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Development and Validation of Analytical Method for Qualitative and Quantitative Determination of Glibenclamide in Different Brands of Tablet Dosage form Using UV-Visible Spectroscopy

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Abstract

This paper describes a methodology to develop a new method for qualitative and quantitative determination of glibenclamide (GLB) in three brands of GLB tablets. We validated the developed method for linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) according to the guidelines of the International Conference on Harmonization. Finally, we estimated the qualitative and quantitative amount of GLB in three brands of GLB tablets using validated method. A non significant difference was observed in the dissolution profiles of GLB in these tablets. Validation of developed method showed that a linear relationship (r^2>0.999) was observed at maximum absorbance (λ_{max}) at 229.5 nm with concentration range of 3-15 µg/ml GLB. Accuracy of developed method was found to be within the limit of 95-105%. The percent relative standard deviation (%RSD) and percent relative error (%RE) for precision was found to be <3%. In three brands of GLB tablets, values of LOD and LOQ were 10 ng/ml and 35 ng/ml respectively. We also found that the amount of GLB in each tablet corresponded to the requirements of 95-105% of the label claimed in tablet. From the results of validation of developed method, it concluded that developed method showed satisfactory linearity, precision and accuracy for analysis of active ingredients of commercially available pharmaceutical products.

Keywords: Glibenclamide; Analytical methods; UV-visible spectrophotometeric analysis; Anti-diabetic agents

Introduction

Glibenclamide (GLB) also known as glyburide belongs to the second generation of oral anti-diabetic class of sulphonylurea. The chemical name of glibenclamide is 5-Chloro-N-(2-{4-[(cyclohexylcarbamoyl) sulfamoyl] phenyl} ethyl)-2-methoxybenzamide and chemical formula is $C_{23}H_{28}ClN_3O_5S$ (Figure 1). Therapeutically, it is more potent as compared to that of the first generation of sulphonylureas [1].

There are two types of diabetes mellitus i.e. type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). T1DM is known as insulin-dependent diabetes mellitus which is usually leads to the autoimmune destruction of β-cells of pancreatic islets due to which the production of insulin is impaired. T2DM is known as non-insulin-dependent diabetes mellitus in resistance to insulin and/or abnormal insulin secretion from β-cells of pancreatic islets usually occur [2-4]. Glibenclamide is most frequently used as a drug of choice for the treatment of T1DM in which diet and insulin are failing to control the symptoms of T1DM. Moreover, it is also frequently used to normalize the glucose levels during mild to moderately severe T2DM that does not require insulin but diet can control the levels of glucose in T2DM. Glibenclamide works by inhibiting the sulphonylurea receptor in β-cells of the pancreatic islets that ultimately leads to the opening of voltage-dependent calcium channel. This results in the increased concentration of intracellular calcium in β-cells that subsequently leads to the stimulation of insulin release [5].

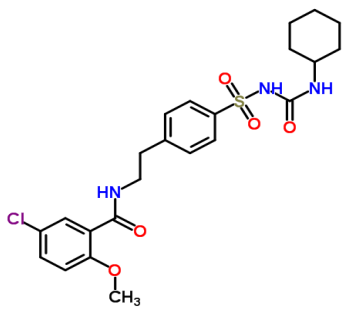

Figure 1: Chemical Structure of Glibenclamide (Source: PubChem-CID 3488).

Method development and Validation for the analysis of active pharmaceutical ingredients in pharmaceutical dosage forms is very important as it gives the precision and accuracy of the developed method [1,6- 8]. Till now, various methods have been developed and validated for the analysis of various anti-diabetic agents [9-15]. High quality of the active ingredient in final pharmaceutical dosage form is very important and it needs a simple, accurate, precise and fast method for the qualitative and quantitative estimation of active ingredient in its specific pharmaceutical dosage form [16-20]. Among various analytical methods, UV-visible spectrophotometric method fulfills these demands and it may be the instrumental technique of choice for

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the analysis of active pharmaceutical ingredients in industrial and as well as laboratory scale [21,22].

The purpose of our study was to develop and validate new method for qualitative and quantitative estimation of GLB in three different brands of tablet dosage form using UV visible spectrophotometer. Moreover, we also performed *in vitro* dissolution studies of GLB in these tablets. Our results indicate that the developed method is precise, cost-effective and can be commercially used for the analysis of active ingredients in commercially available pharmaceutical products.

Materials and Methods

Materials, reagents and chemicals

GLB was kindly gifted by Sanofi-Aventis Pakistan Ltd., Karachi, Pakistan. Daonil (Sanofi-aventis Pakistan Ltd., Karachi, Pakistan), Diabeta (Siza International Pvt., Ltd., Lahore, Pakistan) and Euglucon (Roche Pakistan Ltd., Karachi, Pakistan) tablets were purchased from the local pharmacy, Lahore, Pakistan. All other chemicals and reagents were analytical grade. All chemicals were used without further purification. Samples used in the present study were commercially available tablets containing 5.0 mg GLB/tablet.

UV-visible spectrophotometric method

Qualitative and quantitative determination of GLB in different brands of GLB tablets was determined using UV-visible spectrophotometer (Shimadzu Model 160-A, Kyoto, Japan). Suitable conditions for UV-visible spectrophotometeric analysis were as followed; $\Delta\lambda$= 6.4 nm, range of λ 200-400 nm, absorbance of methanol as at 239 nm (zero-peak) were set as blank.

Preparation of stock solution and calibration standards: Accurate amount of GLB was weighed and transferred to 100 ml volumetric flask. Methanol was added into the flask and the flask was shaken manually till complete dissolution occurred. Final volume was made up to obtain the final concentration 100 µg/ml of GLB. An appropriate aliquot portion of GLB solutions (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 ml) from the standard stock solution of GLB was transferred to 10 ml volumetric flasks and methanol was added in all flasks to obtain the final concentration of 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 µg/ml of GLB. All the solutions were scanned separately between 200 nm to 400 nm in UV-visible spectrophotometer and λ_{max} for GLB was recorded.

In vitro dissolution studies of glibenclamide in different tablet dosage forms

In vitro dissolution studies were performed using 3 brands of GLB tablets with the help of Erweka DT-6 dissolution tester using paddle method (USP apparatus II) at 37 ± 0.0.5 °C with stirring speed 50 rpm as described previously [23]. 900 ml of monobasic potassium phosphate USP buffer (pH 7.6) was used for *in vitro* dissolution studies of GLB tablets. At 10 minute's intervals, 5 ml aliquot was removed using 0.45 µm syringe filter and equal volume of fresh medium was added to maintain the total volume of dissolution medium. The removed aliquot was then analyzed for GLB content determination by measuring the absorbance at 229.5 nm using developed UV-visible spectrophotometric method. Cumulative percentages of drug release into the dissolution medium were then calculated using DDSolver as described previously [24,25].

Validation of UV-visible spectrophotometric method

UV-visible spectrophotometer method was validated according to the guidelines of the International Conference on Harmonization (ICH) [26].

Linearity: To validate the UV-visible spectrophotometer method, calibration curve was obtained at 10 different concentrations of GLB. Triplicate readings were obtained for each concentration and least square regression method was used to analyze the linearity and slope. Intercept and correlation coefficient were also determined.

Accuracy: Recovery studies at three different levels (80%, 100% and 120%) to judge the accuracy of developed method of UV-visible spectroscopy was assessed. For analysis of drugs at three different levels, the standard addition method was followed as described previously [27]. In order to make the concentration of GLB in linearity range, accurately weighed amount of pure GLB was added to finally powdered samples of three brands of GLB tablets. To calculate the %Recovery, %Relative Error (%RE), standard error of the mean (SEM) and relative standard deviation (RSD), experiments were performed in triplicates.

Precision: The precision of the developed UV-visible spectrophotometric method was also determined as inter-day, intra-day and repeatability. Inter-day precision was determined by performing experiments on three different concentrations of GLB in each brand of GLB tablet on three different days of the week whereas, intra-day precision of developed method was determined by performing three individual experiments on three different concentrations of GLB in each brand of GLB tablet at different times of same day. Repeatability was also determined by repeating the analysis of GLB samples for 6 times.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): We also calculated the LOD and LOQ of the developed UV-visible spectrophotometric method using the slope of the calibration curve and standard deviation of the response according to the guidelines of the ICH [26].

Analysis of GLB in three brands of GLB tablets

Finally, we used the validated method to estimate the amount of GLB in 3 different brands of GLB tablets. Contents of 20 tablets from each brand were weighed and crushed to make powder using porcelain mortar and pestle. GLB content in different brands of tablets was determined as described in previous section. Briefly, a quantity of powder having an equivalent amount of 200.0 mg of GLB was weighed and dissolved in methanol in 100 ml volumetric flask and filtered through Whatmann filter paper. After complete dissolution, 5.0 ml aliquot was withdrawn and then diluted with methanol to make the final concentration of GLB 20 µg/ml. The absorbance of GLB was measured at 229.5 nm in UV-visible spectrophotometer and concentration of GLB in three brands of GLB tablets was determined accordingly.

Analysis of excipients influence on developed assay method

We studied the possible influence of excipients on the %recovery of GLB by adding the known amount of pure GLB in stock solutions and determined the %recovery of GLB in these solutions by developed method as described previously [28]. Briefly, 20 tablets were weighed and crushed with porcelain mortar and pestle. An amount of powder equivalent to the dose of GLB in one tablet was weighed and transferred to the volumetric flask. The same amount of pure GLB was weighed and added to that flask. The mixture was sonicated till its complete dissolution and final volume was adjusted with solvent. The resulting solution was filtered through a 0.45 mm nylon filter. Suitable volume was taken and transferred to another volumetric flask to adjust the final volume of 40 µg/ml of GLB. The final solution was used to calculate the % recovery of GLB using developed UV-visible spectrophotometric method.

Statistical analysis

Microsoft EXCEL® (Microsoft Corporation, USA) was used to compute the Standard regression curve analysis. We also used the same software to calculate the mean, SD, %recovery, %RE, SEM and RSD.

Results

Development of UV-visible spectrophotometric method

We measured the wavelength of different concentrations of standard solution of GLB. The maximum absorbance of GLB in standard solution was observed at 229.5 nm (Figure 2). 229.5 nm wavelength was selected for the analysis of GLB in three brands of GLB tablets and for the validation of developed method.

In vitro dissolution studies of glibenclamide in different tablet dosage forms

We estimated the *in vitro* release pattern of GLB from tablets using USP dissolution apparatus II. We used monobasic potassium phosphate USP buffer (pH 7.6) as dissolution medium. 5 ml aliquot samples were removed at specific time intervals and the volume was equilibrated with fresh medium. The aliquot was then filtered using 0.45 µm syringe filter and quantity of GLB released at specific time point was determined measuring the absorbance of GLB at 29.5 nm wavelength. From the results of *in vitro* dissolution profiles (Figure 3), it was found that non significant difference was observed in the release pattern of GLB from three brands of GLB tablets.

We also calculated the mean dissolution time (MDT), percent dissolution efficiency (%DE), time for the release of 50% GLB ($t_{50\%}$) from the tablets and similarity factor (f_2) from the release pattern of GLB. MDT, %DE, $t_{50\%}$ and f_2 are model-independent approaches [29]. From the results of model-independent approaches (Table 1), it was found that the pattern of *in vitro* release of GLB from different tablets was almost similar as non-significant difference was observed among these model-independent approaches.

Validation of UV-visible spectrophotometric method

We validated the developed UV-visible spectrophotometric method according to the guidelines of ICH [26] for its linearity, accuracy, precision, LOD and LOQ.

Linearity: We adjusted the reliable quantification of GLB from 3 µg/ml to 15 µg/ml for each brand of GLB tablet for estimation of GLB via developed method. The standard curve had reliable reproducibility for the estimation of GLB in three brands of GLB tablets across the calibration range. The data showed a good linear relationship with a correlation coefficient (r^2) greater than 0.999 for all calibrations of GLB in three brands over the wide range (Table 2). From the results of Table 2, it was found that the concentration range of GLB in three brands of GLB tablets was same. A non significant difference was also observed between the values of the slope and intercept of GLB.

Accuracy: We determined the accuracy of developed method by performing %recovery studies at three different levels (80%, 100% and 120%). The %recovery of GLB in three brands of GLB tablets at three different levels was almost found to be 100% (Table 3). %RE and %RSD were less than <3% that were within the acceptable limits (Table 3).

Precision: We studied the precision of the developed method as inter-day, intra-day and repeatability. For inter-day, intra-day and repeatability studies, GLB was studied at three different concentrations. The %RSD value for these three parameters were found to be less than <3% (Table 4) which indicated that the developed method was precise and repeatability of the method was accurate.

Limit of detection (LOD) and limit of quantitation (LOQ): The LOD for GLB was found to be 10 ng/ml whereas; LOQ for GLB was 35 ng/ml. The maximum absorbance of GLB was found to be detected at 229.5 nm without any additional peak. The accuracy and precision of

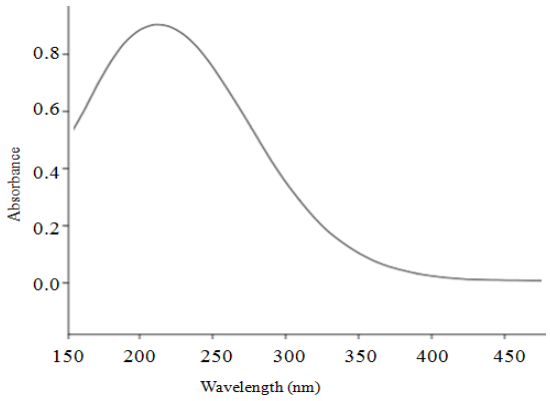

Figure 2: Absorption spectrum of Glibenclamide (GLB).

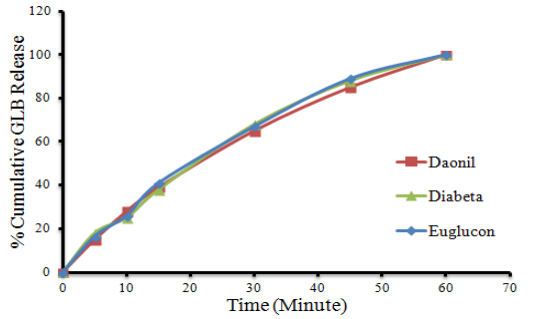

Figure 3: *In vitro* dissolution profile of GLB from three different brands of GLB tablets.

Different Brands of GLB	Parameters				
	MDT (min)	$\%DD_{30}$	%DE (min)	f_2	P Value*
Daonil	14.5	65	30	Reference	0.011
Diabeta	13.21	68	32	85	0.032
Euglucon	13.85	67	28	90	0.013

Abbreviations: GLB: Glibenclamide; MDT: Mean Dissolution Time; %DD: Percent Drug Dissolved Within 30 Minutes; F_2: Similarity Factor; *: Significant difference at P<0.05

Table 1: Model-independent approaches for *in vitro* dissolution profile of GLB.

Parameters	Pure GLB	Brands GLB Tablet		
		Daonil	Diabeta	Euglucon
Absorption maximum (nm)	229.5	229.5	229.5	229.5
Conc. Range (µg/ml)	3-15	3-15	3-15	3-15
Correlation coefficient (r^2)	0.999	0.998	0.989	0.997
Slope	0.118	0.120	0.117	0.119
Intercept	0.0025	0.0024	0.0025	0.0023

Table 2: Linearity parameters for calibration curve of GLB estimated from three different brands of GLB tablets.

GLB at these concentrations were within the acceptable range of %RE and %RSD.

Analysis of GLB in different brands of tablets

We applied the validated UV-visible spectrophotometric method for qualitative and quantitative analysis of GLB in three brands of GLB tablets. The final assay results are elaborated in Table 5. From these results, it was found that the amount of GLB in each tablet corresponded to the requirements of 95-105% of the label claimed in each tablet. The RSD value observed was found to be <3% which indicated the suitability of developed method for routine analysis of GLB in pharmaceutical dosage forms.

Analysis of excipients influence on developed assay method

We also determined the influence of excipients on %recovery of GLB from GLB tablets performing the %recovery studies by adding 100% of the excess of pure GLB in each stock solution of three brands of GLB tablets. The %recovery of GLB in each tablet was almost 100% with RSD value <3% (Table 6) which indicated that the excipients had no influence during the %recovery of GLB from the tablets.

Discussion

T2DM has been considered as one of the major life-threatening disease. Several factors are decisively involved to provoke the pathogenesis of T2DM [3,25,30-33]. Various new therapeutic modalities have been investigated to treat this disease [34-37]. But to combat the symptoms of diabetes, still GLB is among the drug of choice [28].

The developed UV-visible spectrophotometric method was found to be highly specific and reproducible for qualitative and quantitative determination of GLB in three brands of GLB tablets. We also performed the *in vitro* dissolution profile of GLB in three different brands of GLB tablets. From the results, it was found that the *in vitro* dissolution profile of GLB in these brands was almost similar and non significant difference was observed (Figure 3). Moreover, we also estimated the mode of GLB release from its corresponding tablets using model-independent approaches such as MDT, %DE, $t_{50\%}$ and f_2. These approaches are very important to estimate the behavior of drug release from its dosage form [29]. The results of model-independent approaches (Table 1) indicated that the pattern of GLB release from its corresponding tablets were almost similar as there was no significant difference within the model-independent approaches.

The main objective of the validation of the developed method is to obtain the consistent, reliable and accurate data. Validation of the developed method plays its foremost role to achieve these goals. The results obtained from the validation of developed may be used to verify the quality, quantity, accuracy and consistency of the active pharmaceutical ingredient in pharmaceutical dosage form. We validated the developed method by performing linearity, accuracy, precision, LOD and LOQ analysis. Linearity is used to determine the response of different concentrations of drug at specific wavelength. We evaluated the developed method by detecting the linearity using r^2 and intercept values (Table 2). From the analysis of validation parameters, we found that the developed method showed highest linearity (r^2>0.999) in the range of 3-15 µg/ml.

Accuracy is used to measure the closeness of measured value with that of the true value of active ingredient in dosage form [38]. Accuracy is usually calculated in the form of % recovery. In the present study, we calculated the accuracy of the developed method measuring % recovery of GLB at 80%, 100% and 120% (Table 3). The % recovery of GLB at 80%, 100% and 120% was found to be almost 100%. We also measured the %RE and %RSD at all levels. We found that %RE and %RSD were <3% that were within the acceptable limits (Table 3). The developed method was found to be enabling for the accurate quantitative estimation of GLB in tablets. Similarly, we also found a non significant difference between the %RSD values in inter-day, intra-day and repeatability precision which indicated that the developed method for the estimation of GLB was accurate.

LOD and LOQ are the most important parameters for the validation of the developed method. LOD is the lowest amount of active ingredient present in the dosage form that can be detected and it cannot be quantified as an exact value. LOD is a point at which measured value is greater than the uncertainty associated with it [39,40]. LOQ is the lowest amount of active ingredient that can be quantitatively determined with accuracy. It is used to determine the quantity of the ingredient with known concentrations by establishing the minimal level at which the active ingredient can be quantified with suitable accuracy and precision. In the present study, the value of LOD was 10 ng/ml whereas for LOQ, it was 35 ng/ml. The lowest values of LOD and LOQ made the developed method more suitable for the

Amount of GLB added (%)	Theoretical Content of GLB (µg/ml)	Conc. found (µg/ml) Mean ± SD	Recovery (%)	SEM	RE (%)	RSD (%)
Daonil						
0	20	19.84 ± 0.386	99.70	0.223	0.80	1.26
80	40	40.64 ± 0.439	101.27	0.256	1.25	1.46
100	50	50.94 ± 0.519	101.85	0.235	1.95	0.86
120	60	60.74 ± 0.736	101.38	0.358	1.85	1.45
Diabeta						
0	20	20.15 ± 0.437	101.22	0.399	1.36	1.46
80	40	40.36 ± 0.569	101.15	0.368	2.05	1.16
100	50	50.84 ± 0.361	101.87	0.225	1.76	1.48
120	60	60.04 ± 0.687	100.06	0.336	1.81	0.98
Euglucon						
0	20	20.76 ± 0.261	101.95	0.287	0.56	1.56
80	40	40.34 ± 0.325	100.85	0.318	0.85	1.45
100	50	50.54 ± 0.684	101.28	0.367	1.08	1.18
120	60	60.14 ± 0.514	100.89	0.298	0.96	1.59

Abbreviations: GLB: Glibenclamide; SD: Standard Deviation; SEM: Standard Error Mean; RE: Relative Error; RSD: Relative Standard Deviation

Table 3: Percent Recovery studies for accuracy of developed method.

Amount of GLB in each Brand (µg/ml)	Parameters*		
	Inter-day (n = 3)	Intra-day (n = 3)	Repeatability (n=6)
Daonil			
10	1.53	0.98	0.61
20	1.43	0.87	0.60
30	1.28	1.08	0.62
Diabeta			
10	1.43	1.11	0.60
20	1.36	0.99	0.63
30	1.39	1.15	0.61
Euglucon			
10	1.23	1.16	0.62
20	1.32	1.20	0.61
30	1.18	1.09	0.62

Abbreviations: GLB: Glibenclamide; RSD: Relative Standard Deviation *; RSD

Table 4: Results from precision of developed UV-visible spectrophotometric method.

Brand names of GLB	Parameters			
	Label claimed (mg/tablet)	Amount detected (mg/tablet)	RSD (%)	Assay (%)
Daonil	5.0	5.12	0.56	101.09
Diabeta	5.0	5.08	1.18	100.58
Euglucon	5.0	5.02	2.08	100.07

Abbreviations: GLB: Glibenclamide; RSD: Relative Standard Deviation

Table 5: Quantitative and qualitative estimation of GLB in three brands of GLB tablets.

Brand names of GLB	Conc. found (µg/ml)* Mean ± SD	Recovery (%)	RSD (%)
Daonil	20.14 ± 0.319	100.89	2.48
Diabeta	19.87 ± 0.685	99.85	2.08
Euglucon	20.85 ± 1.582	101.15	1.85

Abbreviations: GLB: Glibenclamide; SD: Standard Deviation; SEM: Standard Error Mean; RE: Relative Error; RSD: Relative Standard Deviation; *: 100% excess of pure GLB was added

Table 6: Influence of excipients on developed method for recovery of GLB from its brands.

analysis of GLB in different brands of GLB tablets. The precision results of the validated method were within the acceptable range (Table 4).

We also applied the validated method for the qualitative and quantitative estimation of GLB in three brands of GLB tablets. The amount of GLB estimated by developed method was found to be almost similar as claimed in three brands of GLB tablets (Table 5). We also found that the excipients did not influence the overall assay procedure of validation method (Table 6).

Conclusion

To conclude, statistical analysis of the data depicted that the developed UV-visible spectrophotometric method was found to be accurate and precise. All the parameters enabled the rapid quantitative and qualitative estimation of GLB in three brands of GLB tablets without any excipient interference. Therefore, it can be concluded that the reported method could find practical implementations as an economical quality control tool for the analysis of active pharmaceutical ingredients from their final dosage forms on industrial as well as laboratory scale.

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