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Bromometric Estimation of Gliclazide and Glibenclamide in Bulk and in tablet Formulation.

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ABSTRACT:

Two spectrophotometric methods described for determination of, Gliclazide and Glibenclamide in bulk and pharmaceutical dosage forms using insitu generated bromine as oxidizing agent and either methylene blue or methyl orange as chromogenic agents. Drugs are treated with known excess of bromine and residual unreacted bromine is determined by treating with fixed amount of either methylene blue or methyl orange then measuring absorbance at 669 nm and 508 nm, respectively. The amount of bromine reacted corresponds to the amount of each drug. Effects of acidity, bromate - bromide volume and reaction time, on the absorption were studied. Calibration curves were linear over ranges of 3–10 $\mu g.ml^{-1}$ for Gliclazide, 4- 24 $\mu g.ml^{-1}$ for Glibenclamide in case of methylene blue and of 2–6 $\mu g.ml^{-1}$ for Gliclazide, 4-12$\mu g.ml^{-1}$ for Glibenclamide in case of methyl orange. The methods were validated and satisfactory applied for the determination of drugs in both bulk and in tablet form and results were compared statistically with reported methods.

KEYWORDS: Gliclazide, Glibenclamide, Methylene blue (M.B) and Methyl orange (M.O).

INTRODUCTION

The term diabetes mellitus describes is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. (WHO; 1980) The first widely accepted classification of diabetes mellitus was published by the Expert Committee proposed two major classes of diabetes mellitus and named them, (Insulin dependent diabetes mellitus) IDDM or Type 1, and (non-Insulin dependent diabetes mellitus) NIDDM or Type 2. (WHO; 1997)

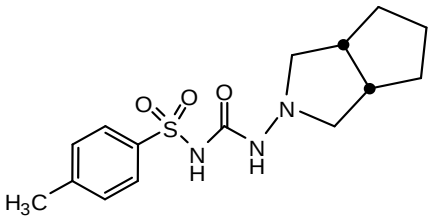
Gliclazide and Glibenclamide are related to Sulphonylurea drugs group, Gliclazide is a second generation Sulphonylurea drug (Fig.1), Gliclazide chemically is 1-(3- Azabicyclo[3.3.0]oct-3-yl)-3-tosylurea;1-(3-Azabicyclo[3.3.0]oct-3-yl)-3-p-tolylsulphonylurea.it is official drug (BP, 2013 Ph. Eur. monograph 1524). Several methods have been reported for determination of Gliclazide either alone or in multicomponent formulations. The methods involves different techniques such as spectrophotometric methods (EL-Enany, 2003; EL-Enany, 2004; Revathi *et al*., 2010; Dhabale and Seervi, 2010; Singh *et al*., 2011), HPLC methods (Gandhimathi *et al.*,2003; Kanij *et al*.,2010; Mansoory and Jain, 2012). HPTLC method (Patil *et al.*, 2014), and L.C. method (Vasudevan *et al*., 2001).

Glibenclamide is also a second generation Sulphonylurea drug (Fig.1). Glibenclamide chemically is 1-{4-[2-(5-Chloro-2-methoxybenzamido) ethyl]benzenesulphonyl}-3-cyclohexylurea, it is official drug (BP, 2013 Ph. Eur. monograph 0718) . Several methods have been reported for determination of Glibenclamide either alone or in multicomponent formulations as UV methods (Patil and Bonde, 2009; Epan *et al*., 2012; Godse *et al*.,2012; Parameswararao *et al.*, 2012; Bilal *et al*., 2013), HPLC methods (Ioannis and Athanasios , 2002; Rajendran, 2007; Venkata, 2011; Angshuman *et al.*, 2012; Jayanthi *et al.*, 2012; Tengli *et al.*, 2013; Narmad *et al*.,2014; SaiThanuja *et al*, 2014), HPTLC methods (Shweta and Sunil, 2010; Sanjay and Mulla , 2104) and T.L.C method.(El-Kousy, 1998).

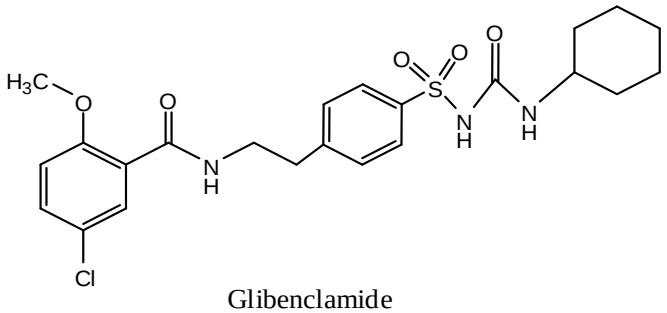
Redox reactions are employed in determination of inorganic cations and anions as well as organic substances. They have also been used as indicator reaction for kinetic catalytic methods. In redox reactions, the reaction products include the oxidized (or reduced) form of the analyte and the reduced (or oxidized) form of the reagent. Change in the absorbance of one of the reactants or products, induced by the reaction, can be employed in the determination.

An example of redox reactions is the oxidation of the analyte by reagent (bromine) and then excess reagent is determined using other spectrophotometric reaction (such as oxidation of methylene blue or methyl orange by excess bromine followed by determination of residual dye).

This method has been widely employed in determination of pharmaceuticals (as a sensitive and rapid method) such as Cyproheptadin (Basavaiah, 2006), Amlodipine (Basavaiah and Chandrashekar, 2006), Salbutamol Sulphate (Somashekar, and Basavaiah, 2007), Gatifloxacin (Basavaiah K. and Tharpa, 2007a), Pantoprazole (Basavaiah and Kumar, 2007b), Simvastatin (Basavaiah, and Tharpa, 2008), Doxcycline (Ramesh *et al*., 2010), levofloxacin HCl, lomefloxacin HCl and sparfloxacin (Elshanawany *et al.*, 2011), and Cefepime ,Cefoperazone and Ceftriaxone (Elshanawany *et al.*, 2014). In this study, Gliclazide, Glibenclamide have been determined Spectrophotometrically through indirect redox method depending on oxidation of drugs by insitu generated bromine and evaluation of excess bromine by using either methylene blue or methyl orang.



Gliclazide



Glibenclamide

MATERIAL AND METHODS

Apparatus

Schimadzu® (1600) UV-VIS Double Beam Spectrophotometer with matched 1 cm quartz cells

Materials and reagents

All chemicals and reagents were of analytical or pharmaceutical grade. Water was always doubly glass distilled and filtered. 5M HCl (El-Nasr Chemicals,

Egypt) was prepared by dilution of 225ml of concentrated HCl (36%) to 500 ml. Methylene Blue and Methyl Orange 60 µg/ ml (Universal Fine Chemicals, India) stock solutions were prepared by dissolving 60 mg of the dye in 20 ml of absolute methanol then completed to 100 ml with bidistilled water. Working solution was freshly prepared daily by dilution of 10 ml of stock solution to 100 ml with bidistilled water (60µg/ml). Bromate / Bromide stock solution was prepared by dissolving 0.1 gm. of potassium bromate (Winlab, England) and 1.0 gm of potassium bromide (Winlab, England) in 100 ml bidistilled water. Working solution was freshly prepared daily by diluting 2.5 ml of stock solution to 100 ml with bidistilled water (25µg/ml in case of methylene blue), or 1.25 ml of stock solution to 100 ml with bidistilled water (12.5µg/ml in case of methyl orange). Gliclazide purity of 99.85% (EPICO) , Glibenclamide purity of 99.4% (ADCO) .Standard stock solutions of all drugs were prepared by dissolving 20mg of each pure drug in 100 ml absolute methanol, working solutions of drugs were freshly prepared daily by diluting 10 ml of stock solution to 100 ml with methanol to get final concentration(20µg/ml)

Pharmaceutical preparations

Diamicron® tablets labeled to contain 80 mg Gliclazide per tablet batch No.1005019 (Servier, Egypt).,Doanil® Tablets labeled to contain 5mg Glibenclamide per tablet batch No. 090235\9869 (Sanofi Aventes, Egypt).

General Spectrophotometric procedures and construction of calibration curves using Methylene Blue method

In 10 ml volumetric flasks, add separately 0.15 – 0.5 ml (in case of Gliclazide), 0.2 – 1.2 ml (in case of Glibenclamide), of woking solution then acidify using 0.4 ml (in case of Gliclazide) or 0.2ml (in case of , Glibenclamide) of 5 M HCl, add 1 ml of bromate - bromide working solution(25µg/ml) close flasks and stand for 3 minutes, add 1 ml dye **M.B** working solution(60µg/ml)then stand for another 3 minutes in both drugs. Complete to mark with bidistilled water then measure absorbance at 669 nm against a reagent blank prepared in the same manner except the addition of the drugs.

General spectrophotometric procedures and construction of calibration curves using Methyl Orang method

In 10 - ml volumetric flasks, add separately 0.1 – 0.3 ml (in case of Gliclazide) and 0.2 – 0.6ml (in case of Glibenclamide) drug solution then acidify using 0.4 ml of 5 M HCl ,add 1 ml of bromate - bromide working solution(12.5µg/ml) close flasks and stand for 5 minutes**,** add 1 ml of **M.O** dye working solution(60 µg/ml) then stand for another 3 minutes(both cases) complete to mark with bidistilled water then measure absorbance at 508 nm against a reagent blank prepared in the same manner except the addition of the drugs..

Preparation and assay of tablet formulations

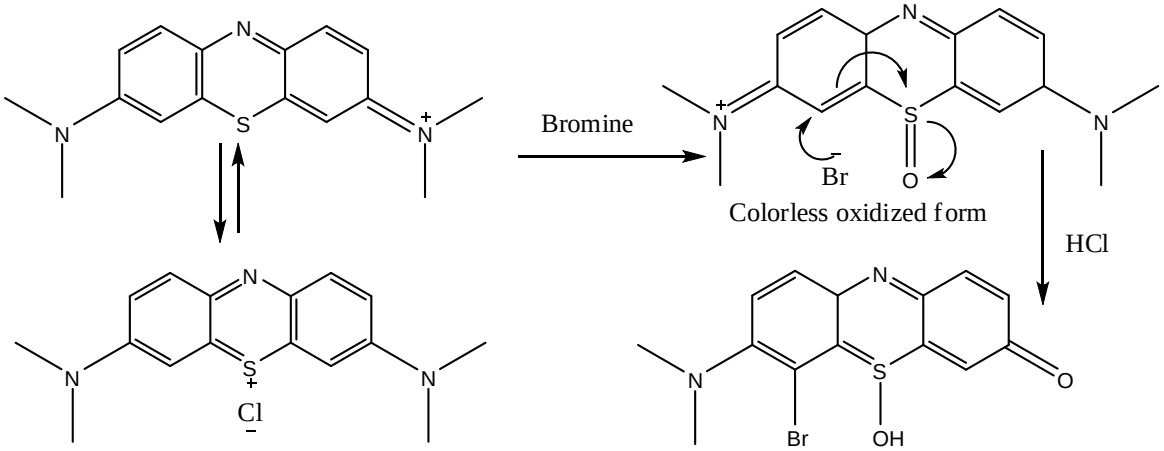
The total content of twenty tablets of Gliclazide (Diamicron®), Glibenclamide (Doanil®) , were accurately weighed and grounded well to a fine powder. A portion of the powder equivalent to 20 mg of each drug was dissolved in the least amount of absolute methanol. The resulting solutions were shaken well for 30 min, filtrated through Whatman Grade No. 41 quantitative filter paper and washed with methanol. The filtrate and the washings of drugs were collected in 100 mL volumetric flask, diluted to volume with methanol and the general procedure mentioned above was followed over the calibration range of each compound.

RESULTS and DISCUSSION

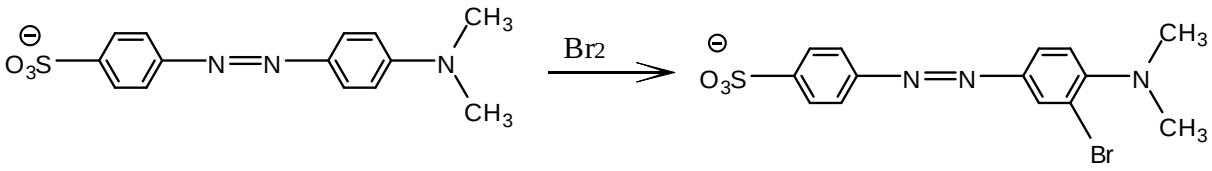
The proposed spectrophotometric methods are indirect and are based on the determination of the residual bromine (insitu generated) after allowing the reaction between each drug and a measured amount of bromine to be complete. The excess bromine was determined by reacting with a fixed amount of either methylene blue or methyl

orange dye .The methods rely on the bleaching action of bromine on the dyes due to oxidative destruction of these dyes as shown in (Scheme1) (in case of methylene blue) Plater and Arkivoc. (2003), and the suggested structure of methyl orange before and after oxidation as shown in (Scheme 2). (Basavaiah, and Tharpa, 2008), Gliclazide and Glibenclamide, when added in increasing amounts to a fixed amount of insitu generated bromine, consume the latter proportionately with a concomitant fall in the concentration of bromine. When a fixed amount of dye is added to the decreasing amounts of bromine, a concomitant increase in the concentration of dye results, Consequently, a proportional increase in the absorbance at the respective λ_{max} is observed with increasing concentration of each drug .The insitu generation of bromine is carried out using a mixture of potassium bromate and potassium bromide in presence of 5 M HCl according to the following equation:

$$5Br^- + BrO^{-3} + 6H^+ \longrightarrow 3Br_2 + 3H_2O$$



Scheme (1) Proposed structures of different forms of methylene blue before and after bromination



Scheme (2) Reaction scheme of methyl orange oxidation by the residual unreacted bromine

Spectral features.

Absorption spectra for determination of Gliclazide and Glibenclamide were studied over range of 200 - 800 nm. After oxidation of all drugs and portions of dyes with bromine, residual un-oxidized methylene blue and methyl orange are absorbed at 669 nm and 508 nm respectively (Fig.1 and Fig.2).

Effect of Acidity:

5 M HCl was used throughout experiments and it was found that 0.4 ml (in case of Gliclazide) and 0.2 ml (in case of Glibenclamide) in M.B method and using 0.4ml (in both Gliclazide and Glibenclamide) In M.O. method.

Effect of bromate - bromide volume:

Bromate - bromide volume was studied by varying the reagent volume

while other factors were held constant. It was found that for both methylene blue and methyl orange methods 1 ml of bromine solution is sufficient for its bleaching action using these stated concentrations (25, 12.5 µg /ml for methylene blue and methyl orange respectively) for all drugs studied.

Effect of time:

Time required for bromination and oxidization the drug before addition of dye and time required to irreversibly oxidize dye after its addition was studied. The bromination reaction was found to be complete in both drugs in 3 minutes with methylene blue and 5 minutes with methyl orange while contact times up to 25 minutes had been examined and no further bromination was detected .A contact time of 3 minutes after addition of dye in both (Gliclazide and Gibenclamide) was necessary for bleaching of dye color by residual bromine and the color of residual dye remain stable (up to 2 hr.) in case of methylene blue and methyl orange respectively.

Method validation

Under the optimum experimental conditions previously described at procedure section the standard calibration graphs for Gliclazide and Glibenclamide were constructed by plotting the absorbance, against concentration using the Microsoft Excel® spreadsheet program. The parameters, Beer's law limits, regression equations, and correlation coefficient for each drug were presented **(Table 1).**

Table (1) Analytical parameters for the determination of Gliclazide and Glibeneclamide using proposed methods

PARAMETERS	**Methylen Blue Method**		**Methyl Orange Method**	
	Gliclazide	**Glibeneclamide**	**Gliclazide**	**Glibeneclamide**
λmax, nm	669	665	508	508
Volume of dye , ml(60µg/ml)	1	1	1	1
Volume of 5M HCL, ml	0.4	0.2	0.4	0.4
Volume of Bromate/Bromide mixture , ml	1ml (**25µg/ml**)	1ml (**25µg/ml**)	1ml(**12.5µg/ml**)	1ml(**12.5µg/ml**)
Time before dye addition, min	3	3	5	5
Time after dye addition, min	3	3	3	3
Beer's law limits, µg/ml	3-10	4-24	2-6	4-12
Regression equation.	y = 0.072x - 0.0929	y = 0.027x + 0.058	y = 0.1584x - 0.080	y = 0.0762x - 0.064
Correlation Coefficient	0.9992	0.9998	0.9994	0.9993

y = a + bx, where **y** is the absorbance, **a** is the intercept, **b** is the slope and **x** is concentration in µg/ml.

Mean, S.D, RSD, LOD, LOQ and Sandell's sensitivity values (*S*) for each drug were presented **(Table 2)**. The correlation coefficient, slope and intercept are described by the regression equation **(Miller, 1991**) $Y = a + b\,x$ (where *Y* is the absorbance of a 10 mm layer, *b* and *a* are the slope and the intercept, respectively, while, *x* is the concentration of the drug measured solution in (µg mL^{-1}) obtained by the linear least-squares method. Regression analysis reveals a satisfactory correlation for the two methods. Low Sandell's sensitivity values for the two drugs indicate the high specificity and sensitivity of the proposed method. This is also supported by the calculated values of the limit of detection (*LOD*) and the limit of quantitation (*LOQ*). The *LOD* is the

smallest concentration of the analyte that is capable of giving a measurable response.

Table (2) Results of the analysis for the proposed methods

Parameter	Methylen Blue Method						Methyl Orange Method					
	Gliclazide*			Glibeneclamide*			Gliclazide*			Glibeneclamide*		
	Taken µg/m	Found µg/ml	Recovery %	Taken µg/ml	Found µg/ml	Recovery %	Taken µg/ml	Found µg/ml	Recovery %	Taken µg/ml	Found µg/ml	Recovery %
	3	3.03	101.14	4	4.08	102.12	2	1.99	99.55	4	3.97	99.47
	4	3.98	99.55	8	8.01	100.13	3	2.96	98.98	6	5.94	99.12
	5	5.05	101.07	12	11.86	98.85	4	4.05	101.23	8	8.07	100.91
	6	5.91	98.64	16	15.9	99.37	5	5.02	100.55	10	10.09	100.94
	7	6.93	99.07	20	20.12	100.61	6	5.96	99.36	12	11.90	99.21
	8	8.07	100.94	24	24.01	100.04						
	10	10.06	100.67									
Mean			100.01			100.19			99.94			99.93
±SD			1.0419			1.1314			0.9247			0.9187
±RSD			1.0412			1.1292			0.9253			0.9193
±SE			0.3683			0.4620			0.4135			0.41090
Variance			1.0859			1.2802			0.855			0.84418
Slope			0.0727			0.027			0.1584			0.0762
L.D.			0.8949			1.1249			0.4294			1.07096
L.Q.			2.6832			3.379			1.43146			3.2369
S.S.			0.0180			0.0205			0.00749			0.00991

Accuracy and precision

The accuracy of an analytical method is the closeness of the test results to the true value. Whereas, precision is an expression for the degree of scattering between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions, Precision could be examined in two aspects; firstly, the intra-day precision (repeatability) referring to the use of analytical procedure within a laboratory over a short period of time, at random, by the same operator with the same equipment. The inter-day precision (intermediate precision) involves estimation of variations in analysis when a method is used within a laboratory on different days **(Table 3)** for M.B and M.O methods respectively.

Both the precision and accuracy of the proposed method were tested by means of recovery test. Both the percentage relative standard deviation (R.S.D. (%)) and percentage relative error (E_r *(%)*) were calculated and summarized in **(Table 4)**.

The accuracy was evaluated by measuring both drug recoveries through the standard addition technique and the drug recoveries in the synthetically prepared mixtures.

The percentage relative error can be calculated using this equation; E_r *(%) = [(found – added)/added] x 100*. **(**Suslu, *et al.* 2002). Both the low values of the inter- and intra-day precision and the near unity values of the accuracy, indicate the high repeatability and reproducibility possessed by the proposed method for determination of Gliclazide and Glibenclamid using M.B or M.O. proposed method.

Table (3). Results of the intraday and interday precision for the determination of Gliclazide and Glibeneclamide using proposed methods *(n=3)*

Method	Drug	Added µg mL^{-1}	Intra-day variation			Inter-day variation		
			Found [a] (µg mL^{-1})	Recovery (%) ± S.D. [a]	R.S.D.[a] (%)	Found[a] (µg mL^{-1})	Recovery (%) ± S.D. [a]	R.S.D.[a] (%)
M.B. Method	Glic.	3	2.979	99.31±1.023	1.030	3.009	100.32±1.270	1.266
		6	5.962	99.38±1.171	1.179	5.965	99.42±1.068	1.075
		10	10.064	100.64±0.654	0.650	10.081	100.81±0.686	0.681
	Glib.	4	4.003	100.09±1.781	1.779	4.0108	100.27±1.464	1.459
		12	11.95	99.66±1.226	1.231	11.973	99.78±1.618	1.622
		24	23.99	99.98±1.579	1.580	24.016	100.07±1.071	1.070
M.O. Method	Glic.	2	2.003	100.18±1.282	1.279	1.991	99.55±0.972	0.977
		4	4.017	100.44±0.600	0.598	4.026	100.66±0.711	0.706
		6	5.960	99.34±0.939	0.945	5.962	99.38±0.722	0.727
	Glib.	4	3.974	99.36±0.886	0.892	3.986	99.67±0.790	0.792
		8	7.928	99.11±0.624	0.630	7.994	99.93±0.602	0.603
		12	11.994	99.95±0.349	0.349	11.947	99.56±0.697	0.700

[a] Means, S.D. and R.S.D. (%) for three experiments carried out on three constitutive days.

Table (4). The accuracy data for the microanalysis of, Gliclazide and Glibeneclamide, using proposed techniques (n = 3)

Drug		Name of the dosage form	Initial tablet sample (µg mL^{-1})	Pure amount added (µg mL^{-1})	Total amount found [a] (µg mL^{-1})	Recovery (%) ± S.D. [a]	R.S.D[a] (%)	*Er* [a] (%)
M.B. Method	Glic.	DIAMICRON® Tablet	6	6	12.07	100.58 ± 0.009	0.009	1.166
				8	13.97	99.78 ± 0.026	0.026	-0.375
				10	15.94	99.62 ± 0.013	0.013	-0.600
	Glib.	DOANIL® Tablet	12	8	19.96	99.80± 0.019	0.019	-0.50
				12	23.86	99.41 ± 0.028	0.028	-1.166
				16	27.63	98.67 ± 0.033	0.033	-2.312
M.O. Method	Glic.	DIAMICRON® Tablet	4	4	8.03	100.38 ± 0.009	0.009	0.750
				6	9.99	99.92 ± 0.017	0.017	-0.166
				8	11.98	99.83 ± 0.023	0.023	-0.250
	Glib.	DOANIL® Tablet	8	8	15.77	98.65± 0.023	0.023	-2.875
				12	20.13	100.87 ± 0.024	0.024	1.083
				16	23.58	98.25 ± 0.007	0.007	-2.625

[a] Means, R.S.D. (%) and *Er* (%) for three replicates

Robustness and ruggedness

Robustness of the method was determined by making slight deliberate changes in the operation parameters, such as hydrochloric acid, bromate/bromide mixture ,and dye volumes (±0.2ml), and standing time during the process,. It was observed that there were no marked changes in the color intensity and that is why the developed methods are declared to be robust **(Tables 5, 6)** for M.B method and M.O. method respectively. On the other hand, the method ruggedness was assessed through comparing the intra- and inter-day precision results that has been performed in different analytical laboratory with different spectrophotometric devices by two different analysts. R.S.D. (%) values for such intermediate precision did not exceed 2 %, indicating the ruggedness of the method.

Table (5) Results of the robustness for determination of Gliclazide and Glibeneclamide using methylene blue method.

Methylene blue				
Parameters	**Gliclazide**		**Glibeneclamide**	
	Volume ml	% of recovery ± SD	Volume ml	**% of recovery ± SD**
HCl (5M)	0.38	99.42±0.769	0.18	99.66±1.226
	0.42	99.15±0.847	0.22	99.78±1.618
Br_2 (25µg/ml)	0.98	100.16±0.819	0.98	100.21±1.187
	1.02	98.83±0.862	1.02	99.29±1.082
Dye (60 µg/ml)	0.98	98.23±0.250	0.98	98.42±0.833
	1.02	100.755±0.298	1.02	100.95±0.736

Table (6) Results of the robustness for determination of Gliclazide and Glibeneclamide using methyl orange method

Methyl orange				
Parameters	**Gliclazide**		**Glibeneclamide**	
	Volume ml	% of recovery ± SD	Volume ml	**% of recovery ± SD**
HCl (5M)	0.38	99.49±0.558	0.38	99.11±0.624
	0.42	101.07±0.460	0.42	99.93±0.602
Br_2 (12.5µg/ml)	0.98	101.04±0.437	0.98	100.75±0.624
	1.02	98.61±0.454	1.02	98.42±0.391
Dye (60 µg/ml)	0.98	98.67±0.303	0.98	98.09±0.213
	1.02	101.42±0.768	1.02	101.11±0.511

Analysis of pharmaceutical formulations

Both the applicability and validity of the proposed colorimetric method were tested through its application for the determination of Gliclazide, and Glibenclamid in pharmaceuticals manufactured by local Egyptian Companies, at each concentration level, five replicate determinations were performed over a two day design. The recoveries were calculated with reference to the calibration graphs, and satisfactory results were obtained, as presented in **(Table7)** for M.B and M.O methods respectively. The results obtained were judged and statistically compared with the reported spectrophotometric methods (Singh et al., 2011; Cicy *et al.*, 2012) using Student's t-test and the one-way analysis of variance (ANOVA test). Both the t-test values and the variance ratio F-values obtained at the 95 % confidence, did not exceed the theoretical tabulated values. Therefore, there is no significant difference between the proposed and the reported methods indicating that the proposed method is as accurate and precise as the reported methods

Conclusion

The proposed method is simple, sensitive, economic, rapid and practically applicable for the determination of Gliclazid and Glibenclamid in bulk and tablet form without any interference from commonly used pharmaceutical excipients. The proposed method possesses simple cheap equipment, more color stability and wide ranges of analytical determination. There is also, no involvement of any critical and hazardous experimental conditions, expensive reagents or sophisticated instruments (as in HPLC and gas chromatography techniques), which an ordinary analytical laboratory cannot afford .For all of the above, the developed method can be recommended for the routine Q.C. analysis of the drugs, in pure form and in formulations, with speed at low cost without losing the accuracy.

Table (7). Analysis of Gliclazide and Glibeneclamide, in pharmaceutical formulations using the proposed reported spectrophotometric techniques.

Drug		Name of the dosage form	[Drug] taken (µg mL^{-1})	Recovery (%) ± R.S.D. [a] (%)		*t*-test	*F*-test
				Proposed	Reported [b]		
M.B. Method	GLIC.	DIAMICRON ® Tablet	3	100.22±0.963	99.71± 1.294	1.19	0.770
			6	99.92±0.867	99.42± 1.487	0.509	4.73
			9	100.11±0.707	100.04 ± 1.316	1.771	3.982
	GLIB.	DOANIL ® Tablet	4	99.42±0.949	99.36± 0.816	0.496	1.625
			8	100.04±0.795	100.86± 0.869	1.32	0.563
			12	100.13±0.571	99.36± 0.827	1.953	0.589
M.O. Method	GLIC.	DIAMICRON ® Tablet	2	100.14±1.324	101.77± 1.684	0.931	2.025
			4	99.96±1.17	100.64± 1.701	1.08	1.588
			6	99.13±0.545	99.42± 1.487	0.281	4.523
	GLIB.	DOANIL ® Tablet	4	99.80±0.773	99.36± 0.816	0.254	0.712
			8	99.93±0.620	100.86± 0.869	0.242	3.97
			12	100.02±0.538	99.36± 0.827	0.919	3.42

a. Average of five determinations.
b. Reported techniques (Singh *et al*.; 2011) for Gliclazide (tablet) and (Cicy E. *et al.* ;2012)for Glibenclamid (tablet)
Tabulated *t*-value at 95 % confidence limit = 2.13; degree of freedom = 4.
Tabulated *F*-value at 95 % confidence limit = 6.388; degrees of freedom = 4.

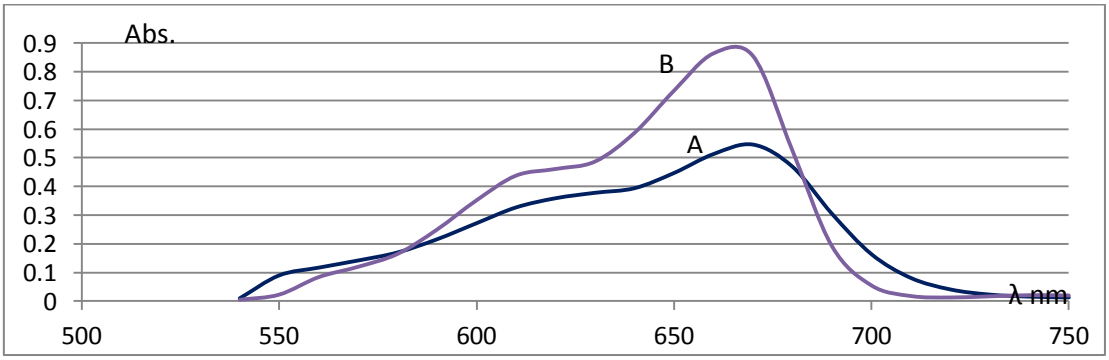


Fig. (1) Absorption Spectra of methylene blue in presence of 9 µg/ml of Gliclazide (A) and 24µg/ml of Glibeneclamide (B).

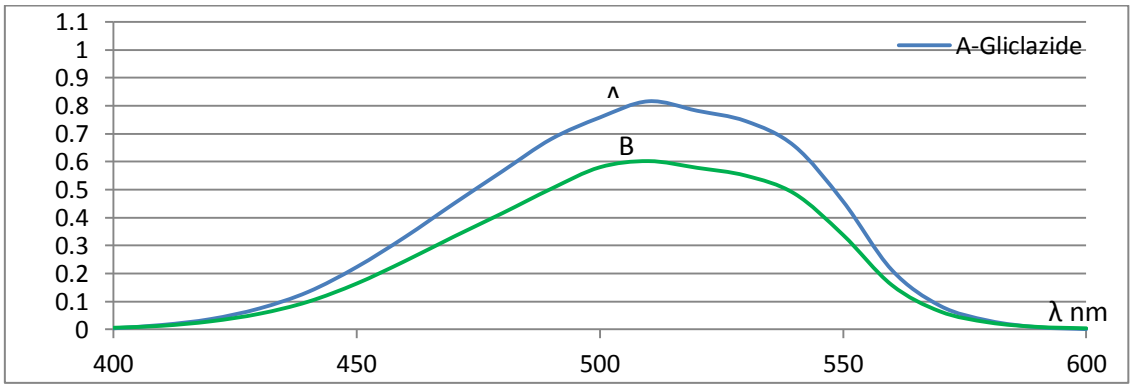


Fig. (2) Absorption Spectra of methyl Orange in presence of 12µg/ml Gliclazide (A) and 8µg/ml Glibeneclamide (B).

REFRENCES

Basavaiah, K. (2006) Application of bromate-bromide mixture and methyl orange in the titrimetric, spectrophotometric and kinetic assay methods for cyproheptadine in pharmaceuticals. Indian journal of Chemical Technology, 13 (4):360-366.

Basavaiah, K., Chandrashekar, U., Nagegowda, P (2006). Titrimetric and modified spectrophotometric methods for the determination of amlodipine besylate using bromate-bromide mixture and two dyes. *Sci. Asia.* 32, 271-278.

Basavaiah, K., Kumar A R. (2007a). Sensitive Spectrophotometric Methods for the Determination of Gatifloxacin in Pharmaceuticals Using Bromate-Bromide, Methylene Blue and Rhodamine-B as Reagents *Eur. J. Chem.* 4(2), 154-161 .

Basavaiah, K., Kumar A R. (2007b). Titrimetric and Spectrophotometric Assay of Pantoprazole in Pharmaceuticals Using Permanganate *Ind. J. Chem. Tech*. 14, 611-615.

Basavaiah, K., Tharpa, K. (2008) Investigation and Optimisation of the Use of Spectrophotometry for the Assay of Simvastatin with in situ Bromine and Three Dyes as Reagents J. Mex. Chem. Soc. 52(3), 193-200.

Bilal A, Rehman K, Akash MSH, Hussain K, Ibrahim M. (2013) Development and Validation of Analytical Method for Qualitative and Quantitative Determination of Glibenclamide in Different Brands of Tablet Dosage form Using UV-Visible Spectroscopy. J Mol Genet Med 7(3): 80. doi:10.4172/1747-0862.1000080

British pharmacopeia, 2013 (Ph. Eur. monograph 0718) pp (1021-1023).

Dhabale P.N. and Seervi C. R. (2010) Simultaneous UV Spectrophotometric Method for Estimation of Gliclazide and Metformin Hydrochloride in Tablet Dosage Form. International Journal of ChemTech Research. 2(2): 813-817.

E1- Enany N. (2004): Spectrophotometric determination of Gliclazide in pharmaceuticals and biological fluids through ternary complex formation with eosin and palladium (II). 11Farmaco, 59 (1): 59-63.

Eapen C, Prasanth V G, Amita R. (2012), Development of UV Spectrometric Method of Glibenclamide (Glyburide) in Bulk and Pharmaceutical Formulations .Int. J. Chem Tech Res., 4(1): 356-360.

EL-Enany N. (2003): Spectrofluorimetric and Spectrophotometric Determination of Gliclazide in Pharmaceuticals by Derivatization with 4-Chloro-7 nitrobenzo-2-oxa-1, 3-diazole. Journal of AOAC International. 86(2): 209-214.

El-Shanawany A A., El-Adl S M., Abdel-Aziz L M, Hassan A F. (2014). Bromatometric Estimation of Cefepime, Cefoperazone, and CefotriaxoneIn their Bulk and Dosage Forms. .Asian Journal of Pharmaceutical Analysis. 4(1): 17-27

El-Shanawany A. A., El-Adl S. M., Abdel-Aziz L. M., Sebaiy M M. (2011). Bromatometric Estimation of Levofloxacin HCl, Lomefloxacin HCl and Sparfloxacin in Bulk and Dosage Forms Asian J. Res. Pharm. Sci. 1(4):131-139

Gandhimathi M, Anandakuma K, Cheriyan A and Ravi, TK (2003) Simultaneous estimation of Metformin and Gliclazide in tablets using RP-HPLC, IJPS. 65: 530-531.

Godse A. M., Pishawikar S.A., Purake R. R., Killedar S.G., Puri U. A. (2012) Spectrophotometric method development for Glibanclamide in bulk and Pharmaceutical dosage form using extract of *Beta vulgaris* root. Asian Journal of Research In Chemistry. 5(5): 591- 594.

Havele SS and Dhaneshwar SR (2010), Determination of Glibenclamide in Tablets by Densitometric HPTLC, Der Pharmacia Letter, 2(4): 440-446.

Ioannis N. and Athanasios C. D., (2002) A validated high-performance liquid

chromatographic method for the determination of Glibenclamide in human plasma and its application to pharmacokinetic studies, Journal of Pharmaceutical and Biomedical Analysis. 28: 653–657.

Jayanthi M., Thirunavukkarasu S.V., Nagarajan V., Elangovan S., Raja S. (2012) Development and Validation of RP-HPLCMethod for Determination of Glibenclamide in Pharmaceutical Dosage Forms. International Journal of Chem Tech Research. 4 (2): 593-601.

John Plater (2003), a degradation product of methylene blue ARKIVOC. 2003: 37-42.

Kanij F., Rahman M Z, Haque T., Azad M A, and Reza M S, (2010) Development and validation of a simple method for simultaneous estimation of Metformin hydrochloride and gliclazide in tablets by using RP-HPLC, Dhaka Univ J.Pharm Sci. 9(2): 83-89.

Kumar A D., Kumar D A., Angshuman B. (2012): Simultaneous estimation of Metformin hydrochloride and Glibenclamide by RPHPLC method from combined tablet dosage form. IJSIT. 1(2):98-105.

Mansoory N. M, Jain A. (2012) Simultaneous estimation of metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide by validated RP-HPLC method in solid dosage form, international journal of pharmacy and pharmaceutical sciences. 4(5):72-76.

Miller J.N. (1991). Basic statistical methods for Analytical Chemistry. Part 2. Calibration and regression methods. A review. Analyst 116: 3-14.

Miller J.C. and Miller J.N., (1993) Significance tests. In statistics in analytical chemistry, third ed.,Ellis Horwood, pp. 101-139, Chichester, UK,.

Naglaa M. El-Kousy (1998), Stability indicating Densitometric determination of some antidiabetic drugs in dosage forms,Using TLC. Microchimica Acta.128, 65-68.

Narmada D., Krishna P.V. Murali, Yusuf S. Mohammad, Ranganayakulu B., Praveen K. Uday, Abhilash P. Raja (2014) RP-HPLC method development and validation for estimation of Glibenclamide in tablet dosage form Asian Journal of Pharmaceutical Analysis. 4(3): 125-128

Parameswararao K., Satynarayana M. V., Raju T. Naga and Ramana G.V. (2012), Novel spectrophotometric methods for the assay of Glibenclamide in pure and dosage forms. Der Pharma Chemica, 4(6):2449-2452.

Patil S. S. and Bonde C. G. (2009), Development and Validation of analytical method for Simultaneous Estimation of Glibenclamide and Metformin HCl in Bulk and Tablets using UV – visible spectroscopy, International Journal of ChemTech Research, 1(4): 905-909.

Rajendran S D, Philip B K, Gopinath R, Suresh B. (2007) .RPHPLC method for the estimation of glibenclamide in human serum. Indian J Pharm Sci; 69:796-9.

Ramesh, J., Basavaiah, K., Divya, R. (2010).Titrimetric and Spectrophotometric Determination of Doxycycline hyclate using bromate-bromide, methyl orange and indigo carmine *CICEQ*. 16(2), 139−148

Revathi R. Saravanan V. S., Ganesan V. (2010): Spectrophotometric Estimation of Gliclazide in bulk and pharmaceutical dosage forms. International Research journal of Pharmacy (IRJP) 1(1) 277-281.

SaiThanuja V, Chandan R S., Anandkumar R Tengli, Gurupadayya B M, Prathyusha W (2014), Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Metformin Hydrochloride, Pioglitazone Hydrochloride and Glibenclamide in Bulk and Pharmaceutical Dosage Forms. IOSR Journal of Pharmacy and Biological Sciences. 9(1): 124-133.

Sanjay S. M. and Mulla S. (2014) Validated HPTLC Method For Simultaneous Determination Of Metformin Hydrochloride And Glibenclamide In Combined Dosage Form, IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS). 9(2):54-59.

Singh P., Kumar R. and Singh H. (2011): Application of UV Spectrophotometric method for analysis of Gliclazide in Pharmaceutical dosage forms. International Journal of Pharmacy and Pharmaceutical Sciences. 3(4): 259-260.

Somashekar B., Basavaiah K. (2007): Sensitive bromatometric methods for the determination of salbutamol Sulphate in pharmaceuticals. J. Anal. Chem. 62(5), 432-437.

Subhashini E and Syama B.S (2014): New analytical method development and validation for the simultaneous estimation of Metformin and Glibenclamide in bulk and tablet dosage form using RP-HPLC.RASAYAN J. Chem. 7(1): 55-63

Suslu I, Tamer A (2002). Spectrophotometric determination of enoxacin as ion-pairs with bromophenol blue and bromocresol purple in bulk and pharmaceutical dosage form J. Pharm. Biomed. Anal. 29: 545-554

Tengli AR, Gurupadayya BM, Neeraj S and Vishwanathan B**.** (2013): Method Development and Validation of Metformine, Pioglitazone and Glibenclamide in Tablet Dosage Form by using RP-HPLC, Biochem Anal Biochem. 2(2): 1000130 *doi:10.4172/2161-1009.1000130*

Venkata R. I., Lakshman Rao A. and Ramana M.V. (2011). Validated RP - HPLC Method for the Estimation of Glibenclamide in Formulation and Serum. International Journal of Research in Pharmaceutical and Biomedical Science. 2 (2): 856-862.

Weetman SC. Martindale (2006) the Complete Drug Reference, thirty fifth ed. Pharmaceutical Drug monographs SE-1702.

WHO Expert Committee on Diabetes Mellitus (1980). Second Report, Geneva: WHO Technical Report Series 646.

WHO. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997; 20:01 83–97.

استخدام البرومين في تعيين كل من الجليكلازيد والجليبنكلاميد فى صورهم النقية وفى الأقراص

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يصف هذا البحث طريقتين لتحليل كل من الجليكلازيد والجليبنكلاميد فى صورهم النقية وفى الأقراص وتعتمد الطريقتين على الانتاج اللحظى للبرومين كعامل مؤكسد واستخدام اما الميثيلين الازرق او الميثيل البرتقالى ككاشف طيفى. فتتم اكسدة تلك الادوية باستخدام البرومين المنتج لحظيا حيث تستهلك جزء من ذلك العامل المؤكسد والجزء المتبقى يؤكسد جزء من الكاشف (الميثلين الازرق او الميثيل البرتقالى) تاركا جزءا اخر يتم قياسه طيفيا عند طول موجى 669 و508 نانو متر على التوالى حيث ان الزياده فى الامتصاص للكاشف المتبقى تتناسب تناسبا طرديا مع تركيز الدواء المؤكسد. وقد تمت دراسة العوامل المختلفه التى تؤثر على التفاعل كالحامضية ، تركيز العامل المؤكسد والوقت وقد اتبع قانون بيير على مدى تركيز قدره (3-10) ميكروجرام /ملليلتر لماده الجليكلازيد ،(4- 24) ميكروجرام /ملليلتر لماده الجليبنكلاميد فى حاله الميثلين الازرق وقدره (2 - 6) ميكروجرام /ملليلتر لماده الجليكلازيد ، (4- 12) ميكروجرام /ملليلتر لماده الجليبنكلاميد فى حاله الميثيل البرتقالى. وقد استخدمت الطرق فى تعيين هذه الادويه فى بعض المستحضرات الصيدليه وتمت مقارنة النتائج احصائيا مع الطرق المرجعيه.