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Research Article

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Spectrophotometric Determination of Glibenclamide in Pharmaceutical Formulation using charge Transfer Complexation with Picric Acid

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Abstract A new, simple and low cost spectrophotometric method for the determination of Glibenclamide in pharmaceutical preparations was developed. The method was based on the interaction of Glibenclamide as n-electron donor with Picric acid as the π – acceptor. The absorbance maximum (λ_{max}) of the resultant complex was at 419 nm. Different variables affecting the reaction were carefully studied and optimized. Of the different solvents tried, Acetonitrile was found to be the most suitable solvent. Beer's law was obeyed in concentration range of 20 – 100 µg/ml Glibenclamide. The correlation coefficient was found to be (r = 0.9920). The limit of detection and limit of quantification were 1.40 µg/ml and 4.12 µg/ml, respectively. The reaction ratio between Glibenclamide and Picric acid was studied and found to be 1:1. The method was validated and results obtained for the assay of three different brands of Glibenclamide tablets were compared with the BP method (titrimetric). The repeatability and reproducibility of the developed method were evaluated and the obtained results quoted. The proposed method was successfully applied to the determination of Glibenclamide with good accuracy and precision.

Keywords Glibenclamide, Pharmaceutical analysis, Charge transfer complex, Method development, Spectrophotometry

Introduction

Glibenclamide (glyburide) is a second generation oral sulfonylurea anti-diabetic agent widely used for the treatment of type II diabetes mellitus and gestational diabetes mellitus [1,2]. It is completely and rapidly absorbed from the gastrointestinal tract and possesses a potent and long lasting anti-diabetic effect [3]. Glibenclamide acts mainly by stimulating endogenous insulin release from beta cells of pancreas. Chemically, Glibenclamide is 5-chloro-n-[2-[4[[[(cyclohexylamino)carbonyl]-amino]sulphonyl]phenyl]-ethyl]-2-methoxy benzamide.

Type II diabetes (formerly noninsulin-dependent diabetes mellitus or adult-onset diabetes) is a metabolic disorder characterized by hyperglycemia due to cellular resistance to insulin, combined with insufficient pancreatic secretion of insulin. Over 300 million people suffer from diabetes worldwide with type II diabetes representing between 90–95% of all reported cases in adults [4]. In Nigeria, as at 2013, it was estimated that about 5 million individuals are affected by diabetes [5] with a mean national prevalence of about 5 % [6].

Sulfonylureas are oral antidiabetic drugs that increase insulin release from pancreatic beta cells. Gliclazide, glibenclamide and glimepiride are second-generation sulfonylureas used as initial treatment of type II diabetes in patients who cannot control hyperglycemia with diet and exercise [7].

Several analytical methods have been described for the determination of glibenclamide such as TLC [8, 9]; UV Spectrophotometry [10-13]; Spectrofluorimetry [14]; HPLC–UV [15-16]; RP-HPLC [17-18]; HPLC–fluorescence [19-21]; CE–UV [22]; LC/API (MS) [23]. Some methods have also been developed for measurement of

Glibenclamide using liquid chromatography–tandem mass spectrometry (LC–MS/MS) [24-27]. Radi [28] described a voltametric method for the drug while Aggarwal and Sunshine [29] utilized a GLC method for the drug. Radio immuno assay methods have also been developed for the assay of Glibenclamide [30].

The challenge of counterfeit and sub-standard pharmaceuticals is a global one but the severity of the problem varies widely between countries, ranging from less than 1% in developed nations to 50% in some poor countries [31]. This, together with the fact that aging or unsuitable storage conditions of the drugs may lead to their degradation, engenders the need to develop alternative analytical methods that are simple and require only readily available equipment in modest laboratories of the developing world.

Materials and Methods

Materials and Equipment

Glibenclamide tablets were commercial products. The following reagents were obtained from Sigma-Aldrich, Germany: Methanol, Acetone, Dichloromethane, 1,4-dioxane, Ethanol. Acetonitrile (Lobachemie, India) and Picric acid (JT Baker Chemical Company). Pure Glibenclamide reference powder was obtained from Prof. Johnson Ogoda Onah, of the Department of Pharmaceutical Chemistry, University of Jos, Nigeria. All the chemicals and reagents used were of analytical grade and were used without further purification. The aqueous solutions were freshly prepared with double distilled water. A Shimadzu UV-Visible double beam spectrophotometer (Model 1250, Japan) with matched 1 cm quartz cells was used for the measurements.

Method

Preparation of 0.01% w/v Picric acid solution

Picric acid (0.025 g) was weighed on an analytical balance and dissolved in some acetonitrile in a 250 mL volumetric flask and the volume made up to mark.

Preparation of Glibenclamide stock solution

A 100 mg mass of the pure reference material was weighed and dissolved in some acetonitrile in a 250 mL volumetric flask and the volume made up to mark to form the stock solution (1 mg / mL). Working solutions were prepared by making appropriate dilutions of this standard Glibenclamide stock solution.

General Procedure

Aliquots of the stock solution were transferred to 10 mL-volumetric flasks followed by addition of 2 mL of freshly prepared Picric acid solution (0.01% w/v) to each of the flasks in order. The flasks were shaken and allowed to react for 15 minutes before their volumes were made up to 10 mL with Acetonitrile to give final drug concentration in the range of 20 – 100 µg/mL. A blank solution was prepared in the same way but excluding the analyte (Glibenclamide). The above solutions were all prepared in triplicates. The absorbance of each solution was measured at 419 nm against a blank.

Effect of reaction conditions

In accordance with the general procedure, the conditions influencing the charge transfer reaction were optimized commonly by single factor test method, in which the studied condition was changed only. These conditions include solvent, reaction temperature and time, reagent concentration.

Stoichiometric study

The Job method for continuous variation was employed to determine the stoichiometric ratio [32].

Validation of the proposed method

Under the specified optimum reaction conditions, the calibration curve for the drug was constructed. Regression equation for the data was derived with the aid of Microsoft Excel software program using the least-squares method. Each concentration of standard solution was assayed in triplicates and the mean absorbance obtained was then plotted versus concentration.

Determination of accuracy and precision of the proposed method

The precision and accuracy of the method were investigated based on inter-day variation (repeatability) assessment by analyzing Glibenclamide using six replicates within the limit of quantification range. The precision and accuracy

of the method were expressed as % RSD and recovery of the measured concentration, respectively. Also, reproducibility (within day or intraday variation) was investigated.

Determination of Glibenclamide content in tablet formulation using the proposed method

Five different brands of Glibenclamide tablet formulation were assayed using the developed method. For each brand, the contents of 20 tablets were weighed, ground into a fine powder. An accurately weighed portion of the powder equivalent to 100 mg Glibenclamide was transferred into a 100 mL volumetric flask. Next, 25 mL of acetonitrile was added and after some minutes of mechanical shaking, the suspension was made up to mark with the solvent. After filtration, suitable amounts of the filtrate were then taken and the analytical procedure was applied as previously described. The content of each label claim was verified by comparing the concentrations obtained from the validated curves with the actual concentrations of the drug taken. Standard deviations were also calculated for each brand.

Statistical Analysis

The percentage content of the various brands determined by the developed method and the official assay method were compared using student's t- test.

Results and Discussion

Interactions between electron donors and acceptors are usually accompanied with the formation of intensely coloured charge transfer complexes and this type of reaction has been utilized for the determination of many drugs. The theoretical basis and application of charge-transfer complex formation has been extensively studied [33]. Glibenclamide absorbs radiation in the UV range with a λ_{max} of 230 in methanol. The drug can react with a suitable chromogen to produce a derivative with improved light absorbing capacity usually at a longer wavelength which can be useful for the spectrophotometric determination of the drug in bulk and dosage forms. This served as the motivation to use Picric acid to form a charge transfer complex suitable for the determination of Glibenclamide. Upon formation of the yellow coloured complex, the maximum absorption was found to have shifted to 419 nm.

Based on the complexation reaction, we investigated the behaviour of Picric acid when allowed to react with Glibenclamide under different conditions that can influence the colour formation, intensity and stability. These factors include diluting solvent, the reagent concentration, the reaction time and temperature.

The effect of using different solvents with a range of dielectric constants (DE) for dilution was evaluated. Some of the solvents employed were: Acetone (DE 20.7), Acetonitrile (DE 37.5), Methanol (DE 32.7), Dichloromethane (DE 8.93) and 1, 4-dioxan (DE 2.25). It has previously been established that non-polar solvents enhance $n\rightarrow\pi^*$ transition while polar solvents enhance $\pi\rightarrow\pi^*$ transition.The interaction of Glibenclamide with Picric acid as π acceptor in non-polar solvents (low DE) such as 1, 4-dioxan and dichloromethane produced colored charge-transfer complexes with low molar absorptivity values. However, this improved with the use of higher polarity solvents and acetonitrile was found to be the most suitable diluting solvent. This can be attributed to its high dielectric constant which promotes maximum yield of radical anions and high solvation power for the acceptors [33, 34]. It can also be inferred that in this solvent, complete electron transfer from Glibenclamide (D), as an electron donor, to the acceptor moiety (A) took place with the formation of intensely colored radical ions with high molar absorptivity values, according to the following:

$D + A \rightleftarrows (D\text{-}A) \rightleftarrows D^{\cdot +} + A^{\cdot -}$

Complex radicals ions

The effect of the reaction time on the formation of product was evaluated at 25 ^oC by allowing the reaction to progress for different time periods. The reaction was complete within 15 minutes and longer reaction times did not affect the absorbance as shown by the plateau in Figure 1.

Attempts to accelerate the reaction by heating at different temperatures was not successful as the absorbance values obtained began to decline at temperatures beyond room temperature (Figure 2) and it can therefore be concluded that the optimal temperature for the stability of the complex is 25 ^oC.

The effect of time on the stability of the charge transfer complex was examined by monitoring the absorbance of the complex at different time intervals. The absorbance was stable for 12 hours, which implies that the method is

reliable and will be useful for the processing of a large number of samples. To optimize the concentration of the reagent, 2 ml of varying concentrations (0.004, 0.006, 0.008, 0.010 and 0.012 % w/v) of Picric acid in acetonitrile were used for colour development The results revealed that 2 mL of 0.01 %w/v Picric acid was sufficient to generate the maximum, reproducible colour intensity of the complex (Figure 3).

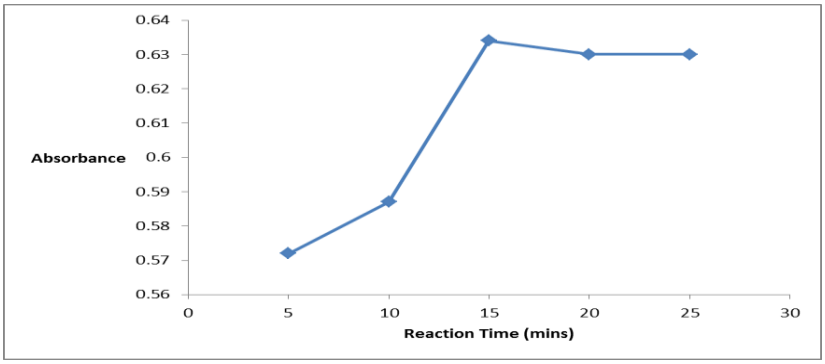

Figure 1: Effect of reaction time on Glibenclamide-Picric acid charge transfer complexation

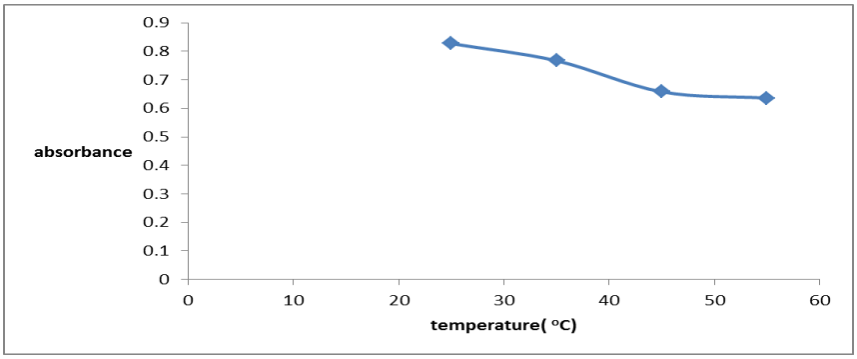

Figure 2: Effect of temperature on Glibenclamide - Picric acid charge transfer complexation

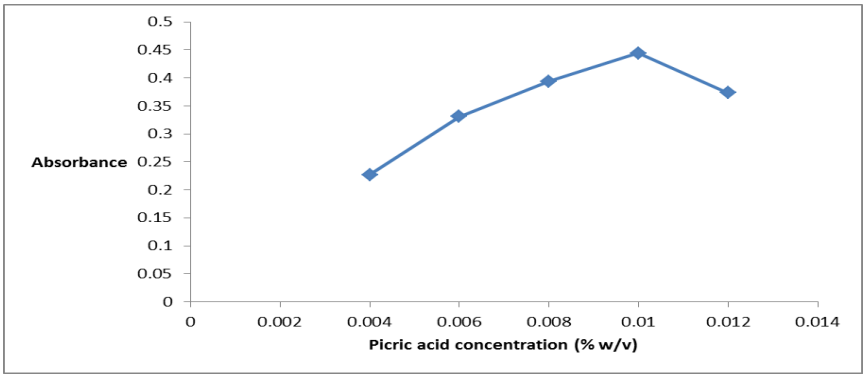

Figure 3: Effect of Picric acid concentration on Glibenclamide -Picric acid charge transfer complexation

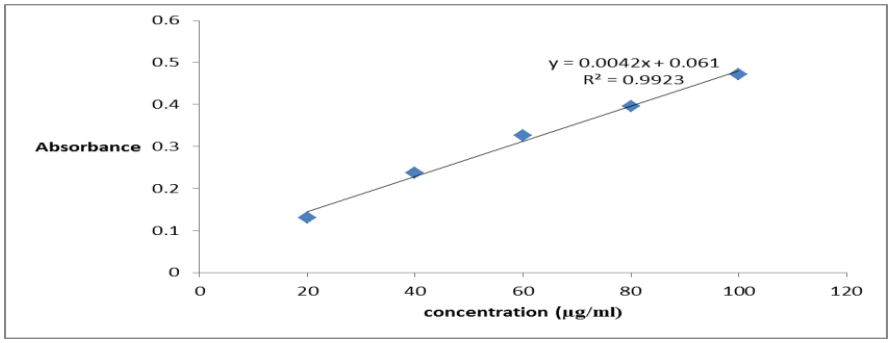

Figure 4: Calibration curve for Glibenclamide -Picric acid charge transfer complex

Under the optimized reaction conditions, calibration curve was obtained by plotting Absorbance versus concentration which showed a linear relationship. Beers Law was obeyed in the range of 20-100 µg/mL. The molar absorptivity, Sandells sensitivity, regression equation and correlation coefficient were determined and are shown in Table 1.

Table 1: Summary of analytical parameters for the proposed method

S/N	Parameter	Result
1.	Absorption maximum	419nm
2.	Regression equation	Y_{abs}=0.0042x + 0.061
3.	Correlation coefficient (r^2)	0.9923
4.	Beers Law Limit	20 – 100 µg/mL
5.	Molar Absorptivity	2.63 x 10^4 L/mol/cm
6.	Sandells sensitivity	0.534µg/cm^2
7.	Limit of detection	1.40 µg/mL
8.	Limit of Quantification	4.12 µg/mL

The limit of detection (LOD) and the limit of quantification (LOQ) for Glibenclamide using the proposed method were found to be 1.40 µg/mL and 4.12 µg/mL, respectively. The statistical calculation of these limits was performed using the following equation:

LOD = 3 *s*/*k* and LOQ = 10 *s*/*k*, where *s* is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte, and *k* is the slope of the calibration curve.

Molar absorptivity and Sandells' sensitivity were similarly calculated to be 2.63x10^4 L mol^{-1} cm^{-1} and 0.534 µg cm^{-2} respectively. The inter-day variation ranged between 1.22-1.84 % and the intra-day variation ranged from 1.39-2.11 % for the method.

Investigation of the stoichiometry of the reaction using Job's method revealed a 1:1 ratio for the complexation between Glibenclamide and Picric acid (Figure 5 below).

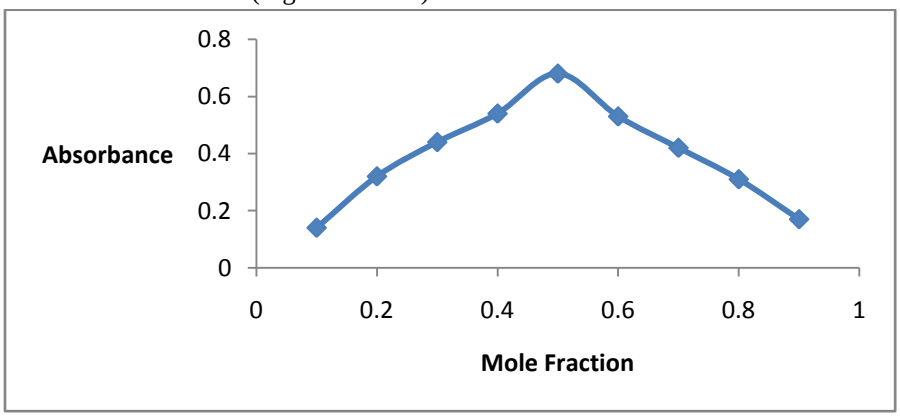

Figure 5: Continuous variation plot for Gliebnclamide- Picric acid charge transfer complexation

Table 2 shows the results obtained for the analysis of three brands of Glibenclamide tablets using the developed method as compared to the BP method. The results obtained from comparisons using the student's t-test suggest that there are no significant differences between the proposed method and the B.P method.

Table 2: Percentage Content (%) of Glibenclamide ±SD in tablets using proposed method Compared to the BP method

Brand	Label Claim	Titrimetric Method [35]	Developed Method
Glemids®	5 mg	103.11 ± 0.49	104.16 ± RSD 0.37
Diabeta®	5 mg	100.19 ± 0.84	101.80 ± RSD 0.43
Glanil®	5 mg	102.44 ± 0.92	103.02 ± RSD 0.20

n (number of replicates) = 3; * indicates significant difference at p<0.05.

Most of the reported methods for the assay of Glibenclamide require expensive or sophisticated instruments or involve procedures with intricate control of experimental conditions. This is in contrast to the developed method which is quite simple and accurate and it can therefore be concluded that the developed method is suitable for routine analysis of Glibenclamide, even during field work.

Conclusion

The proposed charge transfer method using the π acceptor picric acid has been demonstrated to be suitable for the spectrophotometric analysis of Glibenclamide in formulated products. The method has the advantage of being simple, accurate, sensitive and suitable for routine quality control of Glibenclamide in dosage form without any interference from excipients.

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