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Novel spectrophotometric methods for the assay of glibenclamide in pure and dosage forms

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ABSTRACT

A simple, sensitive and selective method for the determination of glibenclamide in bulk and in pharmaceutical formulations is described. The method is based on extraction of this drug into chloroform as ion-pair with sulphonphthalein dyes as bromocresol purple (BCP) and bromothymol blue (BTB). The optimum conditions of the reactions were studied and optimized. The absorbance of the yellow products was measured at 418nm for Glibenclamide-BCP and 424nm for Glibenclamide-BTB. The calibration curves obeyed Beer's law over the concentration range of 2.5-12.5µg/mL for Glibenclamide-BCP and Glibenclamide-BTB with correlation (r^2=0.9996 & 0.9998) respectively. The result of the proposed methods were compared stastically (F & t-test) with those of the reference method reported in the literature and was found no significant difference with good recovery.

Keywords: Glibenclamide, bromophenolblue, bromothymol blue, ion-pair complex

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INTRODUCTION

Glibenclamide(Fig-I) [1] is chemically known as 5-chloro-N-[2-[4[(cyclohexylamino) carbonyl] amino] sulfonyl] phenyl] ethyl]-2-methoxy benzamide is second generation sulphonyl ureas drug widely used in treatment of type 2 diabetic patients. It acts by inhibiting ATP-sensitive potassium channels in pancreatic beta cells causing cell membrane depolarisation(increasing intracellular calcium in the beta cell) which stimulates the insulin release.

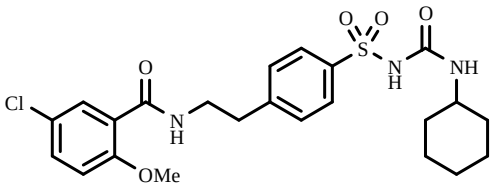

Fig-I: Structure of glibenclamide

Several assay techniques have been reported for quantitative determination of glibenclamide in biological fluids which high performance liquid chromatography (HPLC)[2],LC-MS[3-5] in plasma and UV-Spectropthometric techniques[6,7] in dosage forms.

The present paper describes two new highly sensitive, rapid, simple, economical visible spectrophotometric methods for the determination of glibenclamide in pure and in tablets by exploiting its analytically useful functional groups and its ability to form ion-pair complex with two acidic dyes BCP and BTB.

MATERIALS AND METHODS

Apparatus:
A double beam UV-Visible Double beam spectrophotometer (Shimadzu 160 A) with matched 1.0cm quartz cuvettes was used for all absorbance measurements. A digital pH meter Model Elico L1 120 was used for pH measurements.

Chemicals and Materials:
Glibenclamide (pure drug) used was obtained from Micro Labs Ltd as gift sample with 99.9% w/w assay value. All the Solvents and other chemicals used were of analytical grade and double distilled water was used throughout the investigation. Solutions of BCP 0.25% (w/v) BTB 0.10% (w/v) were prepared separately in double distilled water freshly. Phosphate buffer of pH-3.4 (hydrochloric acid, Merck Specialities Pvt Ltd, Mumbai, India, Sp. gr. 1.18) was prepared usually in distilled water.

Preparation of standard stock solution:
Standard drug solution of glibenclamide(stock) was prepared by dissolving 10mg in 10ml methanol (1mg/ml). The working standard solution of glibenclamide (50µg/ml) was obtained by appropriately diluting the standard stock solution with the same solvent.

Assay procedure for tablets:
Ten tablets were weighed and pulverized to a fine powder. The amount of tablet powder equivalent to 10mg of glibenclamide was weighed accurately and transferred 100ml volumetric flask. Then 10ml methanol was added and kept for 15min with frequent shaking and volume was made upto mark with methanol. The solution was then filtered through Whatmann filter paper #41.The filtrate was evaporated to dryness and the residue was dissolved as under standard solution preparation and was proceeded as stated in "recommended procedure"

Assay Procedures:
With BCP: Aliquots (0.5-2.5ml) of standard solution of glibenclamide(50µg/mL) were transferred to 60ml stoppered separating flasks. The final volumes in each separating flasks was adjusted to 5.0ml by adding phosphate buffer solution (pH 3.4). To the above flasks then add 2.0ml of BCP solution and were shaken for 5 min. Latter 10ml of chloroform were added to the tubes and the mixtures were shaken for 2 min and allowed to stand for 5 min for separation of the chloroform layer. The absorbance of the chloroform phase was measured after an equilibrium time of 10min in 1.0cm quartz cells at 418 nm against blank solution, which was prepared similarly calibration curve was plotted using absorbance-values versus concentration.

With BTB: Same procedure described in the above method(BCP) was followed for varying aliquots of standard solution of glibenclamide(50µg/mL) and the absorbance of glibenclamide-BTB complex was measured at 424nm against the reagent blank as reference respectively. The concentration of the unknown was read from the calibration graph or computed from the respective regression equation derived using the absorbance concentration data.

RESULTS AND DISCUSSION

The Proposed methods were based on the formation of an ion-pair complex between glibenclamide(drug) and the two acidic dye bromocresol purple (BCP) and bromothymol blue (BTB) at pH(3.4±0.01) followed by extraction of the complex chlorofom, and measuring the absorbencies of yellow drug-dye complexes at 418 and 424nm for BCP and BTB respectively[Fig-II].

Calibration graphs were constructed from five points covering the concentration ranges 2.0-12.5µg/mL for BCP and BTB methods respectively. Regression analysis of the Beer's law data indicated a linear relationship between absorbance and concentration (Table-I) which is corroborated by high values (close to unity) of the correlations coefficients. The calculated molar absorptivity and Sandell sensitivity values are summarized in Table-I. The limits of detection (LOD) and quantification (LOQ) were calculated using the formulae:

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

(where S is the standard deviation of blank absorbance values, and b is the slope of the calibration plot), calculated according to the ICH guidelines(2007), and were also summarized in Table-I. The high values of "ε" and low values of Sandell sensitivity and LOD indicate the high sensitivity of the proposed methods.

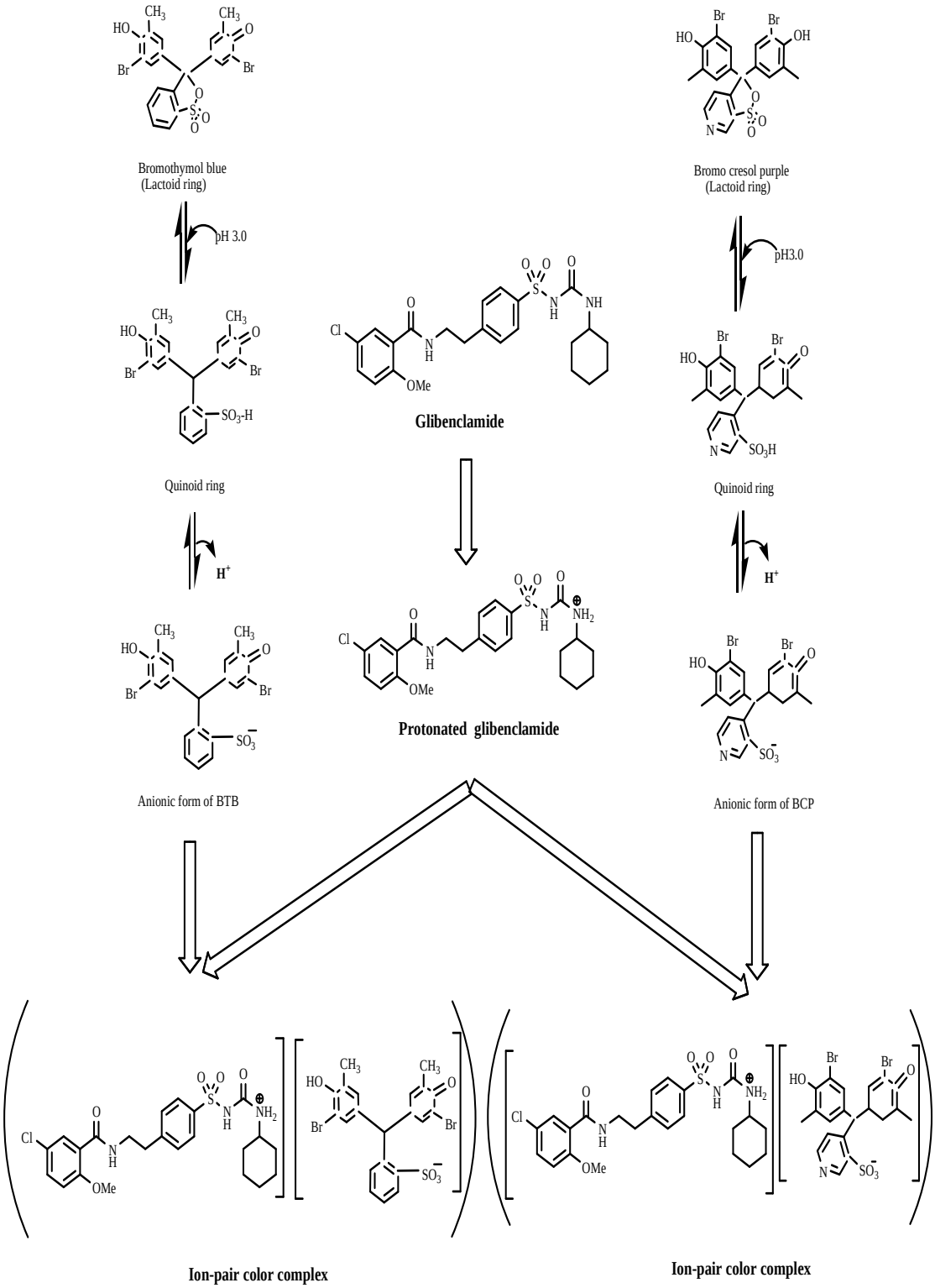

Fig-II: Reaction scheme of Glibenclamide with BTB and BCP

Precision and accuracy of the proposed methods were determined by carrying out the determination of six replicates of pure samples of the glibenclamide, whose concentration was within Beer's law range. Values of the standard deviation (SD), relative standard deviation (% RSD) and range of error at 95% confidence level were calculated and the results are summarized in Table -II. The percentage relative standard deviation (% RSD) values were 0.581 for BCP and 0.8948 for BTB ≤ 1.00% (inter-day) indicating high precision of the proposed methods.

Table-I: Results of optical characteristics, precision and accuracy of the proposed methods for Glibenclamide assay

Parameter	BCP	BTB
λ_{max} (nm)	418	424
Beer's law limits (µg/ml)	2.5 –12.5	2.5 –12.5
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	1.195×10^4	9.924×10^3
Sandell's sensitivity (µg.cm^{-2}/0.001 absorbance unit)	0.0103	0.0124
Optimum photometric range (µg/ml)	3.0 – 10.5	2.5 – 12.0
Regression equation (Y=a+bc) ;slope (b)	0.0469	0.0398
Standard deviation on slope (S_b)	7.225×10^{-4}	3.354×10^{-4}
Intercept (a)	7.3×10^{-3}	2.9×10^{-3}
Standard deviation on intercept (S_a)	4.160×10^{-4}	1.935×10^{-4}
Standard error on estimation (S_e)	5.710×10^{-3}	2.652×10^{-3}
Correlation coefficient (r)	0.9996	0.9998
Relative standard deviation (%)*	0.5831	0.8948
% Range of error (confidence limits)		
0.05 level	0.4876	07483
0.01 level	0.7213	1.1069
LOD	0.0266	0.0142
LOQ	0.266	0.142

** Average of six determinations considered*
*** Average of three determinations*

Analysis of pharmaceutical formulations

The proposed methods were applied for the quantification of glibenclamide [MICRONASE] in commercial tablets. The results were compared with these obtained by a published method[7]. Statistical analysis of the results did not detect any significant difference between the performance of the proposed methods and reference method with respect to accuracy and precision as revealed by the Student's t-value and variance ratio F-value. The results of assay are given in Table-II.

Table-II: Estimation of Glibenclamide in Pharmaceutical formulations(MICRONASE)

Sample	Labelled amount (mg)	Amount obtained (mg) Proposed methods*		UV method	%Recovery of Proposed methods**	
		BCP	**BTB**		**BCP**	**BTB**
MICRONASE(Tablets)	5.0	4.94 ± 0.14 F=1.300 t=0.461	4.96 ± 0.16 F=1.00 t=0.216	4.98 ± 0.16	99.19±0.45	99.59±0.22

**Average of six determinations*
*** Mean and standard deviation of six determinations*

CONCLUSION

The present paper describes new validated visible spectrophotometric methods for the determination of glibenclamide in pure and in tablets. The statistical parameters and the recovery data reveal good accuracy and precision of the methods. The methods are quite selective as the drug contains basic moiety which preferentially interacts with bromocresol purple and bromothymol blue and the drug-dye ion-pairs can be extracted into the organic solvent before measurement. Hence, the proposed methods can be used in routine analysis of glibenclamide in quality control laboratories.

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