

2.5 Disintegration time

The disintegration times for six tablets per brand were determined using a disintegration tester (Eagle Scientific Limited, Nottingham, UK) at (37.0 ± 0.5) °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 2 mm 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 ± 0.5) °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 ± SD.

2.6 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±0.5°C. Tablets of each brand of Glibenclamide were placed one each in the vessel of the dissolution apparatus with a paddle rotation of 50 rpm for 30mins. The samples were filtered and 5ml of the filtrate was withdrawn at intervals which were subsequently replaced with an equivalent volume of the dissolution medium that was maintained at 37.0±0.5°C. The samples that were withdrawn were diluted with an equivalent volume of phosphate buffer and their absorbance measured at a λmax of 276nm using a UV spectrometer

(Shimadzu, Japan). The concentration and the percentage of Glibenclamide released was evaluated using the formula below;

%Released = [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 (KH_2PO_4) 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 0.7mL/min, a detection wave length 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 standard solutions

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 175 µg/ml were made for calibration concentrations used for the linearity study.

2.7.1.1 Reference Solution (Sulfonamide)

50 mg 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 0.5 mg/ml (500µg/ml) stock solution was obtained. From the stock solution, serial dilutions of 25, 50, 75, 100 and 125 µg/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). The sample was analyzed using HPLC using a 20µl volume injected six times for each brand using three separate preparations. Also, six injections of a standard solution of Glibenclamide were injected. The area of the Glibenclamide peaks obtained was quantified with the area of the standard Glibenclamide peaks in order to determine

the percentage of Glibenclamide present in each brand. Empower 2 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 was determined using the method previously described. Twenty 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 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 R^1 radicals regulate their pharmacological and pharmacokinetic profiles. They also possess a $–SO_2$-NH-CO-NH- moiety which is hydrophilic in nature. Their aryl and R portions are lipophilic in nature and are responsible for the differences in their potencies i.e their sulfonylurea receptor binding properties, metabolism, and routes of elimination [23].

In this study, Fourier Infrared Spectroscopy (FTIR) was used to ascertain if chemical functional groups in the aryl-sulfonyl-urea sequence were present in the different brands of Glibenclamide compared with the functional groups present in a pure sample of Glibenclamide which was used as a 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 impurity

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

3.0 Results and Discussion

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

Table 2: Physicochemical properties of Glibenclamide brands.

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

3.1 Uniformity of weight

Uniformity of weight which is an important quality control parameter for solid dosage forms must correspond to compendial requirements as it determines the uniformity of dosage units. Uniformity of dosage units can be estimated through the evaluation of weight variations or drug content uniformity. Weight variations estimate directly or indirectly the variations in the amount of active pharmaceutical ingredients and excipients present in drugs. Variations of active ingredients can affect the *in vivo* and *in vitro* performance of the drug and cause adverse side effects while variations in excipients can affect drug delivery, patient compliance, bioavailability and stability of drugs. 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%.

3.2 Hardness or crushing strength

The hardness or crushing strength of a tablet can affect the rate of disintegration of tablets. 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, the method of granulation employed, or high compressive force used during the compression of tablets. The crushing strength requirement for a satisfactory tablet as recommended by BP is between 5-8kg [26]. All the brands failed the non-official hardness test, Diatab tablets had the highest crushing strength of all the four brands with a mean hardness or crushing strength of 15.4 kg.

3.3 Friability

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. 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 Test

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 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, for the identification of critical manufacturing parameters and serves as an aid in evaluating 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 tests revealed that all the brands released more than 70% of

active pharmaceutical ingredient Glibenclamide in 30mins and were found to follow the following dissolution order: D < C < B < A or A > B > C > D.

3.5 Disintegration Test

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 and it occurs before the dissolution of tablets in the body [32]. It is the rate determining step in drug absorption [33]. Disintegration tests of tablets isn't an important test for controlled and sustained release drug products. The test is influenced by certain factors such as crushing strength, quality of disintegrants, compactness, among others. Also, the bioavailability of the active pharmaceutical ingredients present in the drugs may be influenced by the amount and quality of excipients used for its formulation [34]. The disintegration times of the samples met the BP 2009 requirements of within 15 min for uncoated tablets [26] and they range from 1.34 to 2.20 mins.

3.6 Identification using IR spectroscopy

Fourier Infrared Spectroscopy was used to identify the chemical functional groups present in the samples and they were compared with that of the reference standard. The IR spectrum obtained from the reference standard used (pure Glibenclamide) and all the brands, revealed 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 in all the samples. This indicated that all the samples possessed components present in the aryl-sulfonyl-urea sequence of hypoglycemic sulfonylureas such as Glibenclamide. Table 3 below gives a summary of the peaks and their functional groups observed form the IR spectrum of pure Glibenclamide and all the brands of Glibenclamide tested and figures 2-6 shows the spectra obtained for them.

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, 3312.9, 3117.9	3516.3, 3313.4 3248.4	3522.2, 3315.7	3521.8, 3314.2	3521.8, 3315.2 3266.2
N-H bend	1614.9, 1590.7, 1563.1, 1519.0	1656.8, 1617.9, 1529.3	1618.0, 1522.5	1617.3, 1520.9	1617.4
C-H stretch	2930.3, 2854.7	2931.8, 2899.5	2932.5, 2899.9	2931.9, 2899.9	2931.3, 2899.4
C=O bend	1713.4	1712.3	1714.5	1714.5	1714.1
C-Cl bend	839.7, 819.4,	758.0, 716.5,	758.9,	757.2,	754.2,
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, 1093.4, 1011.4,	1140.5, 1114.3, 1070.7, 1056.4,	1201.1, 1140.7, 1114.4, 1070.7,	1141.5, 1115.0, 1071.3, 1029.9,	1201.0, 1165.3, 1138.9, 1115.1,

		1016.9, 987.89	1017.4,		1091.4, 1057.9, 1031.8
Aromatic C-H out of plane bend	684.9	716.5	899.4,	987.9	754.2

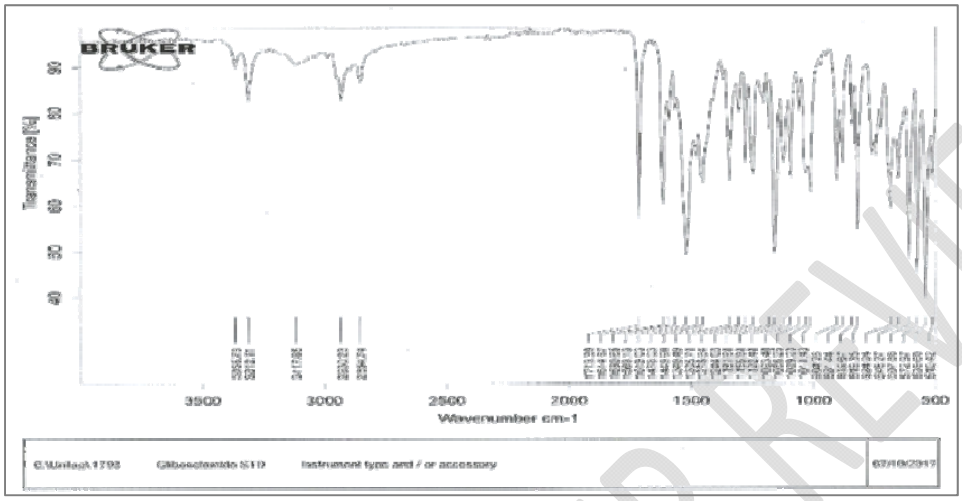

Figure 2: FTIR Spectra of Glibenclamide Reference Standard

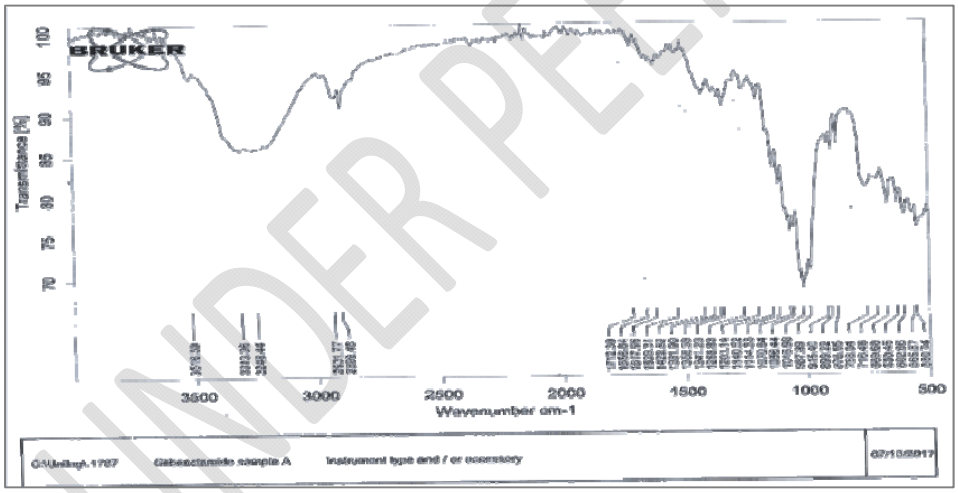

Figure 3: FTIR Spectra of sample A revealing the functional groups present in the sample

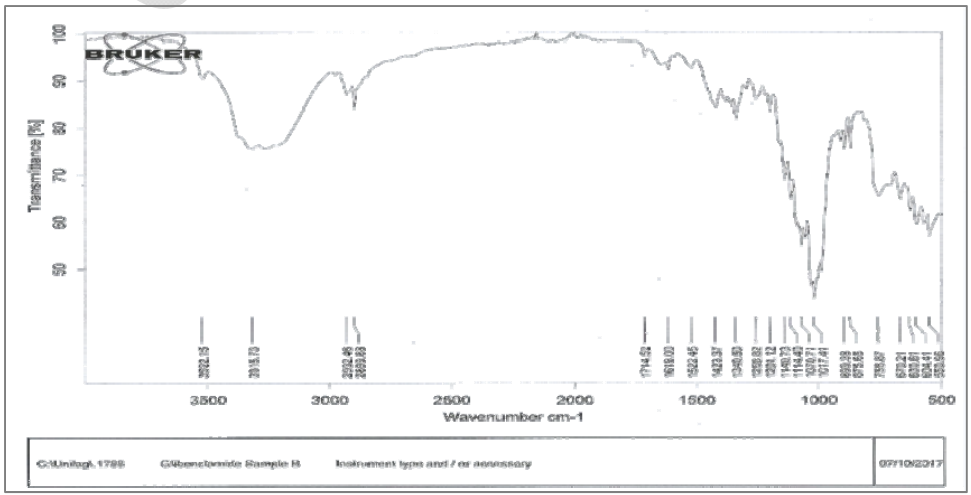

Figure 4: FTIR Spectra of sample B revealing the functional groups in the sample

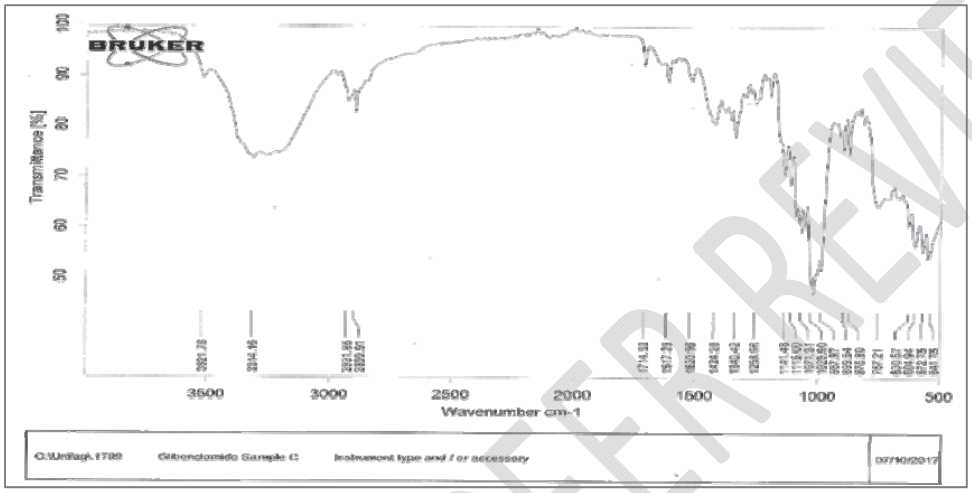

Figure 5: FTIR Spectra of sample C revealing the functional groups in the sample

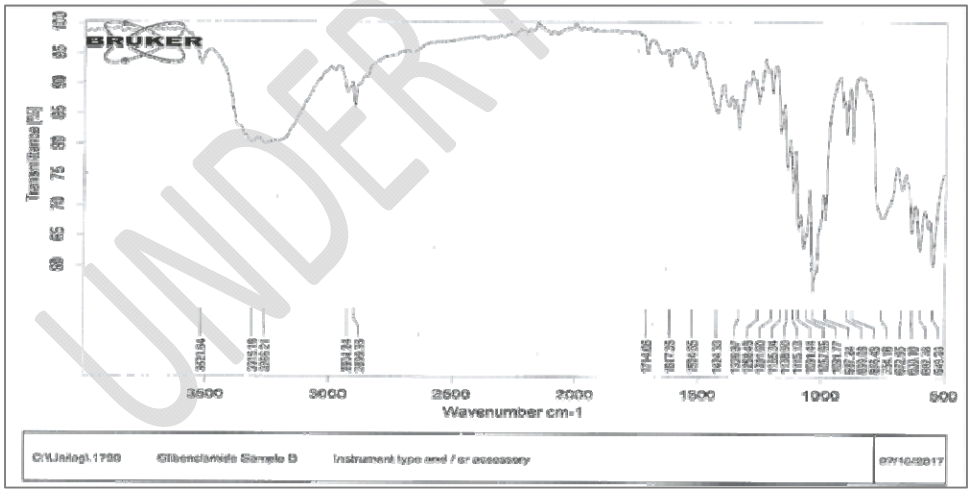

Figure 6: FTIR Spectra of sample D revealing the functional groups in the sample

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 figures 7-12.

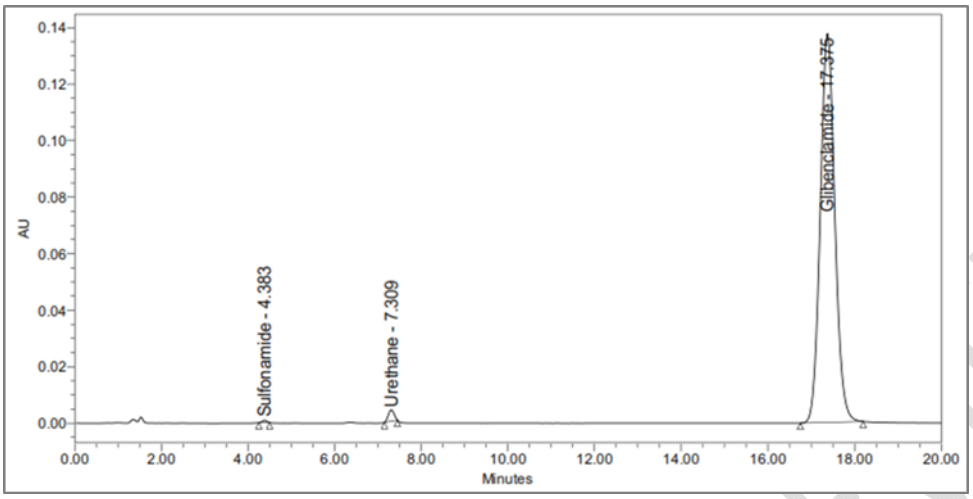

Figure 7: HPLC Chromatogram of Glibenclamide and related impurities

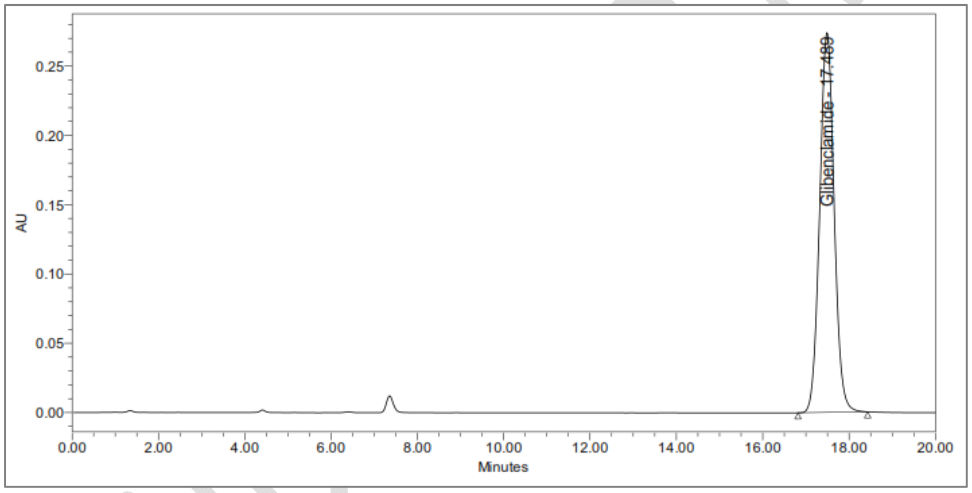

Figure 8: HPLC Chromatogram of Glibenclamide Reference Standard

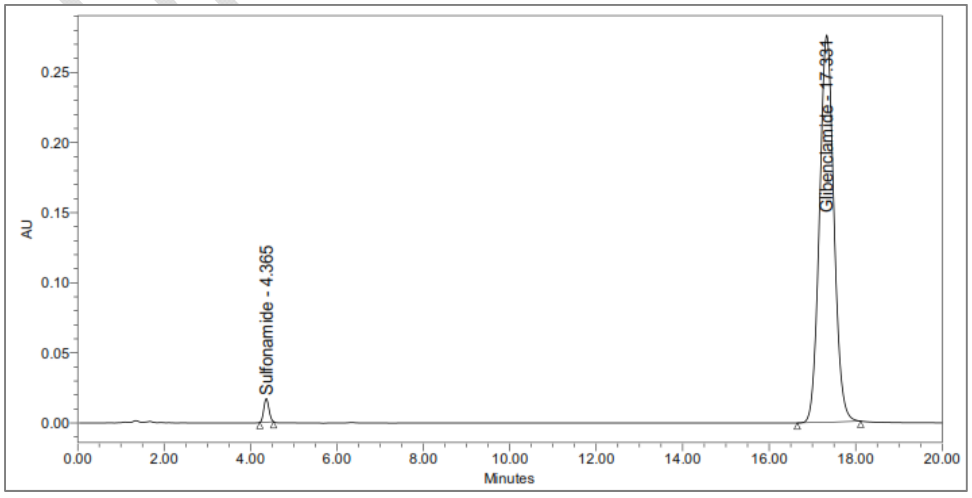

Figure 9: A HPLC Chromatogram of sample A revealing the presence of Glibenclamide and Sulfonamide impurity

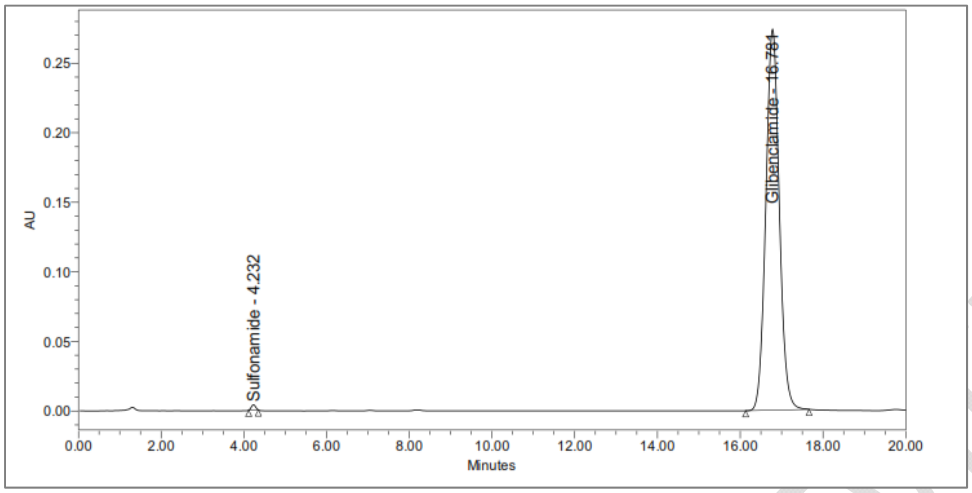

Figure 10: A HPLC Chromatogram of sample B revealing the presence of Glibenclamide and Sulfonamide impurity

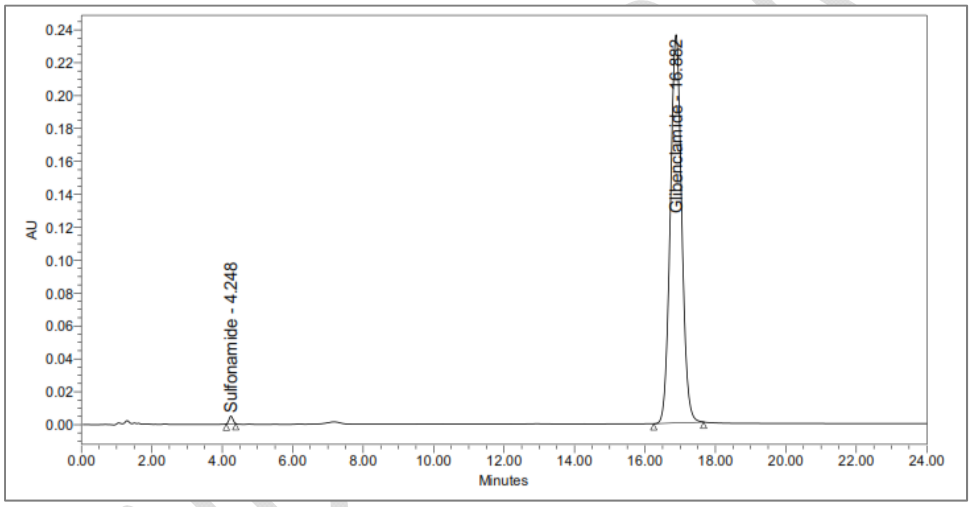

Figure 11: A HPLC Chromatogram of sample C revealing the presence of Glibenclamide and Sulfonamide impurity

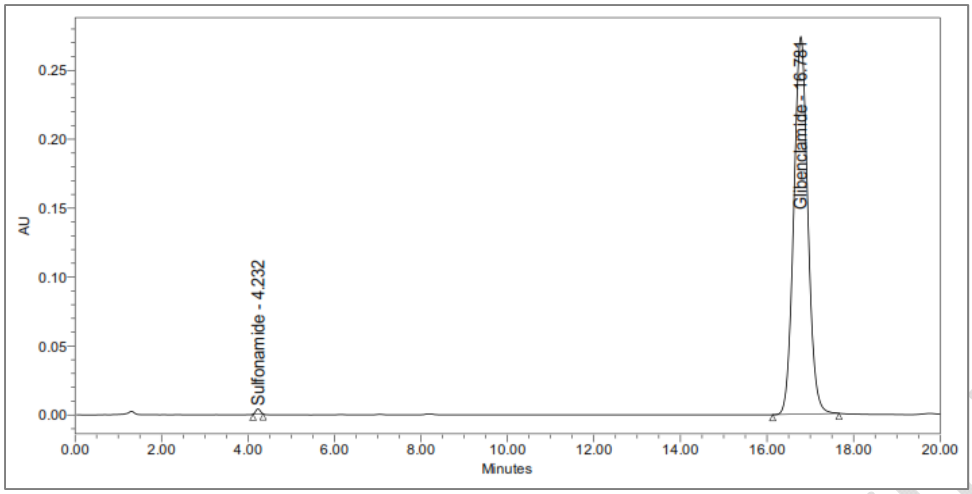

Figure 12: A HPLC Chromatogram of sample D revealing the presence of Glibenclamide and Sulfonamide impurity

3.7.1 Linearity

Linearity which involves the tendency of getting test results that are in direct proportion to the concentration of the analyte was estimated by injecting a series of five to six injections of different concentrations of Glibenclamide (50-175µg/ml) and Sulfonamide (25-125µg/ml) using a standard calibration curve. The calibration curve for Glibenclamide reflected linearity with a regression coefficient (R^2) of 0.9993 and a linear regression equation of Y=30338x-19975 while that of Sulfonamide reflected linearity with a regression coefficient (R^2) of 0.9991 and a linear regression equation of Y=35.268x+519. Figure 14 and 15 below shows the calibration curves for Glibenclamide and Sulfonamide respectively while table 4 gives a summary of the results obtained from the linearity and sensitivity studies that were carried out

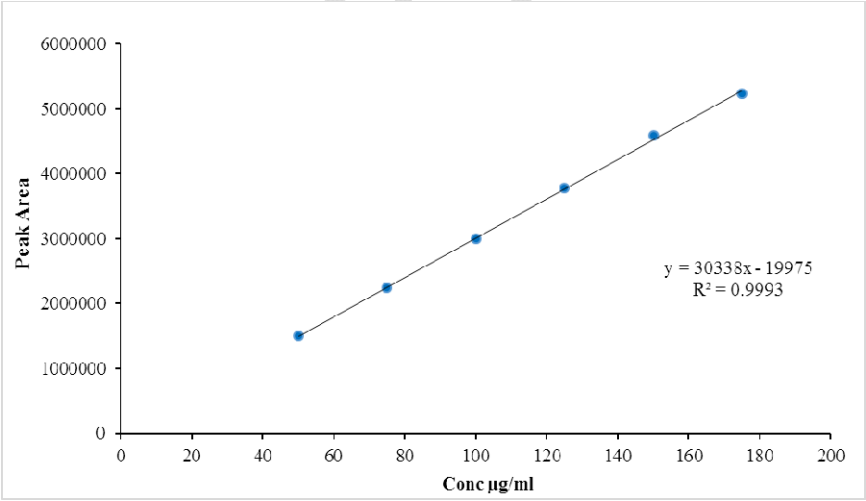

Figure 14: Standard calibration curve for Glibenclamide

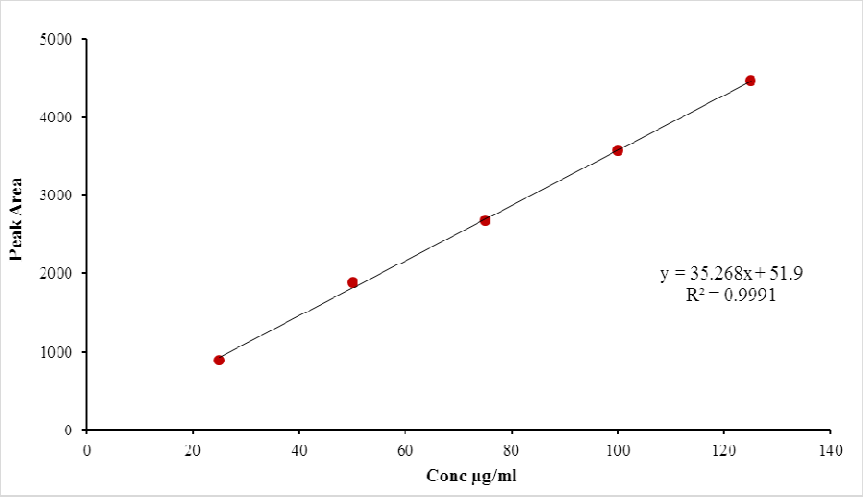

Figure 15: Standard calibration curve for Sulfonamide impurity

Table 4: Linearity and sensitivity data for Glibenclamide and Sulfonamide impurity

Parameters	Glibenclamide	Sulfonamide
Regression Equation	Y=30338x-19975	Y=35.268x+519
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

3.7.2 Precision

The precision of the assay method in terms of the measure of 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 100 µg/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 75 µg/ml and was found to be 0.383%. Table 5 below shows the precision data obtained.

Table 5: Precision data for Glibenclamide and Sulfonamide impurity

Determination of precision (repeatability)		
	Glibenclamide (100 µg/ml)	**Sulfonamide (75 µg/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

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 (δ/ s) and LOQ = 10 (δ/ s) according to ICH guidelines [24]. Where δ 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 0.075µg/ml and 0.227µg/ml respectively for Glibenclamide while 0.114µg/ml and 0.345 µg/ml was obtained respectively for Sulfonamide impurity as shown in Table 4.

3.7.4 Accuracy

The Accuracy (% Recovery) of the method which refers to the nearness of agreement between an accepted reference value and the obtained value was determined by calculating the percentage recovery of the recovered analyte. This was done at three different concentrations of 50, 100 and 150 µg/ml of the standard Glibenclamide solution and 25, 50 and 75 µg/ml of the standard solution of Sulfonamide. The data obtained were statistically analyzed using the formula %Recovery = [(Found/Recovered concentration ÷ the injected concentration)*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%. The %RSD at all levels for both Glibenclamide and Sulfonamide impurity was < 3%, which is within the acceptable limits (Table 6).

Table 6: Percentage recovery studies for Glibenclamide and Sulfonamide impurity analysis

Content of Glibenclamide and sulfonamide in Daonil									
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									
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									
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									
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.

3.7.5 Analysis of Glibenclamide and Sulfonamide impurity in each brand

The test results of the amount of Glibenclamide present in each brand were in good agreement with the BP specification range of 95%-105% for the active drug content [25] expect for brand A (Daonil) which exceeded the specification by having 107% of the drug content. This might be due to the interference with excipients used for its formulation. The results are presented in Table 7

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% with sample C (Diatab) having the highest impurity content and sample A (Daonil) having the lowest impurity content. These were within acceptable limits [22]. The results are presented in Table 8.

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

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 with the BP specifications for hardness/crushing strength. The FTIR spectra of all the brands, when compared with the reference standard, revealed that they had functional groups that were present in the aryl-sulfonyl-urea sequence of hypoglycemic sulfonylureas. One of the brands failed the assay test by having above the 95-105% drug content limit given by the British Pharmacopeia with all the brands having less than the 1% acceptable limit for impurities. The physicochemical evaluation of the brands of glibenclamide tablets tested attests the need for constantly monitoring the physicochemical equivalence of marketed drug products for their efficacy, quality, and safety.

Also, the results obtained from the validation of the developed method showed that it is a simple, accurate, precise, sensitive and reproducible analytical technique that can be used for the quantification of Glibenclamide and Sulfonamide impurity found in Glibenclamide tablets. Therefore, the developed method can be found useful as an economical quality control tool for the determination of active pharmaceutical ingredients in final dosage forms and their related impurities as well as for the identification and elimination of counterfeit or adulterated tablets.

REFERENCES

[1] Sattar N, Preiss D, Murray H.M, Welsh P, Buckley B.M, de Cream A.J, Seshasai S.R, McMurray J.J, Freeman D.J, Jukema J.W, Macfarlane P.W et al., 2010. Statins and risk of incident diabetes: a collaborative meta-analysis of randomized statin trials. Lancet, vol 375 no 9716 735-742 DOI: https://doi.org/10.1016/SO140-6736(09)61965-6

[2] International Diabetes Federation. Diabetic atlas eighth edition 2017, Brussels. Available at http://www.idf.org/diabetesatlas. (Accessed 20th July 2018).

[3] Zhao Y, Jiang Z, Guo C. New hope for type 2 diabetics: Targeting insulin resistance through the immune modulation of stem cells. Autoimmune Rev. 2011; 11: 137–142

[4] Thomas ER, Brackenridge A, Kidd J, et al. Diagnosis of monogenic diabetes: a 10-Year experience in a large multi-ethnic diabetes center. J Diabetes Investig. 2016; 7: 332–37; DOI: http://dx.doi.org/10.1111/jdi.12432.

[5] Tripathi BK, Srivastava AK. Diabetes mellitus: complications and therapeutics. Med Sci Monit. 2006; 12(7): RA130–147.

[6] Ogunmodede SO, Oseni SO, Adenmosun OO, et al., *Dracaena Arborea* Extracts: A Phytotherapeutic Option for Ameliorating Oxidative Stress-mediated Testicular Disorders in Alloxan-induced Diabetic Rats, J. Coastal Life Med. 2015; 3: 930-935.

[7] Ismail-Beigi F. Pathogenesis and glycemic management of type 2 diabetes mellitus: a physiological approach. Arch Iran Med. 2012; 15(4): 239–246.

[8] Ripsin CM, Kang H, Urban RJ. Management of blood glucose in type 2 diabetes mellitus. Am Fam Physician. 2009; 79 (1):29-36.

[9] Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, et al. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. N Engl J Med. 2001; 345 (11):790-797

[10] Prevalence of overweight and obesity among adults with diagnosed Diabetes United States, 1988-1994 and 1999-2000"Centers for Disease Control and Prevention (CDC) (November 2004) MMWR. Morbidity and Mortality Weekly Report; 53(45): 1066-1068.

[11] Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature. 2001; 414(6865): 782-787.

[12] Hu FB, Manson JE, Stampfer MJ, et al. lifestyle, and the risk of type 2 diabetes mellitus in women. N Engl J Med. 2001; 345(11): 790–797.

[13] Manson JE, Ajani UA, Liu S, et al. A prospective study of cigarette smoking and the incidence of diabetes mellitus among US male physicians. Am J Med. 2000; 109: 538–542.

[14] Cullmann M, Hilding A, Östenson CG. Alcohol consumption and risk of pre-diabetes and type 2 diabetes development in the Swedish population. Diabet Med. 2012; 29(4): 441–452.

[15] Jack L Jr, Boseman L, Vinicor F. Aging Americans, and diabetes. Public health and clinical response. Geriatrics 2004 Apr; 59 (4):14-17.

[16] Kerr. D., Patridge.H, Knott.J. and Thomas, P.W. HbA1c 3 months after diagnosis predicts premature mortality in patients with new-onset type 2 diabetes. Diabet. Med. 2011; 28 (12), 1520-1524

[17] World Health Organization. WHO Essential Medicines and Health Products Annual Report 2015. World Health Organization, 2016.

[18] Figueroa-Valverde, L., Díaz-Cedillo, F., López-Ramos, M., GarcíaCervera E, Pool-Gómez E, Cardena-Arredondo C, Ancona León G. Glibenclamide-pregnenolone derivative has greater hypoglycemic effects and biodistribution than Glibenclamide-OH in alloxan-rats. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc. Czech. Repub. 2012; 156, 122-127.

[19] Kunte H, Schmidt S, Eliasziw M, Delzoppo GJ, Simard JM, Masuhr F, Weih M, Dirnagl U. Sulfonylureas improve outcome in patients with type 2 diabetes and acute ischemic stroke. Stroke. 2007; 38(9): 2526-2530.

[20] Bennett W. L. Comparative effectiveness and safety of medications for type 2 diabetes- An update including new drugs and two drugs combinations. Annals of Internal Medicine; 2011; 154(9): 602-613

[21] Quality Assurance of Pharmaceuticals: A Compendium of guidelines and related materials. (2006). Vol.2, Good Manufacturing Practices and Inspection 2nd edition.

[22] ICH Harmonised Tripartite Guideline Q3A (R2). (2006) Impurities in New Drug Substances.

[23] Thomas J.A and Thomas M.J. Insulin and Hypoglycemic drugs. In: Bittar E. Molecular and Cellular Pharmacology. Elsevier Science. 1997; 601-622.

[24] International Conference on Harmonization. Validation of analytical procedures: Test and methodology. ICH, London, 1996; 342.

[25] British Pharmacopoeia. (2007). The Pharmaceutical Press, London. Volume II.

[26] British Pharmacopoeia Commission. British Pharmacopeia Vol.III. London: The Stationery Office Limited; 2009, p. 6578-85.

[27] Shirsand SB, Suresh S, Para MS, Swammy PV and Kumar DN. *Planatago ovata* mucilage in the design of fast disintegration tablets. *Indian Journal of Pharmaceutical Sciences*, 2009; **71**(1):41-45.

[28] Allen LV, Popovich NG, and Ansel HC. Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems. 9th Edn. Philadelphia, PA: Lippincott Williams & Wilkins. 2011; 231-236.

[29] Dissolution testing of tablets and capsules (2017). The International Pharmacopeia. 7th Edition. Accessed on 1st June 2018

[30] Adegbolagun OA, Ololade OA, Osamah SE. Comparative evaluation of the biopharmaceutical and chemical equivalence of some commercially evaluable brands of ciprofloxacin hydrochloride tablets. Tropical Journal of Pharmaceutical Research. 2007; 6:737-745.

[31] British Pharmacopeia (2004b). Monograph A-Z. The Pharmaceutical Press, Her Majesty's Stationary Office, London. pp. 1-4:361, 1306, 2141.

[32] Karmakar P and Kibria MD. *In vitro* comparative evaluation of quality control parameters between paracetamol and paracetamol tablets available in Bangladesh. *International Current Pharmaceutical Journal*. 2012; **1** (5):103-109.

[33] Abrahamsson B, Albery T, Erisson A, Gustaffson I and Sjoberg M. Food effects on Tablet disintegration. *European Journal of Pharmaceutical Sciences*. 2004; **22**(2):165-172.

[34] Kwan KC, Swart FO and Mattocks AM. Factors affecting Tablet disintegration. *Journal of the American Pharmaceutical Association.* 2006; **46**(4):236-239.