Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(15):94-102
(http://derpharmachemica.com/archive.html)

Development of a Validated Initial Rate Method for the Assay of Gliclazide in Drug Formulations

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ABSTRACT

The objective of this work was to develop a simple, sensitive and kinetics based spectrophotometric method for the determination of gliclazide in drug formulations. The method was based on the oxidation of the drug with an alkaline potassium permanganate at room temperature (25±1 ^{0}C). The reaction was followed spectrophotometrically by measuring the rate of change of the absorbance at 602 nm. The initial rate method was adopted for constructing the calibration curve which was linear in the concentration range of 1.5 ×10^{-5} M -1.85 ×10^{-4} M corresponding to 5.0 – 60.0 µg/mL. The limits of detection and quantitation were 0.99 and 3.03 µg/mL, respectively. Statistical comparison of the results shows excellent agreement and indicates no significant difference in accuracy and precision. The method can be used for routine quality control testing of commercial dosage forms.

Keywords: Gliclazide, initial rate method, drug formulations, accuracy and precision, kinetic spectrophotometry

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INTRODUCTION

Diabetes mellitus is a group of metabolic diseases categorized on the response of body cells towards insulin and known as insulin dependent diabetes mellitus (IDDM, Type I) and non-insulin dependent diabetes mellitus (NIDDM, Type II). Gliclazide is an oral antihyperglycemic agent used to improve the defective insulin secretion and may reverse insulin resistance observed in patients with NIDDM, Type II.It is chemically known as 1-[(4-methylbenzene) sulfonyl] -3- octahydrocyclopenta [c] pyrrol-2-yl} urea (M. formula $C_{15}H_{21}N_3O_3S$, M.W. 323.41). Gliclazide is a second generation sulphonylurea binds to the β-cell sulfonyl urea receptor, which subsequently blocks the ATP sensitive potassium channels, results in closure of the channels and leads to a decrease in potassium efflux resulting in depolarization of the β cells. Gliclazide has been described to have antioxidant properties, *in vitro* and in vivo independent of glycemic control, due to its azabicyclo-octyl ring which is believed to act as a general free radical scavenger in vitro [1,2]. Gliclazide is metabolized via three primary methods: oxidation of the tolyl group; hydroxylation of the azabicyclo-octyl ring; and glucuronidation. Gliclazide is metabolized by the liver and the majority of the excreted drug is found in the form of one of the metabolitesin both urine (60-70%) and feces (10-20%).The drug is officially listed in British Pharmacopoeia, which describes a nonaqueous titration method for the assay of gliclazide in bulk and dosage forms [3].In view of the importance of the drug, several analytical methods including high-performance liquid chromatography (HPLC) [4-15], liquid chromatography- mass spectrometry [16], capillary gas chromatography [17], TLC-densitometry [l8], voltammetry [19]and fluorimetry[20] were reported to determine gliclazide in pure, pharmaceutical formulation and biological fluids. Undoubtedly, the above mentioned methods are sophisticated and sensitive. However, they employ lengthy procedures and some of the instruments are unavailable in most labs due to high cost making some of these methods of limited use. These limitations can be eliminated by introducing simple spectrophotometric methods. Literature search revealed few UV spectrophotometric methods [21-24] for the estimation of gliclazidein commercial dosage forms. Few visible

spectrophotometric methods for the assay of gliclazide were also reported in its bulk and formulations based on extraction[25] and direct methods[20, 26].

Kinetics based spectrophotometric methods are of great importance in pharmaceutical industries due to their simplicity, elimination of tedious extraction and filtration steps prior to the absorbance measurement, and improved selectivity. The interference of coloured or turbid samples and certain active ingredients present in commercial dosage forms can also be avoided.This paper proposed a kinetic spectrophotometric method by utilizing the oxidizing property of potassium permanganate in alkaline medium for the determination of gliclazide in drug formulation. The method is based on the reaction of methyl group of gliclazide with alkaline $KMnO_4$ resulting in the formation of green colored product that absorbs maximally at 602 nm. The absorbance increases with time and therefore a kinetic method, i.e., an initial rate method is adopted for the determination of gliclazide in commercial dosage forms.

MATERIALS AND METHODS

Apparatus

Spectral runs were made on Spectronic 200 Visible Spectrophotometer (Thermo Scientific, USA) with 10 mm matched square cuvettes for all spectral and absorbance measurements.

Standard and Reagents

- All chemicals and reagents used were of analytical or pharmaceutical grade.
- Standard stock solution of gliclazide(0.5 mg/mL, 1.54×10^{-3} M) was prepared by dissolving 50 mg in small amount of methanol and finally diluted with distilled water in a 100 mL volumetric flask. This solution was used to prepare the calibration curve and quality control samples.
- Quality control samples were prepared at three concentration levels: 10, 30 and 50 µg/mL. Pharmaceutical formulations of gliclazide such as Diamicron®MR (Les Laboratories Servier, France) and Glaze®(SPIMACO, Saudia Arabia)were obtained from commercial sources.
- A 0.005M $KMnO_4$ (LobaChemie Pvt. Ltd., India) and 2 M NaOH((LobaChemie Pvt. Ltd., India)aqueous solutions were freshly prepared. The solutions were standardized by the recommended procedures.

Recommended procedure for the determination of Gliclazide

Initial rate method

Into a series of 25 mL standard volumetric flasks, 2.4 mL of 0.005 M $KMnO_4$ and 1.7 mL of 2 M NaOH solutions were transferred. Then, a varied amount of 0.5 mg/mL of gliclaize (0.25- 3.0 mL) were pipetted into the standard volumetric flasks and diluted to volume with distilled water at room temperature (25± 1^0C). The contents of each flask were mixed well and solutions were transferred to spectrophotometric quarts cuvettes to record the increase in absorbance at 602 nm as a function of time for 15 min against the reagent blank prepared similarly without drug.

The initial rates of reaction (v) at different concentrations were obtained from the slope of the tangent of the absorbance-time curves. The initial rate of the reaction was plotted against the initial concentrations of gliclazide for the alkaline medium. Alternatively, a regression equation was also developed for the quantitative estimation of gliclazide.

Procedure for the assay of Gliclazide in commercial dosage forms

Two commercially available tablets (Diamicron® MR and Glaze®) each containing 80 mgof gliclazidewere weighed and finely powdered. Weighed quantity of the powder equivalent to 50 mg gliclazide was transferred into a small conical flask and was extracted with 10 mL methanol. The solid was then extracted with distilled water (4× 10 mL) by shaking and filtering through a Whatmman filter paper. The residue was washed well with distilled water for complete recovery of drug and finally the filtrate was diluted with the appropriate volume to give a concentration of 0.5 mg/mL. The assay was completed following the recommended procedure for the determination of gliclazide.

Limit of detection (LOD) and limit of quantitation (LOQ)

According to the International Conference on Harmonization (ICH) guidelines, the following expressions are used to evaluate LOD and LOQ [27]:

$$LOD = 3.3 \times S_0 / b \text{ and } LOQ = 10 \times S_0 / b$$

Where S_0 and b are standard deviation and slope of the calibration line, respectively.

Method Validation

The proposed method has been validated for linearity, precision, accuracy, interference and recovery studies.

Linearity
For evaluation of linearity, determination of gliclazide was done at seven concentration levels: 5.0, 10.0, 20.0, 30.0, 40.0, 50.0 and 60.0 µg/mL. Each concentration was analyzed for five times.

Precision and accuracy
Three concentrations within the linearity range were selected: 10.0, 30.0 and 50.0 µg/mL. Five sample solutions of each concentration were prepared and analyzed within one day. Additionally, this assay was repeated for five consecutive days. The intra and interday precision and accuracy were also determined by analyzing the quality control samples that were tested five times in one day and over five consecutive days.

Interference and recovery studies
The proposed method was applied to the determination of gliclazide in its pharmaceutical formulations. Common tablet excipients such as talc, lactose, starch, avisil, gelatin, and magnesium stearate did not interfere with the assay. The results were compared with those obtained by using a reference method [22].

RESULTS AND DISCUSSION

Spectral studies

The spectrum of the pure drug was scanned in the UV range (200-400 nm) using methanol: distilled water (1:4) as a blank and absorption maxima was found to be 225.5 nm (Fig. 1a). The reagent blank (alkaline $KMnO_4$) shows one peak at 525 nm when measured against methanol: water (1:4) as a reference. The addition of alkaline $KMnO_4$ solution to the drug solution resulted in the shift to a new characteristic peak at 602 nm (Fig. 1b) due to the formation of manganate ion in the presence of drug. The equilibrium is attained in about 40 min. Therefore, a kinetically based method (Initial rate) was developed for the quantitative estimation of glicalzide by measuring the increase in absorbance at 602 nm as a function of time.

Optimization of variables

The spectrophotometric properties of the colored product as well as the different experimental parameters affecting the color development were carefully studied and optimized. The effect of $KMnO_4$ concentrations on the absorbance of the colored product was studied keeping the concentration of drug and NaOH constant and varying $KMnO_4$ concentration. It was found that increasing the volume of $KMnO_4$ resulted in a subsequent increase in the absorbance value of the colored product up to 2.2 mL and remained constant up to 2.6 mL. Therefore 2.4± 0.2 mL which resulted in a final concentration of 3.04×10^{-6} M was used as the optimum concentration of $KMnO_4$ (Fig. 2).
The effect of concentration of NaOH on the absorbance of the colored product was also studied keeping the concentration of drug and $KMnO_4$ constant. It was observed that increasing the molar concentration of NaOH resulted in a gradual increase in the absorbance of the colored product up to 1.12×10^{-1} M and then absorbance remained constant up to 1.60×10^{-1} M. Thus a final concentration of 1.36×10^{-1}M was used as the optimum concentration of NaOH (Fig. 3).

Stoichiometry

The stoichiometric ratio between potassium permanganate and gliclazide in alkaline medium was ascertained by the limiting logarithmic method. Two sets of experiments were used for the analysis. In the first set, gliclazide concentration was varied keeping $KMnO_4$ concentration fixed while in the second set it is the concentration of $KMnO_4$that was varied. The logarithmic of the absorbance, thus obtained, was plotted against the logarithm of the molar concentration of $KMnO_4$ or gliclazide. The slopes of the two straight lines were perceived in each case indicating the combining molar ratio between gliclazide and $KMnO_4$ as 1:1. (Fig. 4).

It has been reported that the oxidative attack occurs at the methyl group on the benzene ring of gliclaizide[28, 29].On the basis of the stoichiometric relationship and literature background, the oxidative reaction is proposed and given in scheme1.

Analytical Data

The initial rates of the reaction were determined from the slope of the initial tangent to absorbance-time curve(Fig. 5).Under the optimized experimental conditions, a pseudo-order reaction condition was worked out by using a large excess of $KMnO_4$ and NaOH solution with respect to the initial concentration of gliclazide. As a result, a pseudo zero order condition was obtained with respect to the reagents; the overall concentration change of $KMnO_4$ and NaOH during the course of reaction would be negligible. The initial rates of the reaction were determined from the slopes of the initial tangent to the absorbance–time curves and are summarized in Table 1. The reaction would obey the following rate equation:

$$v = dA/dt = K'C^n$$

where K' is the pseudo-order rate constant, C is the concentration of gliclazide, n is the order of the reaction.The logarithm form of the above equation is written as:

$$\log(v) = \log \Delta A/\Delta t = \log K' + n \log C$$

The linear regression analysis using the method of least square treatment of calibration data was used to evaluate slope, intercept and correlation coefficient. Under the working experimental conditions, a calibration graph was constructed by plotting log of initial rate of reaction (log*v*) *versus* log of gliclazide concentration (log*C*), which showed a linear relationship over the concentration range of 5.0- 60.0 µg/ml. The regression of log *vversus* log*C* gave the following linear regression equation:

$$\log(v) = 3.6877 + 0.997 \log C$$

With a correlation coefficient (*r*) of 0.9999. The value of *n* in the regression equation confirmed that the reaction is first order with respect to gliclazide. The confidence limits for the slope of the line of regression and intercept were computed using the relation $b \pm tS_b$ and $a \pm tS_a$ at 95% confidence level and found to be $0.997 \pm 3.49 \times 10^{-3}$and $3.687 \pm 1.45 \times 10^{-2}$, respectively. This showed the high reproducibility of the proposed method.

The limits of detection (LOD) and quantitation (LOQ) were evaluated and found to be 0.99 and 3.03 µg/mL, respectively. Linear dynamic range, correlation coefficient, variance, standard deviation and confidence limits for slope and intercept of the calibration line are summarized in Table 1.The low value of variance (S_0^2) indicated negligible scattering of the experimental data points around the line of regression.

Method Validation Parameters

Accuracy and Precision

The accuracy and precision of the proposed kinetic spectrophotometric method was determined in terms of intermediate precision. Five replicates were performed on pure drug solution at three different concentration levels within the specified range and were analyzed during the same day (intraday precision) and for five consecutive days (interday precision). The analytical results obtained by the initial rate method are compiled in Table 2. The percentage relative standard deviation (% RSD) as precision and percentage recovery as accuracy of the proposed method of gliclaizde from the calibration curve showed that the present proposed method has good repeatability and reproducibility. The results of these assays are reported in Table 2. The intraday and interday precision assays were also carried out for gliclazidein pharmaceutical preparations. The results are summarized in Table 3.

Interference and recovery studies

Recovery experiments were carried out by the standard addition method. The concentration of gliclazide was added to pre-analyzed pharmaceutical formulations (Diamicron® MR, Glaze®) at three concentration levels by measuring five replicate analyses following the recommended procedures for the determination of the active drug. The results, summarized in Table 4, showed excellent recoveries (98.46%–101.9%) with low values of relative standard deviations (0.33%– 1.24%). It is also clear from the data that no interference from the common excipients used in tablets was observed.

Table 1Summary of optical and regression characteristics using the initial rate method

Parameters	
λ_{max} (nm)	602
Linear dynamic range (µg/mL)	5.0 – 60.0
Regression equation	log v = 3.6877 + 0.997 log C
S_0[a]	2.71×10^{-3}
Intercept(a)	3.687
S_a[b]	5.24×10^{-3}
$\pm tS_a$[c]	1.45×10^{-2}
Slope (b)	0.997
S_b[d]	1.26×10^{-3}
$\pm tS_b$[e]	3.49×10^{-3}
Correlation coefficient (r)	0.9999
Variance $(S_0)^2$	7.398×10^{-6}
LOD (µg/mL)	0.991

[a]*Standard deviation of the calibration line,*
[b]*Standard deviation of the intercept,*
[c]*Confidence interval of the intercept at 95% confidence level,*
[d]*Standard deviation of the slope,*
[e]*Confidence interval of the slope at 95% confidence level.*

Table 2 Summary of accuracy and precision results in pure form

Proposed methods	Amount (µg/ml)		Recovery (%)	RSD[a] (%)	SAE[b]	C.L.[c]
	Taken	Found ± SD[a]				
Intraday assay	10.00	10.040 ± 0.0008	100.404	0.537	0.0004	0.0010
	30.00	30.260 ± 0.0020	100.867	0.416	0.0009	0.0024
	50.00	49.911 ± 0.0015	99.822	0.196	0.0007	0.0019
Inter day assay	10.00	10.028 ± 0.0011	100.275	0.733	0.0005	0.0014
	30.00	29.976 ± 0.0028	99.919	0.598	0.0012	0.0034
	50.00	49.885 ± 0.0015	99.971	0.192	0.0007	0.0018

[a]Mean for five independent analyses. [b]SAE. standard analytical error. [c]C.L. confidence limit at 95% confidence level and four degrees of freedom (t = 2.776).

Table 3 Summary of accuracy and precision results in pharmaceutical formulations

Proposed methods	Amount (µg/ml)		RSD (%)	Recovery (%)	SAE[b]	C.L.[c]
	Taken	Found ± SD[a]				
Intra day assay						
Diamicron® MR	10.00	9.885 ± 0.00114	0.743	98.853	0.0005	0.0014
	30.00	29.872 ± 0.00385	0.832	99.575	0.0017	0.0048
	50.00	49.924 ± 0.0037	0.479	99.948	0.0016	0.0046
Glaze®	10.00	10.002 ± 0.00148	0.956	100.017	0.0007	0.0019
	30.00	29.717 ± 0.00432	0.939	99.057	0.0019	0.0053
	50.00	49.847 ± 0.00594	0.770	99.693	0.0026	0.0073
Inter day assay						
Diamicron® MR	10.00	9.898 ± 0.0023	1.499	98.982	0.0010	0.0028
	30.00	30.079 ± 0.0031	0.668	100.264	0.0014	0.0039
	50.00	49.963 ± 0.0065	0.821	99.926	0.0028	0.0079
Glaze®	10.00	9.963 ± 0.0026	1.687	99.629	0.0012	0.0032
	30.00	30.273 ± 0.0046	0.971	100.911	0.0020	0.0057
	50.00	49.976 ± 0.0093	1.208	99.952	0.0028	0.0079

[a]SD. standard deviation. [b]SAE. standard analytical error. [c]C.L. confidence limit at 95 % confidence level and four degrees of freedom (t = 2.776)

Table 4 Standard addition method

Proposed method	Amount (µg/mL)			Recovery (%)	RSD (%)	SAE[b]
	Taken	Added	Found ± SD[a]			
Diamicron® MR	5.00	5.00	9.847 ± 0.0015	98.465	0.971	0.0007
	5.00	15.00	20.241 ± 0.0023	101.204	0.734	0.001
	5.00	25.00	29.730 ± 0.0015	99.101	0.329	0.0007
Glaze®	5.00	5.00	9.976 ± 0.0019	99.758	1.243	0.0009
	5.00	15.00	20.283 ± 0.0016	101.915	0.520	0.0007
	5.00	25.00	29.924 ± 0.0044	99.747	0.948	0.0002

[a]Mean for five independent analyses. [b]SAE standard analytical error. RSD,relative standard deviation.

Table 5 Comparison of the proposed methods using point and interval hypothesis tests

Formulations	Proposed method						Reference method[22]	
	Recovery (%)	RSD[a] (%)	t-value[b]	F-value[b]	θ_L[c]	θ_U[c]	Recovery (%)	RSD[a] (%)
Diamicron® MR	100.02	0.99	0.23	1.55	0.994	1.008	99.94	0.80
Glaze®	100.07	1.16	0.23	1.53	0.988	1.010	100.17	0.94

[a]Mean for five independent analyses.
[b]Theoretical t-value ($\nu = 8$) and F-value ($\nu = 4, 4$)at 95 % confidence level are 2.306 and 6.39, respectively.
[c]In pharmaceutical analysis, a bias, based on recovery experiments, of ±2 % is acceptable.

Pharmaceutical applications

The proposed method was further successfully applied to the determination of gliclazide in its drug formulations. Commercial dosage excipients such as talc, lactose monohydrate, starch and magnesium stearate did not interfere with the assay. The result of the initial rate method was compared with the reference method [22] using point and interval hypothesis tests [30] and is summarized in Table 5. The calculated t- (paired) and F- values at 95% confidence level do not exceed the theoretical ones indicating no significant differences between the performance of the proposed method and the reference method. A bias of ± 2%, which is based on recovery experiments, is

permissible by The Canadian Health Protection Branch [31]. Therefore, the acceptable limit lies within θ_L= 0.98 and θ_U= 1.02. It is evident from Table 5 that the true bias of all samples of drug is smaller than ± 2% and thus confirming that the proposed method is reliable with acceptable recovery.

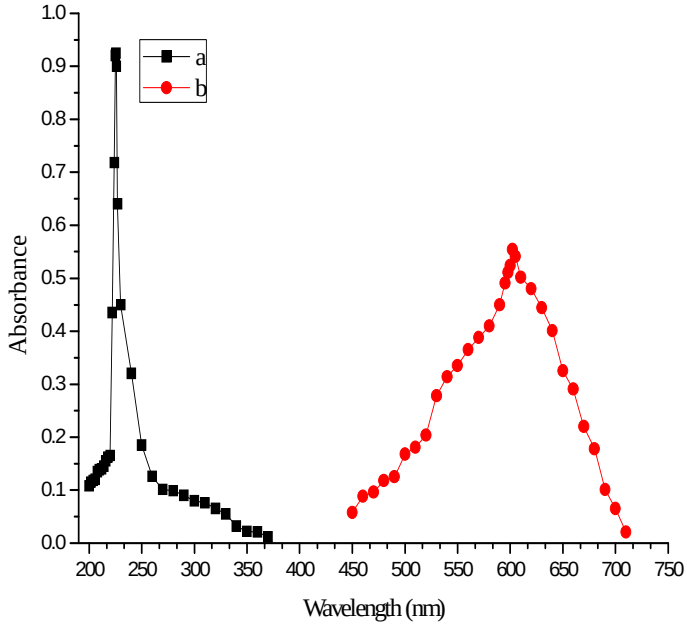


Fig. 1a.Absorption spectra of gliclazide(0.5mg/mL)*vs* methanol: distilled water (1:4) and b. 2.5 mL gliclazide(0.5 mg/mL) + 2.2 mL $KMnO_4$(0.005 M)+ 1.7 mLNaOH (2 M) *vs*. blank [2.2 ml $KMnO_4$ (0.005 M)+ 1.7 mLNaOH (2 M)]in distilled water

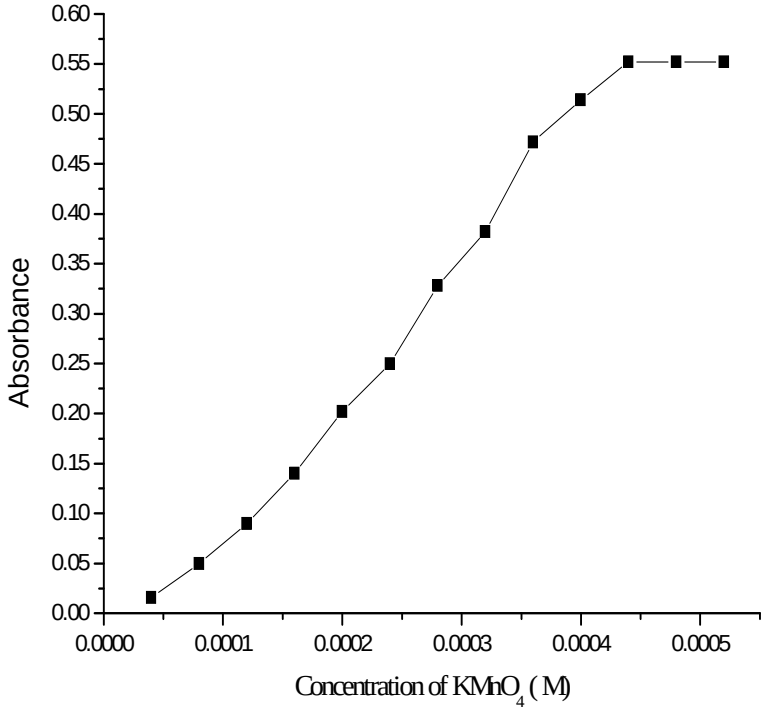


Fig. 2Effect of concentration of $KMnO_4$(0.005 M); keeping constant concentration of gliclazide(50µg/mL) and NaOH (1.36×10^{-1} M) in 25 mL volumetric flask

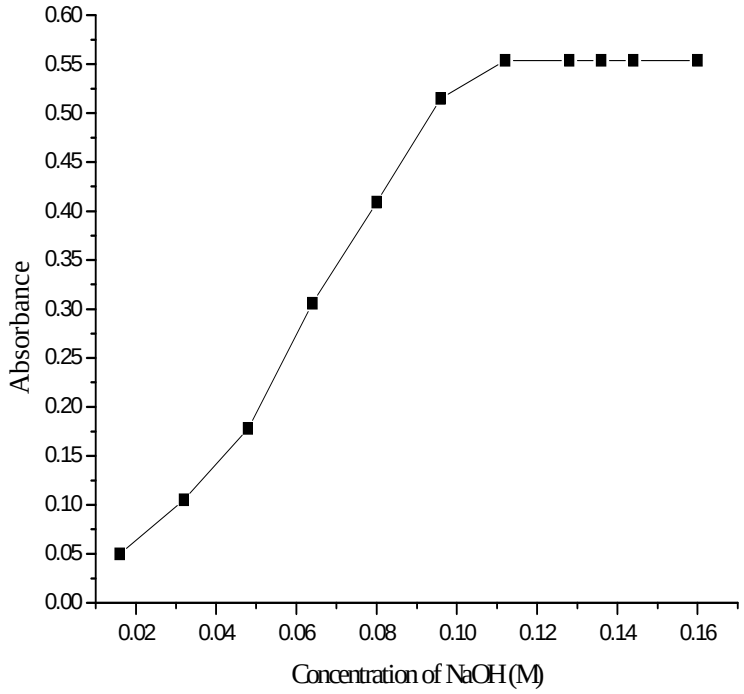


Fig. 3Effect of the concentration of NaOH(2M); keeping constant concentration of gliclazide (50µg/mL) and $KMnO_4$(4.4×10^{-4} M)in 25 mL volumetric flask

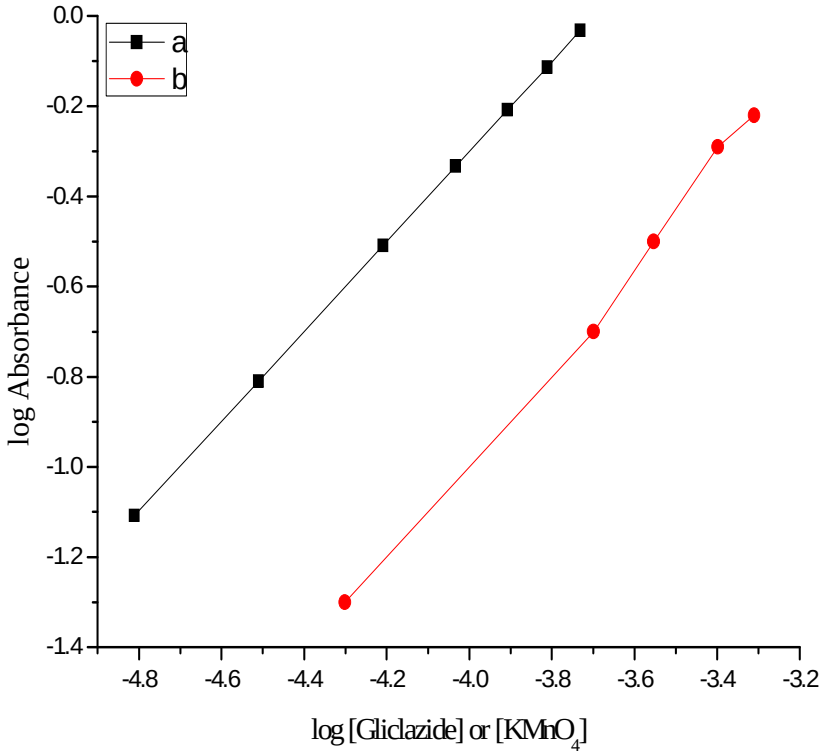


Fig. 4 Limiting logarithmic plot for molar combining ration between gliclazide and $KMnO_4$ in alkalinemedium:a. log A*vs* log [Gliclazide]; b.log A *vs* log [$KMnO_4$]

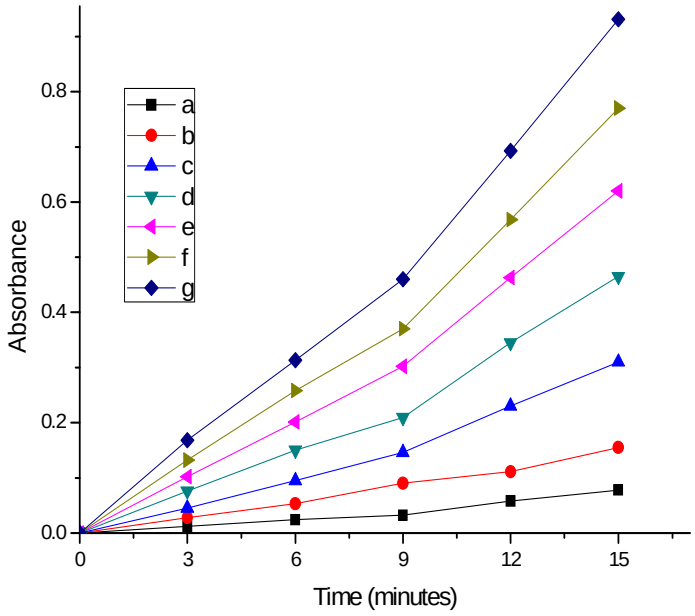
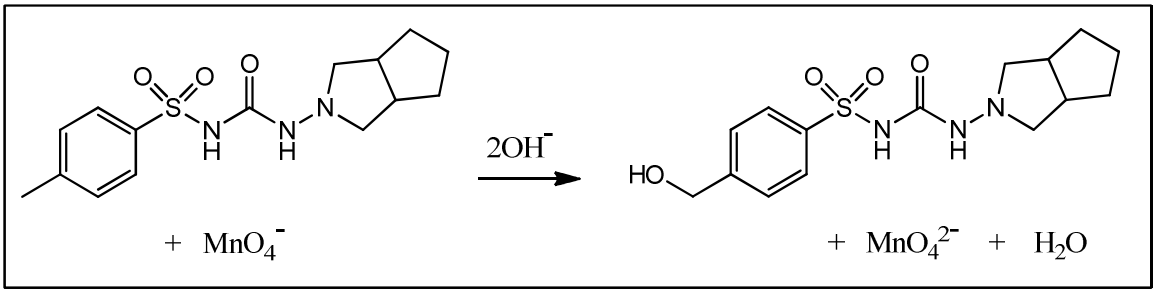


Fig. 5Absorbance -time plot for the reaction between gliclazide and $KMnO_4$ in alkaline medium
a. 5 µg/mL, b. 10 µg/mL, c. 20 µg/mL, d. 30 µg/mL, e. 40 µg/mL, f. 50 µg/mL, g. 60µg/mL



Scheme1.Proposed oxidative reaction of gliclazide

CONCLUSION

The proposed validated kinetic spectrophotometric method provides a simple, cost effective, fast and efficient method that does not require any laborious clean up procedure before measurement. Low-cost reagents enable their frequent application in the research laboratories, pharmaceutical industries and hospitals. In addition, the method has good linear dynamic range with good accuracy and precision. The method shows no interference from the common excipients and additives. Therefore, it is concluded that the proposed method provides a rapid determination of gliclazide and may be used as an alternative method to reported ones for routine determination of gliclazide in bulk and pharmaceutical formulations.

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