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Inclusion Complex of Metformin and β-Cyclodextrin Spectroscopic Study and Analytical Application

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ABSTRACT

The supramolecular interaction of metformin and β-cyclodextrin (β-CD) has been investigated utilizing Uv-visible spectroscopy and Fourier transforms infrared spectroscopy (FTIR). The formation of inclusion complex has been confirmed based on the changes of the spectral properties. The results showed that β-CD reacted with metformin to form 1:2 host-guest complex. In addition, solid inclusion complex was synthesized. Based on the significant enhancement of the absorption intensity of metformin produced through complex formation, a simple, accurate, rapid and highly sensitive spectrophotometric method for the analysis of metformin in pharmaceutical dosage form was developed and validated. The parameters influencing the inclusion complex formation were studied and optimized. At optimum experimental conditions a linear relationship between absorbance and concentration of metformin is observed in range of 2-20 μg mL^{-1} of metformin at maximum wave length 233 nm, with limit of detection (LOD) 0.61 μg mL^{-1} and correlation coefficient of (r= 0.9996). The proposed method was applied successfully to the determination of metformin in pharmaceutical preparations and the results were satisfactory in comparison to official method.

Keywords: Metformin, β-cyclodextrin, inclusion complex, FTIR.

INTRODUCTION

Metformin hydrochloride (MET) Figure 1 with chemical structure 1,1-Dimethylbiguanide hydrochloride [1]. It is antidiabetic drug that is utilized in the medication of type 2 diabetes (non-insulin dependent diabetes) that recovers the control of glycaemia primary by inhibiting hepatic gluconeogenesis and seems to ameliorate hyper glycaemia by improving peripheral sensitivity to insulin, reducing gastro intestinal glucose absorption and hepatic glucose production [2,3]. Currently metformin is accessible for the treatment of polycystic ovary syndrome and has been reported to recover vascular function, prevent pancreatic cancer and other diseases [1,4].

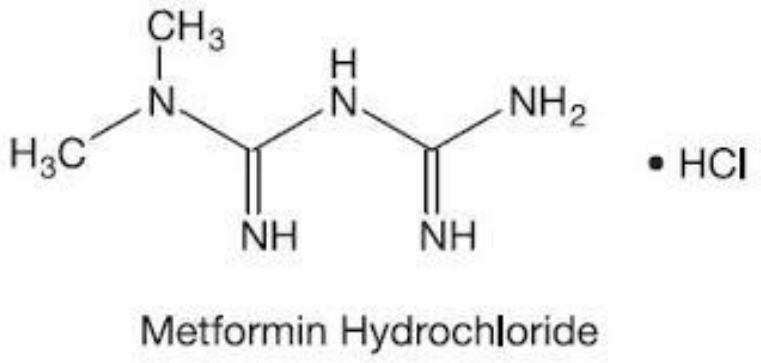

Figure 1: Chemical structure of Metformin.

A number of methods have been reported in the literature for the analysis of metformin hydrochloride in pharmaceutical preparations and biological fluids. These include high performance liquid chromatography (HPLC), gas liquid chromatography (GC), capillary electrophoresis (CE), near infrared spectroscopy, UV-spectrophotometry, conductometry, voltammetry or by visual titration, NMR spectrometry, Chemiluminescence and atomic spectrometry [5-18].

Cyclodextrins are a family of cyclic oligosaccharides, composing of a macrocyclic ring of glucose sub units linkage by α-1,4 glycosidic bonds arranged in " chair conformation " therefore the molecules are shaped like a hollow truncated cone [19]. The outer periphery of the macrocyclic ring is hydrophilic because of the presence of numerous hydroxyl groups. The internal cavity of CDs is relatively hydrophobic and can encapsulate a variety of compounds particularly pharmaceuticals. CDs have an extensive range of application in pharmaceutical, cosmetic, food industry and other industries**.** It uses for a variety of purpose which include solubility enhancement of insoluble substances, stabilization of labile guests against oxidation, light and heat, control of volatility and controlled release of drugs, flavors and various branches of analytical chemistry [20-25]. The three industrially important forms are, alpha (-α), beta (-β) and gamma (γ) CDs, are composed of six, seven, and eight glycopyranose units respectively. The capability of CDs to form inclusion complexes is highly influenced by size, shape, hydrophobicity and form of guest molecule. Inclusion complex between CD host and the guest is determined by several weak forces, including van der Waals, hydrophobic dipole-dipole and hydrogen bonding interactions [26]. From an analytical perspective the formation of inclusion complexes allows to enhance fluorescence intensity [27-30] and induce chiral separation in capillary electrophoresis (CE) [31-34]. Elbashir et al., have made comprehensive review on this topic for determination of several pharmaceutical drugs, pesticides and metal ion [35].

The inclusion complex of metformin with β-CD has been investigated with UV-visible spectroscopy and infrared spectroscopy. Based on enhancement in the absorbance of metformin produce through complexation with β-CD, a

spectrophotometric method for the analysis of metformin in pharmaceutical dosage form was developed and validated. Moreover, solid complex of metformin/ β-CD was prepared by co-precipitation method.

EXPERIMENTAL PROCEDURE

Apparatus

Absorbance was done using UV-visible spectrophotometer model Shimadzu 1800 with quartz cells of 1 cm optical path length. IR was recorded using (FTIR) spectrometer 8400s (Shimadzu, Japan). Samples were pressed into KBr pellets and measured at frequencies from 4000 to 500 cm $^{-1}$. pH meter model HI 255 (Hanna Instruments, Mumbai, India) was used for pH measurement, analytical balance, ultrasonic bath and shaker.

Reagents and solutions

Metformin hydrochloride was kindly gifted from general medicine company (Khartoum, Sudan) and was used as received; its purity was 100%, and β-CD was purchased from sigma-Aldrich (St. Louis, USA), Tablets claim to contain 850, 500 mg per tabs were obtained from a local pharmacy. Other chemicals of analytical grade were used.

Stock and standard solutions: A 100 gml^{-1} stock standard solution of metformin was prepared by dissolving 10 mg of metformin in distilled water into 100 ml volumetric flask and diluted to the mark with the same solvent and mixed well. 0.1134 g of β-CD was dissolved in distilled water transferred into 100 ml volumetric flask and diluted to the mark with the same solvent and mixed well to prepare 1×10^{-3} M. pH 4.0 buffer solution was prepared from 0.2 mol L^{-1} KCl solution and 0.2 mol L^{-1} HCl solution and the pH was adjusted by pH meter. Other buffer solutions having different pH values were also prepared according to the method in the literature.

Procedure

UV-visible spectroscopy measurement: A 0.2 ml portion of 100 µg ml^{-1} metformin hydrochloride standard solution was placed into 10 ml volumetric flask, 2 ml (pH 4.0) buffer solution and 2 ml β -CD were added. The mixture solution was shaken thoroughly, standing for 10 min, diluted to the mark with water and very well mixed. The absorption spectrum that the β-CD reacts with metformin to form host guest complex was measured against reagent blank prepared with same reagent concentration without metformin. The absorbance spectra were measured at 233 nm.

Preparation of solid complex: The solid inclusion complex between metformin hydrochloride and β-CD was prepared using co-evaporation method. Accurately weighed 0.1 g of metformin hydrochloride was transferred and dissolved in 20 ml of methanol. About 0.2597 g of β-CD was dissolved in 30 ml of distilled water. Both the solutions were placed into 250 ml beaker, placed over electromagnetic stirrer for 24 hours at room temperature to evaporate. At this time the formation of white crystal was observed. This is inclusion complex of Met with β-CD, and was then subjected to characterization.

Stoichiometry of the inclusion complex was analyzed using job's method of continuous variation [36]. Equimolar 3×10^{-3} mol L $^{-1}$ solution of metformin and β -CD were mixed to standard volume (1: 9 …., 9: 1), inclusive, varying the molar ratio but keeping the total concentration of the species constant. An analogue dilution set of metformin stock solution was carried out using distilled water, and the solution were allowing to stand for 10 min, the maximal absorbance was measured for all solutions and the difference in absorbance in the presence and in absence of β-CD was plotted against R.

Determination of metformin in pharmaceutical: Twenty tablets of metformin hydrochloride were accurately weighed and powdered. A quantity of the powder incorporating 10 mg of metformin was placed into 100 ml volumetric flask, about 70 ml of water was added, and the mixture was shaken for 10 min and the volume was completed with water to give a concentration of 100 µg ML^{-1}, and the solution was filtered.

RESULTS AND DISCUSSION

Absorption spectra

The absorption spectrum of metformin in the absence and in the presence of 2 × 10-4 M β -CD was first recorded. The result is presented in Figure 2, showed that the wavelength of maximum absorbance of metformin at pH 4.0 was 233 nm, when β-CD was added into the metformin solution, the wavelength of maximum of absorbance did not change however, the absorbance is slightly increasing, giving rise to molar absorptivity coefficient from 1.07×10^4 to 1.6×10^4 L moL^{-1} cm^{-1}.

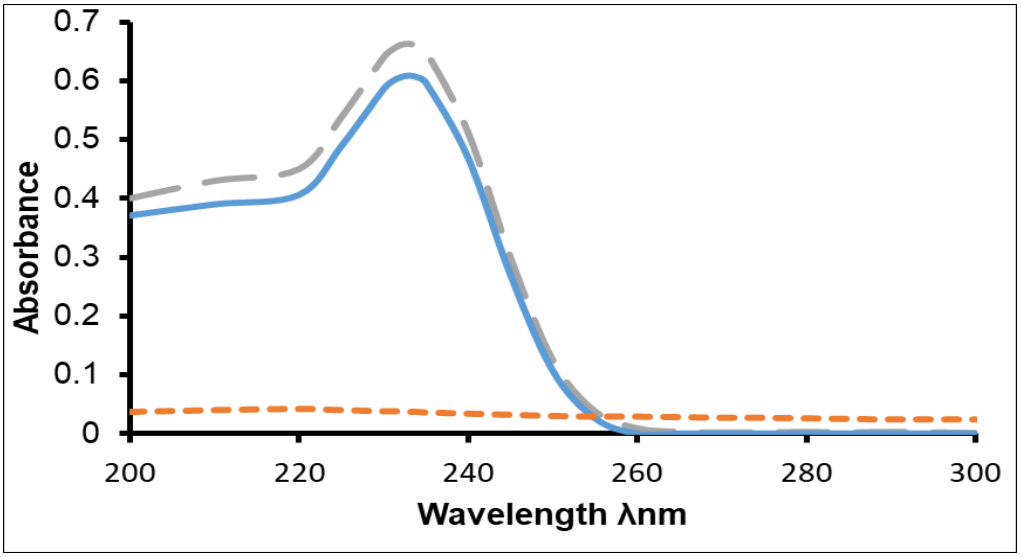

Figure 2: Absorbance spectra of β-cyclodextrin, Metformin and metformin – β-CD, concentration of metformin 8 µg ml^{-1}, β-CD 1×10^{-3} M, at room temperature, time 10 min, pH 4.0.

Infrared spectra studies

The FTIR spectra of the β-CD, metformin and metformin- β-CD inclusion complex are represented in Figure 3. The β-CD exhibited key peaks at around 3400 cm^{-1} to 3200 cm^{-1} (O-H stretching vibration) [37,38], 2932 cm^{-1} (C-H stretching) and 1154 cm^{-1} (CC bending). The frequencies of metformin hydrochloride were at 3370 cm^{-1} and 3298 cm^{-1} which correspond to the (N-H) asymmetric and symmetric stretching vibration respectively. The peak at 3170 cm^{-1} demonstrate (N-H) symmetric stretch, bond lowered due to conjugation. The frequencies at 1628 cm^{-1} and 1541 cm^{-1} correspond to bending vibration of (N-H) [39,40]. The peaks at 1165 cm^{-1}, 1033 cm^{-1} represent (C-N) stretching frequencies [41]. At 1446 wave number, a peak is observed which confirmed the presence of C-H (in-plane bending) [42].

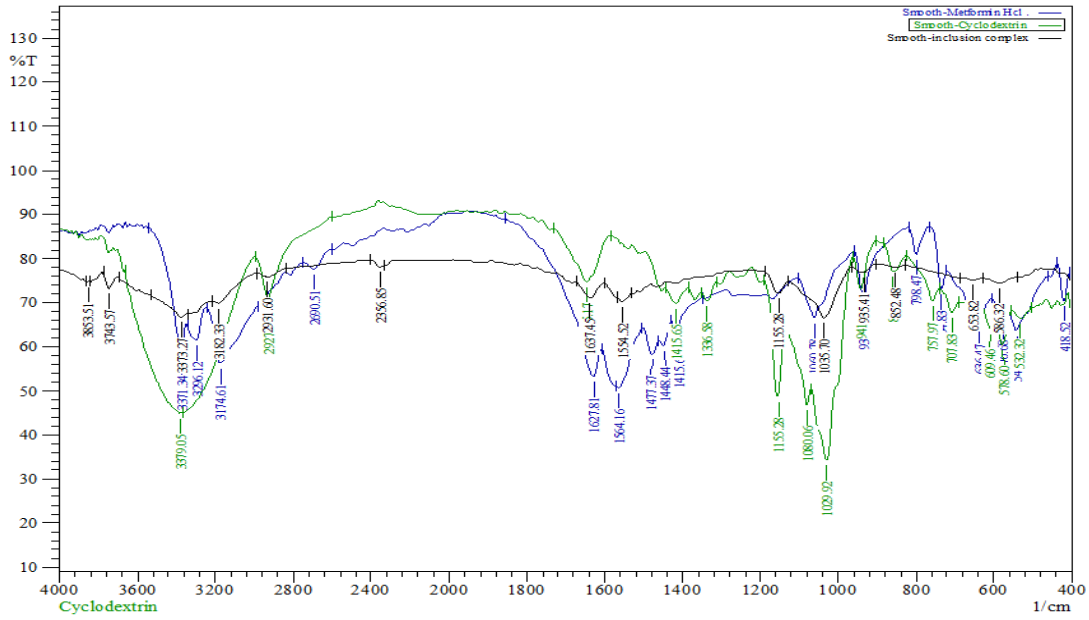

Figure 3: FT-IR spectra of (—) Pure HPβCD, (—) Pure metformin, and (—) metformin-βCD complex.

The inclusion complex between metformin and β-CD had shown shift in the characteristic peaks of parent component. The C-N peak of metformin had shifted to a lower frequency (blue shift); from 1165 cm^{-1} and 1033 cm^{-1} to 1152 cm^{-1} and 1027 cm^{-1}. New peaks were seen in the region of 2934 and 2930 cm^{-1}. The shift in C-N stretching vibration to lower frequency region is assigned to hydrogen bonding interaction between the N-H of metformin and hydroxyl group of β-CD. This clearly confirms that the N-H group is included in the interaction of metformin with β -CD in this inclusion complex. Thus the FTIR spectrum significantly confirms the formation of metformin hydrochloride and β-CD inclusion complex.

Optimization of the reaction conditions

The effect of different factors such as pH, time, buffer volume and concentration of β-CD on the formation of inclusion complex were studied. The optimum conditions for the development of procedure were investigated by varying the parameters one at a time while keeping the others constant.

Effect of pH: Effect of pH on the absorbance of the metformin-β-CD complex has been studied at pH range from 1.0 to 7.0. The results were presented in Figure 4. The absorbance of inclusion complex increased from 1.0 to 4.0 and remains constant after pH 5.0. The tests demonstrated that the absorbance of inclusion complex was maximal at pH 4.0, what is more an excellent linear relationship existed between the absorbance of inclusion complex and the concentration of metformin at pH 4.0. Therefore, pH 4.0, K Cl- HCl buffer solution was selected as an optimum pH.

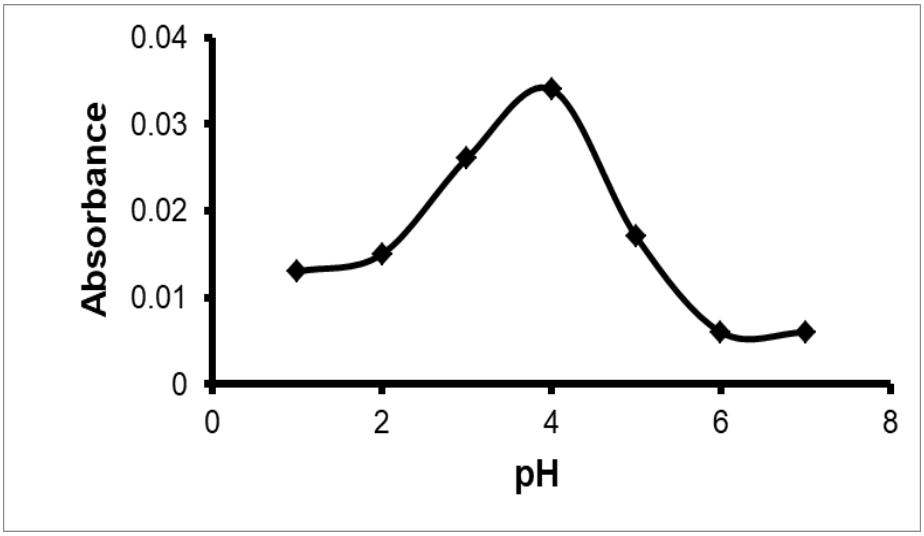

Figure 4: Effect of pH on the reaction of metformin with β-CD, 1 ml of metformin (100 µg ml^{-1}**),** 2 ml β-CD 1X 10^{-3} M, reaction time 10 min.

Effect of temperature and time: The effect of temperature on absorbance of metformin β-CD was tested in the range of 20-70 ° C. A gradual decrease in absorbance was seen as temperature is increased, value 25 ºC was selected as optimum temperature. Effect of time in room temperature was studied, after waiting for different time periods at 25 ºC and the absorbance begin to increase instantly and become constant after 15 min.

Effect of β-CD concentration: The effect of β-CD concentration on the absorbance of metformin- β-CD was examined. The concentration of metformin was fixed at 10 µg ml^{-1} and the concentration of β-CD was varied from (1×10^{-4} to 5×10^{-5} moL L^{-1}). As the concentration of β-CD increase, the absorbance is increased until the stable inclusion complex formed at about 2×10^{-4} M, and then the absorbance decrease.

Effect of amount of the buffer: Keeping pH at 4.0, the effect of amount of buffer solution on the absorbance of metformin-β-CD inclusion complex was also studied. The absorbance enhances rapidly with the rise of amount of the buffer solution, and become maximal when the amount of buffer solution is 2.0 ml. Therefore, a volume of 2.0 ml KCl- HCl buffer pH 4.0 was recorded as optimum in this study.

Furthermore, the stoichiometry of the inclusion complex was studied using job's method of continuous variation [36]. Equimolar 3×10^{-3} mol L^{-1} solution of metformin and β-CD were mixed to standard volume (1:9 …., 9:1), inclusive, varying the molar ratio but keeping the total concentration of the species constant. An analogue dilution set of metformin stock solution was carried out using distilled water, and the solutions were allowing to stand for 10 min, the maximal absorbance was recorded for all solutions and the difference in absorbance in the presence and in absence of β-CD was plotted against R. The job plot of absorption versus molar ratio was symmetrical and proved that 1:1 ratio (drug/reagent) supporting that one molecule of metformin reacts with one molecule of β-CD (Figure 5).

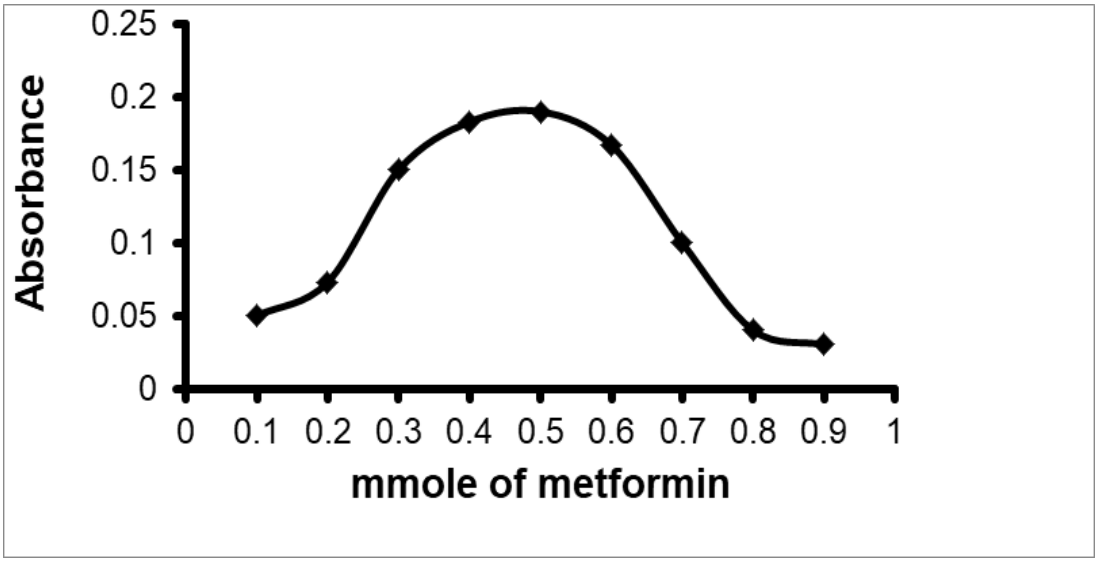

Figure 5: The continuous variation plot for the stoichiometry of the reaction of metformin with β-Cyclodextrin.

Validation of Analytical method

The validity of the procedure was examined with regard to linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and robustness following the International Conference on Harmonization (ICH) guidelines [43].

Linearity and Sensitivity: The limit of detection (LOD) and limit of quantification (LOQ) were measured using the formula LOD = 3.3 × SDa/b and LOQ =10x SDa /b, SDa is the standard deviation of the intercept, and b is the slope. The LOD and LOQ were measured to be 0.61 and 1.8 µg/ml respectively, Table 1. In the developed method linear plot (n=10) with good correlation coefficient were gained in the range 2-20 µg/ml. The regression equation was found to be as y=0.076x +0.049, where y is the absorbance at 233 nm, x is the concentration of metformin in µg/ml in the range 2-20 µg/ml.

Table 1: Summary of quantitative parameters and statistical data using the proposed procedure.

Parameter	Value
Linear range	2-20
Limit of detection (µg ml^{-1})	0.61
Limit of quantification (µg ml^{-1})	1.8
Slope	0.076
Intercept	0.049
Correlation coefficient (r)	0.9996
molar absorptivity of metformin (L mol^{-1} cm^{-1})	1.07×10^4
molar absorptivity of β -CD for inclusion complex (L mol^{-1} cm^{-1})	1.6×10^4
Regression equation	Y=0.076 × +0.049

Accuracy and Precision: The accuracy of the develop method was performed out using standard addition technique. A different quantity of standard solution was added to known concentration of the drug sample. The average percent recoveries obtained in range 98.8 -100.5, Table 2, with corresponding interday precision (RSD) 0.86% and intraday precision 1.13%. This confirming is

high reproducibility of the results and precision of the procedure. This good level of precision was adequate for quality control analysis of metformin in its pharmaceutical formulations.

Table 2: Recovery of the proposed method.

Sample Content (µg ml^{-1})	Standard added (µg ml^{-1})	Found (µg ml^{-1})	Recovery (% ± RSD)
2	6	7.9	98.8 ± 0.43
2	8	10.03	100.3 ± 0.3
2	14	16.09	100.5 ± 0.25
Recovery was calculated as the amount found /amount taken × 100. Values are mean ± R.S.D for three determinations.			

Robustness: Robustness was tested by assessing the effect of small variation in the procedure parameter on its analytical performance. In these experiments, one parameter was varied whereas the others were kept constant, and the recovery percentage was calculated each time. The results obtained indicate that small variation in the method parameters did not remarkably affect the methods; recovery values were illustrated in Table 3.

Table 3: Robustness of the proposed spectrophotometric method.

Recommended Conditions		Recovery % ± RSD
Standard Condition		100.1 ± 0.20
pH	4.2	98.6 ± 0.62
	3.8	99.7 ± 0.54
β-cyclodextrin concentration	2.2×10^{-3}	100.2 ± 0.63
	1.8×10^{-3}	99.5 ± 0.36
Temperature (0C)	30	101.4 ± 0.98
	20	99.2 ± 0.41
Reaction time (min)	15	100.5 ± 0.47
	5	97.9 ± 1.10
Values are mean of three determination		

Application of the proposed procedure to real sample

Metformin hydrochloride tablets were analyzed using the develop method as well as with the official spectrophotometric method (British Pharmacopeia) and the results were statistically compared with each other. The label claim percentage was 99.9% and 100.1% for metformin 500 and 850 mg respectively Table 4. The calculated t- and F-test values, indicates no significant difference between the calculated and theoretical values of both the proposed and the official methods at 95% confidence level. This confirmed similar accuracy and precision in the analysis of metformin in tablets. The proposed method has the advantage of being practically free from interferences by excipients.

Table 4: Results of assay of formulation for the proposed method

Tablet	Label claim Mg/tablet	Assay% of label claim
Glucophage	500	99.9
Metnormin	850	100.1

CONCLUSION

The results obtained in this work obviously demonstrate that β-CD react with metformin to form a 1:1 host-guest complex. The formation of the inclusion complex metformin-β-CD was verified by FTIR, UV-visible spectroscopy. Based on the improvement of the absorbance intensity of metformin resulted from complex formation, a simple, sensitive and accurate method for determination of metformin in the presence of β -CD was proposed. The developed method is fully validated and successfully utilized for the determination of metformin pharmaceutical formulations. Frequently present excipients did not found to interfere during the assay.

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