RESEARCH ARTICLE

Extractive Spectrophotometric Method for the Determination of Glibenclamide by Ion-Pair Complex in Pure Form and Pharmaceutical Formulation

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ABSTRACT

A simple, rapid, and sensitive extractive spectrophotometric method is presented for the determination of glibenclamide (Glb) based on the formation of the ion-pair complex between the Glb and anionic dye, methyl orange (MO) at pH 4. The yellow colored complex formed was quantitatively extracted into dichloromethane and measured at 426 nm. The colored product obeyed Beer's law in the concentration range of (0.5–40) $\mu g.m^{L-1}$. The value of molar absorptivity obtained from Beer's data was found to be 31122 $L.mol^{-1}.cm^{-1}$, Sandell's sensitivity value was calculated to be 0.0159 $\mu g.cm^{-2}$, while the limits of detection (LOD) and quantification (LOQ) were found to be 0.1086 and 0.3292 $\mu g.ml^{-1}$, respectively. The stoichiometry of the complex created between the Glb and MO was 1:1 as determined via Job's method of continuous variation and mole ratio method. The method was successfully applied for the analysis of pharmaceutical formulation.

Keywords: Determination, Extractive spectrophotometric, Glibenclamide, Ion-pair, Methyl orange, Pharmaceutical.

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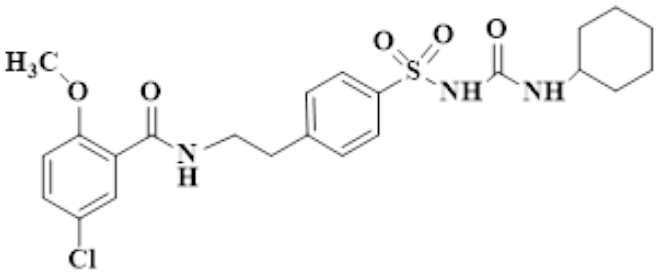
INTRODUCTION

Glibenclamide (Glb) also known as glyburide, the IUPAC name is 5-chloro-*N*-[2-[4-(cyclohexylcarbamoylsulfamoyl) phenyl]ethyl]-2-methoxybenzamide[1], Scheme 1. (Glb) is an anti-diabetic drug in a category of drugs known as sulfonylurea, firmly linked to sulfa drugs. It is utilized for the treatment of diabetes mellitus (DM) type 2, which is not responding enough to a diet. Glibenclamide is metabolized almost entirely via the liver to weakly active metabolites (4-trans-hydroxy derivative and 3-cis-hydroxy derivative) that are ejected in bile and urine. The importance of the activity of these metabolites is clinically determined only in cases of renal failure, where they accumulate and can lead to hypoglycemia.[2] The mode of action simply characterized by increasing insulin secretion from the pancreas, probably by interacting with sulfonylurea receptors on beta cells or by interfering with ATP-sensitive potassium channels on pancreatic beta cells, which increases secretion of insulin, also may increase sensitivity of existing insulin receptors by binding to sulphonylurea receptors (SUR) and blocking potassium channels that rely on ATP (K_{ATP}); the final result is the depolarization which activates voltage-sensitive Ca^{+2} channels, which in turn leads to the entrance of Ca^{+2} ions and secretion of insulin.[3,4] Various methods cited in the literature for its determination in pharmaceutical preparation involve high-performance liquid chromatography (HPLC),[5-10] potentiometric,11 voltammetric,[12] and spectrophotometric methods.[10,13,14]

The extractive spectrophotometric method is well known for its affectability in the assay of medications. Thus, it has gotten extensive consideration for the quantitative determination of numerous pharmaceutical compounds.[15-17] Up until this point, there has been no ion-pair extractive spectrophotometry method reported for the determination of glibenclamide.



Scheme 1: The structural formula of glibenclamide.

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EXPERIMENTAL

Instruments

A Shimadzu UV-Vis 1800 spectrophotometer (Kyoto-Japan) with 10 mm match quartz cells was used for the λ_{max} determination and all absorbance measurements.

WTW GmbH, inolab pH 7110 (Germany) pH meter with a combined glass electrode, which is utilized for checking the pH of buffer solutions.

ACS 120-4 (Kern and Sohn GmbH Germany) analytical sensitive electronic balance (± 0.0001g) was utilized for the weighing of substances.

Materials and reagents

Pharmaceutical grade glibenclamide powder (99.99%) was given from (Arab Pharmaceutical Manufacturing Company, Jordan). The solvents and other chemicals utilized were of analytical grade, and distilled water was utilized during the study period.

Reagents solutions

Methyl orange (MO) (S.D. Fine Chem., Mumbai, India) used without further refining. The stock solution 0.1 % (w/v) was prepared in 100 mL volumetric flask by dissolving precisely weighed 0.1 g in 10 mL methanol and made up to the mark by distilled water. This solution is stable for at least one week if kept in the fridge.

The buffer solution NaOAc-HCl of pH 4 was set by mixing 50 ml of 1M sodium acetate (Merk Pvt, Ltd., Mumbai, India) prepared by dissolving 6.8039 g of sodium acetate in distilled water with a calculated volume of 0.1 N HCl (Merk Pvt., Ltd., Mumbai, India, sp. gr. 1.18) and the pH was modified with the help of pH meter.

Preparation of standard stock solution

A standard stock solution of glibenclamide (Glb) 1000 $\mu g.mL^{-1}$ was set up by dissolving 0.1 g of the drug in 10 mL of methanol (CH_3OH) and was diluted to the mark in 100 mL volumetric flask by distilled water. From the stock solution, working standard solutions were prepared by suitable dilution with distilled water.

Recommended Procedure

To a set of 50 mL separating funnels, 2 mL of buffer solution of pH 4, 2 mL of 0.1 % MO and 1 mL of different concentrations of working standard solution range from (2.5 - 200 $\mu g.mL^{-1}$) were placed to each funnel and mixed well, and then allowed to stand for 6 min. After 6 min, the reaction mixture was extracted with 5 mL dichloromethane by shaking for 3 minutes and then permitted to stand for complete separation of the two phases. The absorbance values of the yellow-colored organic phase were measured at 426 nm versus a blank solution prepared similarly except the addition of the drug substance.

Sample preparation

Ten tablets were precisely weighed separately and crushed to a soft powder, then mixed well, and the mean of the weight was estimated. An amount equivalent to one tablet (each tablet contains 5 mg Glb) was accurately weighed, then 5 mL of methanol was added and stirred for 10 minutes for full dissolution of the medication. The mixture was then moved into a 50 mL volumetric flask and made up to the mark with distilled water to get 100 $\mu g.mL^{-1}$. After that, the solution was filtered by using filter paper and analyzed by the recommended procedure. 0.1 mL of Glb tablet solution was mixed with known serial volumes of active ingredient Glb (0–0.9 mL). The mixtures were mixed with 2 mL buffer solution of pH 4, and 2 mL of 0.1% MO, and the procedure was completed as mentioned above.

RESULTS AND DISCUSSION

Optimization of the analytical procedure

Optimum conditions necessary to achieve fast and quantitative creation of colored ion-pair complex with extreme stability and sensitivity were investigated by several preparatory tests.

Absorption spectra

The absorption spectrum of the yellow color glibenclamide-methyl orange ion-pair complex show λ_{max} at 426 nm, while the colorless blank solution has an essentially negligible absorbance Figure 1.

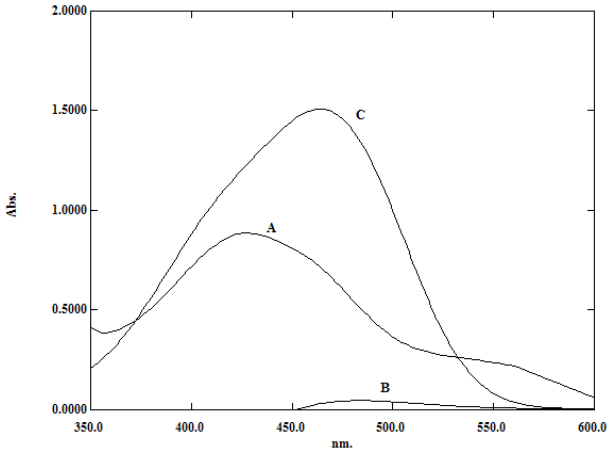


Figure 1: Absorption spectra of (A) Glb-MO ion-pair complex in dichloromethane (10 $\mu g.mL^{-1}$ Glb), (B) blank solution, and (C) methyl orange.

Effect of extraction solvent

The influence of the extraction solvent on the Glb-MO complex was tested. Chloroform, dichloromethane, ethyl acetate, and diethyl ether were examined for efficient extraction of the reaction product from the aqueous phase. Dichloromethane was observed to be the most appropriate solvent for extraction of ion-pair complex, due to its higher competence on color intensity, eclectic extraction of the ion-pair from the aqueous phase, attained the highest absorbance and lesser extraction capacity for the reagent blank Figure 2.

Effect of pH

The ion-pair complex (Glb-MO) depends commonly on the pH of the medium. So, the influence of pH on the creation of ion-pair was investigated using sodium acetate-hydrochloric acid buffer solution in the range (2–5). It became observed that most color intensities became acquired at pH 4, as shown in Figure 3, and thus, 2 mL of buffer solution (pH 4) was used throughout the experiments.

Effect of reagent concentration

The influence of MO concentrations was experienced by taking varied volumes of methyl orange (0.2–2 mL) to a constant amount of glibenclamide (10 μg.mL^{-1}). It was noticed from Figure 4 that the absorbance was constant beyond 0.8 mL. Thus, 2 mL of the reagent solution was necessary to yield a maximum absorbance.

Effect of reaction time

The best time of reaction, which achieves a maximum absorbance of the ion-pair complex, was studied from an immediate measurement to a waiting time of 10 min. It was observed that 6 minutes of mixing glibenclamide with methyl orange is the optimum time for the reaction product to attained maximum absorbance Figure 5.

Effect of order of mixing

The sequence (buffer-reagent-drug-solvent) was found to be the most favorable arrangement for the maximum color intensity, whereas the other orders produce lesser absorbance values.

Effect of shaking time

Ion-pair complex was formed by using vortex mixer, and shaking times ranging from 1–5 minutes was investigated. The optimum time for shaking is the time recommended for the quantitative extraction of the ion-pair complex with dichloromethane was found to be 3 minutes Figure 6.

Stability

The absorbance of the Glb-MO complex was evaluated at interims from the time of extraction, and it was noted a

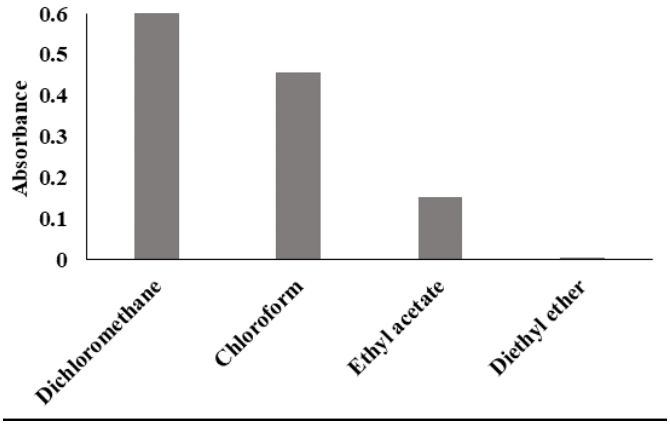


Figure 2: Effect of different extracting solvents on the ion-pair formation.

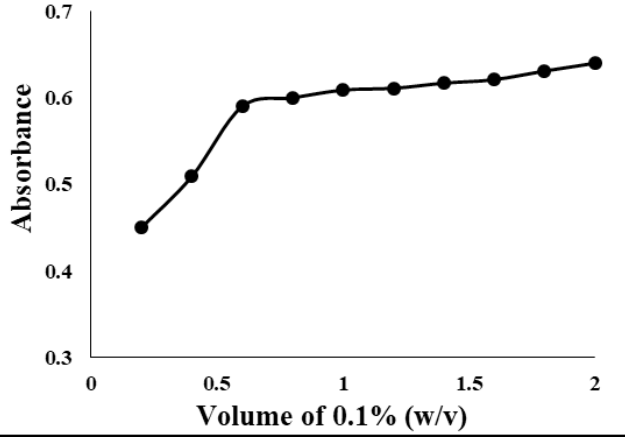


Figure 4: Influence of methyl orange volumes on Glb-MO ion-pair complex.

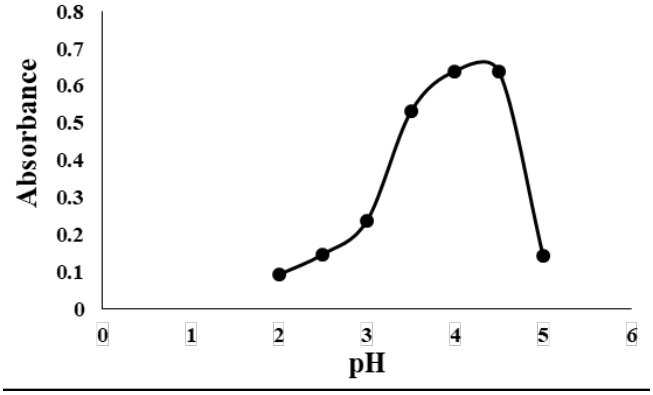


Figure 3: Effect of pH on the absorbance of Glb-MO ion-pair complex.

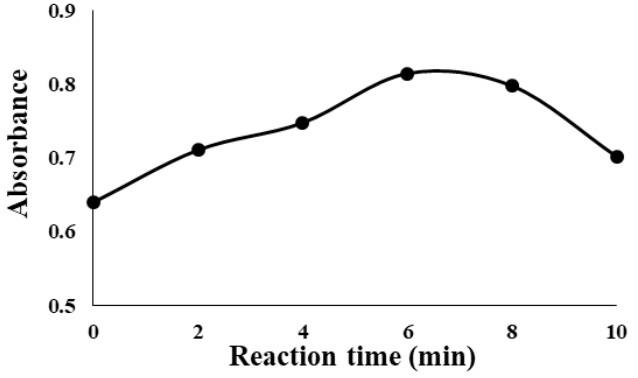


Figure 5: Influence of reaction time on Glb-MO ion-pair complex.

decrease in the absorbance when the reaction product left to stand at room temperature. So, it is recommended to measure absorbance immediately.

Composition of ion-pair complex

So as to determine the molar ratio between the investigated drug substance and MO, Job's method of continuous variations and mole-ratio method were utilized: A 1.0 $\times 10^{-4}$ M of Glb and MO were employed. A set of solutions was prepared where the entire volume of Glb and MO was preserved at 5 mL. The absorbance of the resulting ion-pair complex was recorded at λ_{max} according to the general procedure. The data showed that 1:1 ion-pair [Glb: MO] is created by the electrostatic attraction between them Figures 7 and8.

$$Glb^{+} + D^{-}_{(aq)} \longleftrightarrow Glb^{+}\,D^{-}_{(aq)} \longleftrightarrow Glb^{+}D^{-}_{(org)} \quad (1)$$

Where Glb^{+} and D^{-} illustrate the protonated Glb and the anion of the dye, correspondingly, (aq) and (org) refers to the aqueous and organic phase, correspondingly. The recommended mechanism of Glb-MO ion-pair complex creation is presented in Scheme 2.

Formation constant of ion-pair complex

The formation constant for the ion-pair complex was calculated for the interaction of a drug with the dye by employing the Benesi-Hildebrand equation[18] as presented below:

Where [MO] and [Drug]: the total concentration of methyl orange and drug, respectively. A_c: the absorbance of complex, $\mathcal{E}_c$: the molar absorptivity of the complex, and K_{IP}: the formation constant of (drug-dye) ion-pair complex. The value for K_{IP} was found from the slope of the line attained by plotting [MO]/A_c versus 1/[Drug]. The plot was linear, as shown in Figure 9, and the result is presented in Table 1.

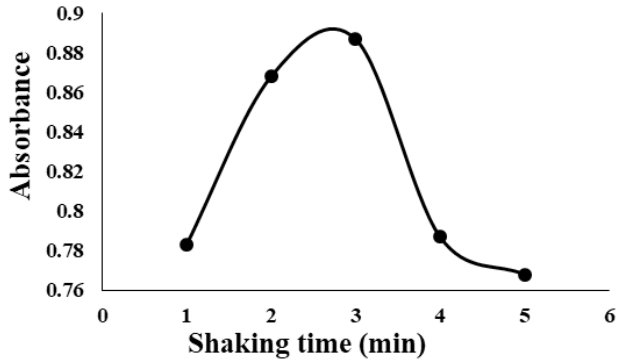


Figure 6: Influence of shaking time on the Glb-MO ion-pair complex.

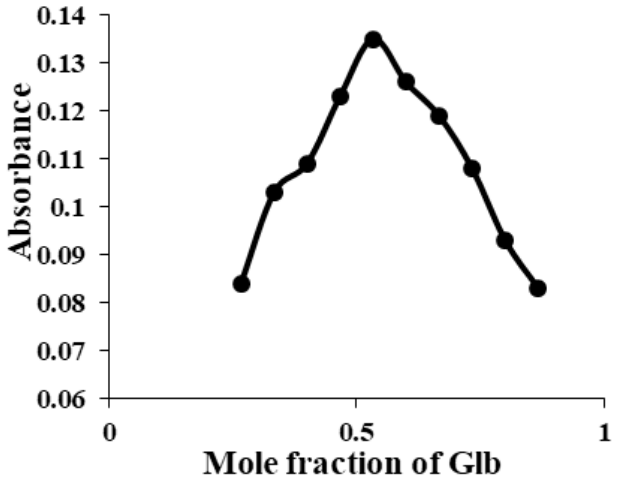


Figure 7: Continuous-variations plot for 1:1 complex

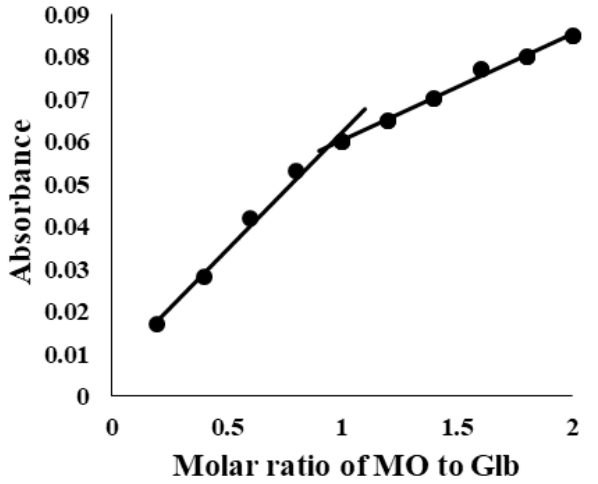


Figure 8: Mole-ratio plot for a 1:1 complex.

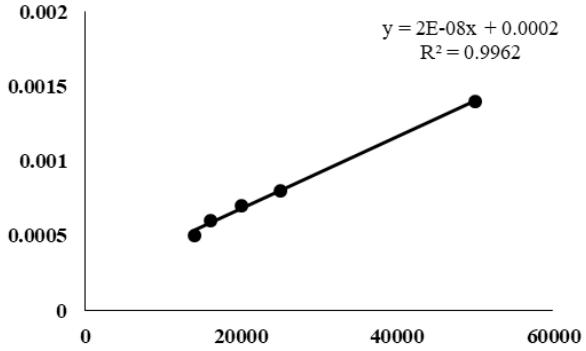
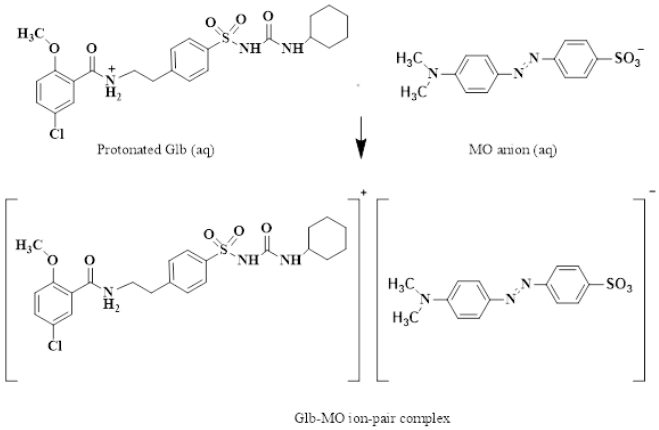


Figure 9: Benesi-Hildebrand plot for the Glb-MO complex.



Scheme 2: a Suggested mechanism for formation ion-pair complex between Glb and anionic dye, methyl orange.

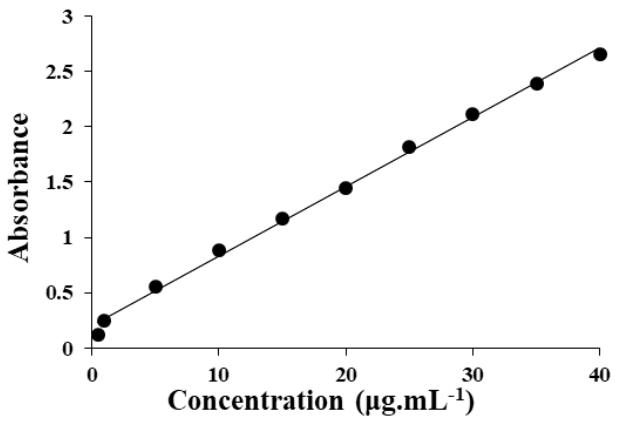


Figure 10: Calibration graph for glibenclamide.

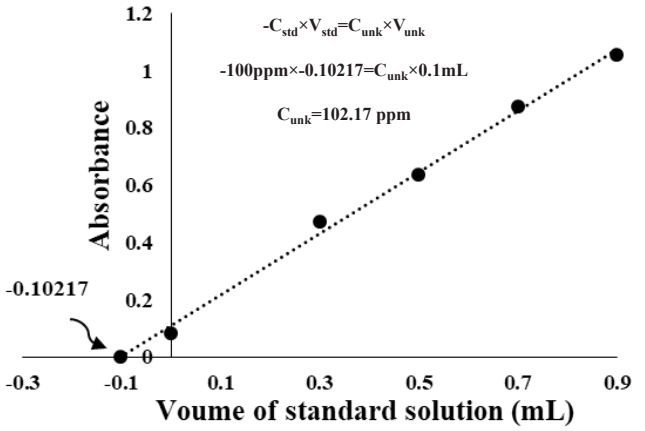


Figure 11: Determination of Glb in tablet (Cyprus) by SAM (0.1 mL of 100 µg.mL^{-1} tablet).

Method validation

Linearity

A linear relationship constructed between the response at λ_{max} and the concentration of the Glb in the range 0.5-40 µg.mL^{-1} Figure 10. The linearity of the calibration curve was demonstrated by the elevation value of the correlation coefficient (r). The high molar absorptivity of the reaction product shows the high sensitivity of the method in Table 2.

Sensitivity

The limit of detection (LOD) and the limit of quantification (LOQ) for the suggested method were calculated in accordance with the ICH guidelines.[19] LOD estimated by the subsequent equation:

Where *σ*: the standard deviation of repeat determination values at similar conditions as in the sample analysis but without of the analyte, and *b*: the sensitivity, in other words, the slope of the calibration curve. According to the formula, the limit of detection was observed to be 0.1086 µg.mL^{-1}. The limit of quantification, LOQ, is defined as:

According to this formula, LOQ was observed to be 0.3292 µg.mL^{-1}.

Table 1: Formation constant of the ion-pair complex between the drug and MO.

Parameter	*Observation*
Intercept	0.0002
Slope	2E-08
Correlation coefficient [r]	0.9981
ε_c [L.mol^{-1}.cm^{-1}]	5000
K_{IP} [L.mol^{-1}]	10000
ΔG [KJ.mol^{-1}]	-22.8234

Precision and accuracy

The precision and accuracy of the proposed method were determined by taking three concentrations of Glb within the Beer's law limit under optimum condition prepared and analyzed in three replicates on the same day. The accuracy of the method is illustrated by the good recovery (99.54-100.16%), and the precision is confirmed by the low (RSD %) < 1% Table 3.

Analysis of pharmaceutical preparation

So as to support the veracity of application of the suggested procedure, standard addition method (SAM) was applied for the analysis of Glb in its pharmaceutical preparation and to eliminate the effect of any matrix that interfere with analyte measurement signals.

The SAM is applied on pharmaceutical preparation containing Glb as an active ingredient. The extrapolation of the line, as shown in Figure 11, intersected with the x-axis at -0.10217 mL with a percent error of 2.17%. The high value of the percent recovery 102.17% reflects the high efficiency of

Table 2: Optical characteristics and statistical data of the regression equation for Glb with MO.

Parameter	*The proposed method*
λ_{max} [nm]	426
Beer's limit [µg.mL^{-1}]	0.5-40
Molar absorptivity [L.mol^{-1}cm^{-1}]	31122
Sandell's sensitivity [µg.cm^{-2}]	0.0159
Slope [mL.µg^{-1}]	0.063
Intercept	0.1971
Correlation coefficient [r]	0.9982
LOD [µg.mL^{-1}]	0.1086
LOQ [µg.mL^{-1}]	0.3292

Table 3: Precision and accuracy data for Glb attained by the suggested method.

Nominal Conc. [µg.mL^{-1}]	*Found* Conc. [µg.mL^{-1}]*	*RSD [%]*	*Er [%]*	*Recovery [%]*
5.000	4.977	0.731	-0.460	99.54
20.000	19.988	0.518	-0.060	99.94
35.000	35.057	0.366	0.163	100.16

*Average of three determinations.

applying standard addition method for determination of Glb in its pharmaceutical preparation.

CONCLUSION

In this study, the ion-pair complex of selected drugs with methyl orange was studied at the optimum extraction conditions. Electrostatic interaction occurs between the secondary nitrogen group present in Glb and the sulfonic acid group of MO. The reagent utilized in this study is inexpensive, easily obtainable, and the method does not include any dangerous reaction conditions or tedious sample preparation. The proposed procedure is selective, fast, and cheap, and the results attained indicated reasonable precision, accuracy, and recovery of the drug. The extensive ability of this method for routine quality control is well confirmed by the analyze of Glb in pure form, and in addition in its respective formulation.

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