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NEW REAGENT FOR SPECTROPHOTOMETRIC DETERMINATION OF METFORMIN IN PURE FORMS AND IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

An accurate, simple, fast and cheap spectrophotometric method has been developed for the determination of Metformin in pharmaceutical pure and dosage forms The method is based on the formation of violel complexe between metformin and chrome azurol S in phosphate buffer at pH=2.5. The resulting complex was measured at λ_{max}=570 nm. The effect of variables such as reagent concentration, and metformin concentration investigated to optimize the procedure. Beer's law was obeyed in concentration 3.31-57.96 µg/ml and the defection limits LOD and quantitation (LOQ) for proposed method are 0.781 and 2.660 µg/ml respectively, Recovary studies and statistical data proved the accuraty reproducbility of the proposed method. The common excipients did not inteffer in this analysis. Hence the methed is useful for routine estimtion of pure metformin and in pharmaceutical formulations.

KEYWORDS: Metformin, chrome azuroL S, complex formation, Spectrophotometric determination.

1- INTRODUCTION

Metformin hydrochloride(Glucophage) (1), chemically is 1,1-Dimethyl biguanide hydrochloride with a molecular formula of C4H12ClN5 (Fig 1).[1]

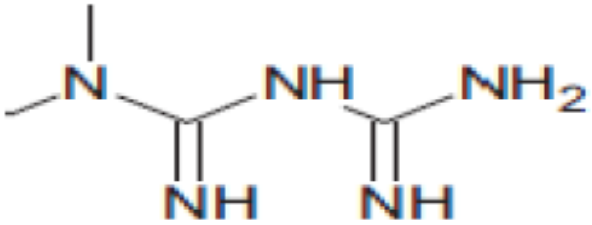

Fig 1: Molecular formula of C4H12ClN5.

It is a drug that enters the body by mouth to combat diabetes, the first drug of its kind chosen for the treatment of type 2 diabetes, especially in people who are overweight or obese and have normal kidney function. Metformin improves hyperglycemia, primarily through repressive action on the production of hepatic glucose (the formation of liver sugar). The number of people with diabetes is constantly increasing worldwide and the need for effective management assumes greater necessity.[2] Metformin hydrochloride has been estimated in pharmaceuticals and biological fluids. The formal method of UV spectra includes a method for estimating it in tablets.[3] Metformin has been known since 1957, but despite its simple chemical composition and multiple detailed investigations, its cellular mechanism remains unknown. Several theories have been developed to explain the work of metformin.[4] Many researchers have identified metformin by using the spectrophotometric method in pharmaceuticals using various reagents such as 1-naphthol oxidaized by sodiom hypochlorite and coupling with metformin, HCl in presence of NaOH, forming a blue-colored complex with a maximum wavelength at 580 nm within a range of 2 -20 µg / ml and molar absorption 3.66×10^4 L / mol.cm.[5] Mubeen G. and others were evaluated metformin in pharmaceuticals and biological fluids. Oxidized by using hydrogen peroxide forming a yellow complex at a 400 nm wavelength and within the field of the Beer's-Lambert law between 4-26 µg / ml and RSD= 1,112.[6] Radhika Bhaskar identified the metformin oxidized by hydrogen peroxide forming a yellow complex, within the field of the Beer's-Lambert Law between 6-24 mg / ml.[7] GUNASEKARAN was applied to both glycazide, pioglitazone and metformin by direct Spectrophotometry at the wavelength of λ = 234 nm with different concentrations and with acceptable accuracy.[8] Valtierra-Alvarado1 Monica A. and others determined a state of equilibrium •Metformin-copper complex in the center of metanole. The field of molar absorption coefficient was between 1.2 × 104-1.3 × 104 L / mol.cm.[9] Srinivas developed a rapid method using RP-HPLC to identify metformin and puglitazone in combination with simultaneous analysis. Metformin and poglitazone retention times were 3.2 ± 0.1 min and 7.3 ± 0.1 min respectively, and relative recovery was 99.4% and 99.6%, respectively.[10] Radhica Radvika identified metformin and glycazide, in a combination using the pseudo-synchronous

analysis and the correlation coefficient was R= 0.9912 and slope 1.0686.[11] Mohamed Salim developed a quick method to determine the cytagelpin and metformin in the case of a combination of the electroploresis, which was used in blood plasma analysis within a linear range of 10-100 µg / ml, 50-500 µg / ml, respectively, with a relative standard deviation RSD% 11.5%.[12] Mouben used optical Spectrophotomety to determination of pure metformin and some of its pharmaceutical preparations by oxidized with hydrogen peroxide where the yellow complex was at a maximum wavelength of λ = 400 nm. The field of the Beier Lambert Law was 4-26 µg / mL and the return value was 99-101.3.[13] The Amruta and et al. determination metformin in binary mixture with cytagalptin by simultaneous analysis. at λ1 = 232nm, λ2 = 266 respectively, and Beer's Lambert's law was 2-12 µg / ml and 25-225 µg / ml, respectively.[14]

Safaa. M. Riad and et al estimation both cytagalptin and metformin in pure state by direct optical Spectrophotometric method and applied it in binary mixture using the Q-Value method within the range of 25-500 µg and 20-1000) µg / ml respectively.[15] Telny Thomas used the simultaneous analysis of metformin and glybrzide in their pharmaceutical preparations at lengths of λ1 = 238 nm and λ2 = 275nm, respectively, within a range of 2-10 µg / ml and 1.2-6 µg / ml, respectively.[16]

2- MATERIALS AND METHODS

- All reagents and chemicals used were of Analytical Reagent: **Grade.** Metformin supplied by GSK Pvt. Ltd. Mumbai, India. Spectral and absorbance measurements were made with UV-VIS spectrophotometer (optima SP3000 from Korea) double beam spectrophotometer with 1 cm matched quartz cell. **Reagent Chrome Azurol S (CAS)**: Chrome Azurol S Is a powder in orange color, [CAS] and its chemical formula $C_{23}H_{13}C_{l2}Na_3O_9S$ and its molecular weight 605.29 g / mole,[7] is shown in figure[2] obtained from BDH. Acids and bases: Hydrochloric acid, Acetic acid, boric acid, phosphoric acid and sodium hydroxide, Inorganic compounds: Sodium Acetate and Mon hydrogen Phosphate from MERCK Company.

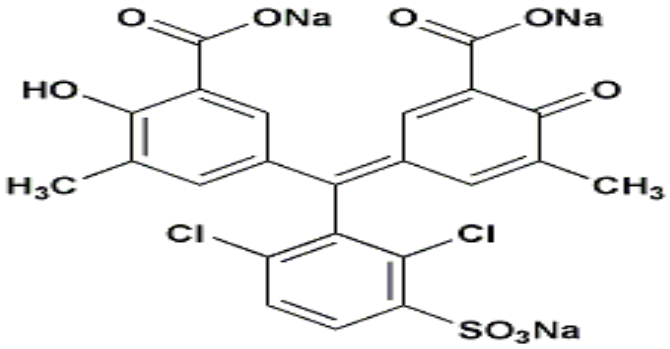

Fig [2] (CAS).

3- Experimental

3.1. Apparatus

All absorbance measurements and spectral runs were made on UV- Vis Spectrophotometer (OPTIMA SP3000 from Korea) double beam Spectrophotometer with 1cm matched quartz cells was used.

3.2. Reagents

All chemicals and reagents used were of analytical grad (Merck, BDH) and used without further Purification Metformin The pH measurement were carried transported with Sartorius Model PB 11 with glass slider.

3.3. Solution

3.3.1. Solution of Metformin

A stock standard Solution was Prepared by dissolved accurately weighed of Pure Metformin in deionized water and diluting to the mark in 100 ml calibrated flask. This stock solution with diluted appropriately with deionized water to obtain Suitable working Solutions. Freshly prepared Solutions were always employed**.**

3.3.2. Stock standard solution of Reagent

stock solution of chromeazurol S (CAS) was prepared by disolved accurately weighed of reagent in deionized Water and diluting to the mark with double distilled water in 100 ml calibrated flask Fig (3).

3.4. General procedure

3.4.1. Procedure for preparatıon of solution

In analysis of working of metformin 2ml $\times 10^{-3}$ mol/L and 1ml buffer phosphate and 2ml$\times 10^{-3}$ Chromeazurol S (CAS) was transferred into 10 ml calibrated flask and diluted by distilled ionized water to the mark.

3.4.2 Procedure for pharmaceutical Formulation

In analysis of Metformin (MET) in tablets by Proposed Method, 20 tablets of (MET) were weighed and pulverized into a fine powder and accurately weighed Portion of the powdered tablets of (MET) was transferred into 100 ml calibrated flask and dissolved in double distilled water filtered through a whatman filter paper (No.42).

Solutions of working range concentration were prepared by proper dilution of this stock solution with double distilled water and followed the above procedure for the analysis. The drug content of the (MET) formulation was the calculated (Table 3).

4- RESULTS AND DISCUSSION

4-1. Absorption Spectra

The absorption spectra of (MET-CAS) complex formed between Metformin and chrome azurol S was measured at (220-700 nm) against the blank Solution prepared under the same conditions as Fig (4).

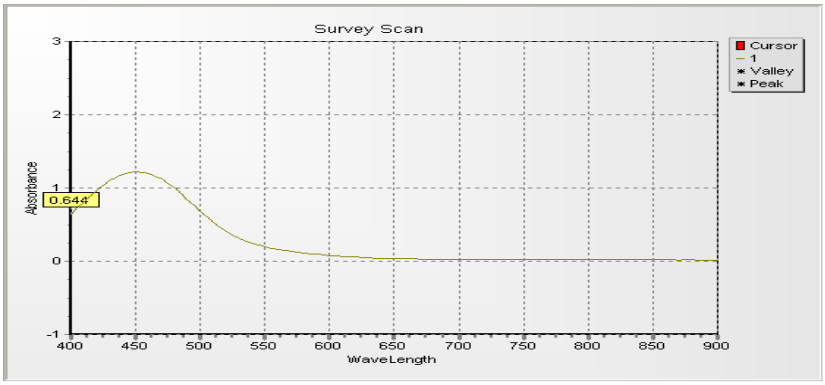

Fig (3*):* Chrome azurol S (CAS).

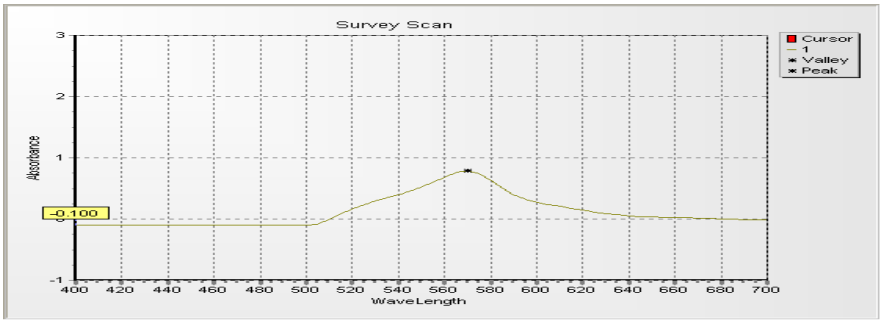

Fig (4): (MET-CAS) Complex.

4-2. Optimum conditions for complex formation

In order of establish the optimum conditions necessary for a rapid and quantitative formation of the Violet colored product and with maximum stability and sensitivity, the absorbance of a series of solutions was measured by varying one parameter while keeping the other constant.

4-2.1. Effect of concentration of reagent (CAS)

The effect of Concentration of Reagent chromeazurol S (CAS) on the formation of absorbance (MET):(CAS) complex was studied within range of concentration 6.05-151.**32** µg/ml.

A Series of Solution were prepared in each calibrated flask 10 ml Contain 33.12 µg/ml from (MET) and increased different concentration from chromeazurol S (CAS). The absorption measured at λ_{max}=570 nm plotted A=f(C) by Concentration of Reagent (CAS) Fig(5).

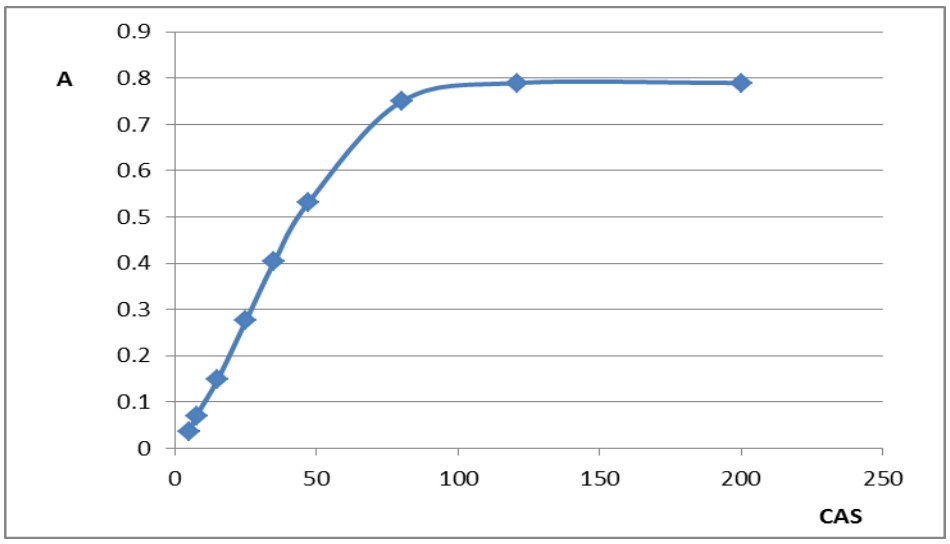

Fig (5): Effect of Reagent Concentration on MET-CAS Complex Absorption.

The concentration of 121.05µg / ml of chromium Azruol S is the optimum concentration of the highest absorption value.

4-2.2. Effect of Metformin Concentration

The effect of (MET) on the formation of absorbance of (MET):(CAS) complex was studied m presence of Concentration Metformin and volume of reagent (CAS) within range of (MET) drug (6.38-79.83 µg/ml). A series of solution were prepared in each calibrated flask contain 121.05 µg/ml from (CAS) reagent and increased different concentration of (MET) drug in calibrated flask 10 ml. The absorption measured at 570 nm. Plotted A=f(C) The relationship was then determined between the absorption changes of the complex formed by the change of the metformin concentration (MET). in Fig (6) shows that the concentration of 33.12µ g / ml of the drug gives better absorption.

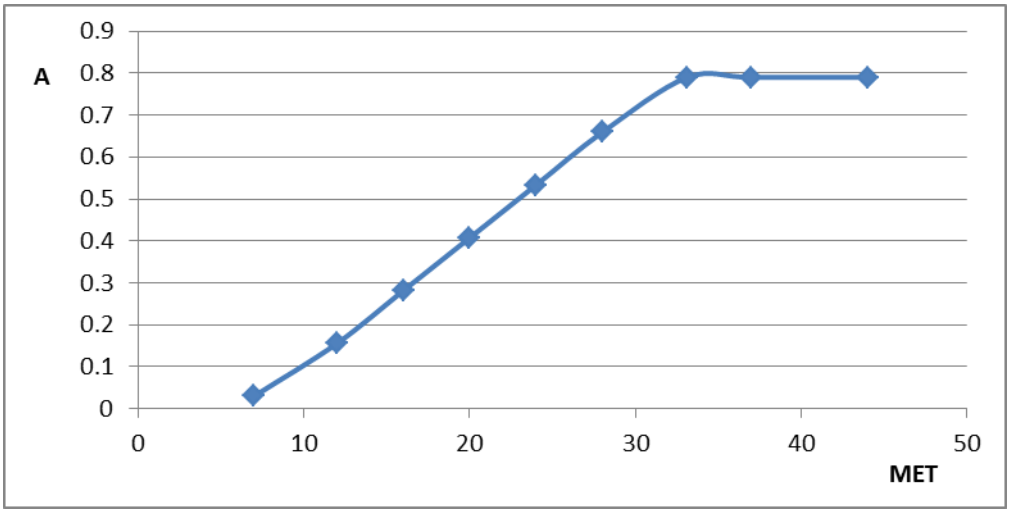

Figure (6): Effect of metformin concentration on MET-CAS complex.

The absorption of the solutions was measured after cooling to the laboratory temperature compared with the solution of the comparison (Blank). Note that the complex is formed directly at laboratory temperature, and remains constant up to 50C.

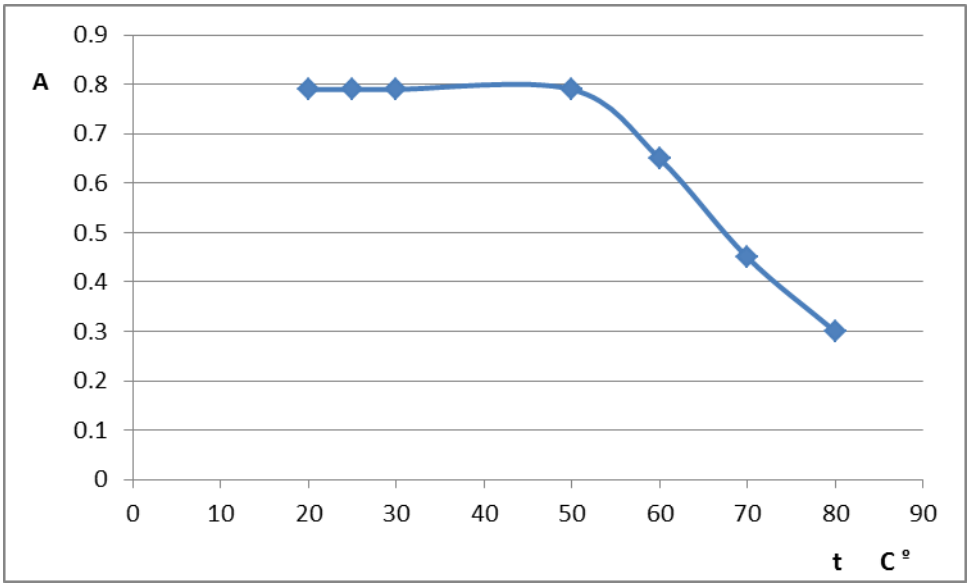

Figure (7): Effect of temperature on the stability of the complex.

4-2.3. Effect of pH

The influence of pH Of buffer solution on the development and stability of complex using different buffer solution Acetate buffer, Briton buffer, phosphate buffer over the P^H range (P^H = (1.0-12). The maximum absorbance observed to Phosphate buffer at pH= 2.5, λ_{max}=570 nm.

And optimum volume of the phosphate buffer solution required to be in the proposed procedure was found. Fig (8).

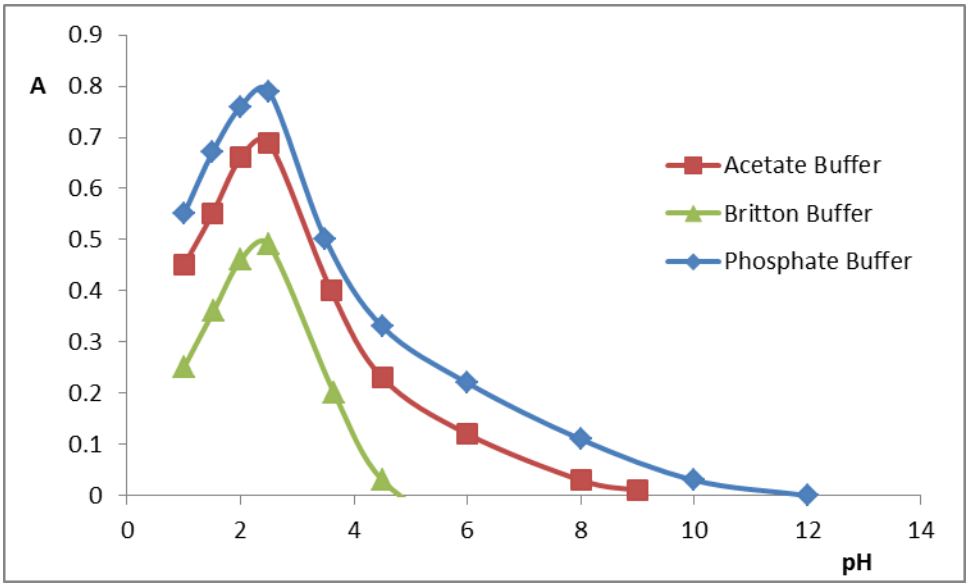

Fig (8): Effect of P^H on formation of (MET):(CAS) complex.

4-2.4. *Stability of complex* and Effect of time

The effect of time on the formation and stability of the complex was studied by measuring the absorbencies of the complex at increasing time intervals, the results show that the complex were formed almost instantaneously in the cases at room temperature (25 ± 2°C). The color of the MET: CAS remained stable for 48 h. after these intervals, a slight decrease in color intensity occurred (Fig9).

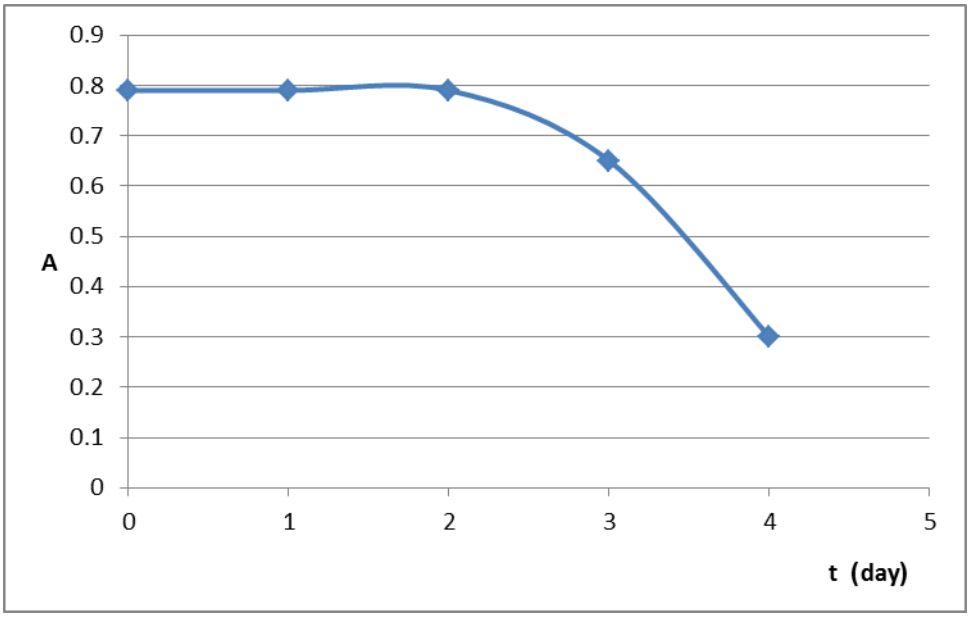

Figure (9): The effect of time on the stability of the complex.

4-2.5. Effect of Sequence of additions

The most favorable sequence is 'drug-reagent-buffer-solvent' for the complete color development and the highest absorbance at the recommended wavelength. Other sequences needed longer reaction time and produced lower absorbance values.

4-2.6. Effect of excipients

The influence of commonly used tablet excipients (lactose, sucrose, glucose and starch) was investigated before the determination of metformin in dosage form. No interference could be observed with the proposed method. This shows that the method is applicable in the case of pharmaceutical preparations of the Metformin.

4 -3. Stoichiometric relationship

The Composition of Complex was established by applying Job's method of continuous Variations a 1×10^{-4} M Standard solution of (MET) and 1×10^{-4} M solution of (CAS) were

used. A series of solutions were prepared in which the total volume of (MET) metformin and chromeazurol S (CAS) was Kept constant.

The reagents were mixed in various proportions in the optimum conditions and diluted to volume in a 10 ml calibrated flask with double distilled water following the above mentioned proceeds. The plots of absorbance against the mole fractions of (MET): (CAS) at λ_{max}=570 nm the curve show maxima of 0.5 the results evidently formed. The molar ratio of the Metformin to Chromeazurol S of the color complex was determined using the molar ratio and continuous variation methods. the ratio were found to be 1:1 for MET:CAS (Fig. 10 -11).

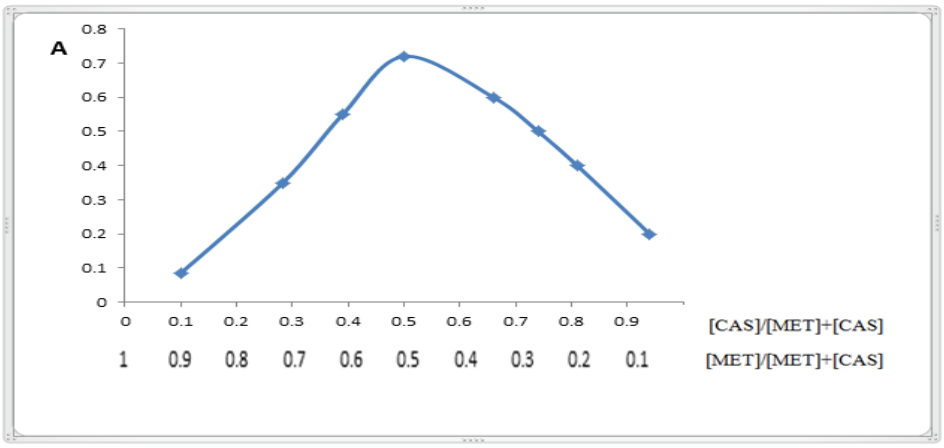

Figure (10): Determination of the correlation ratio of the MET-CAS complex in a continuous variation method.

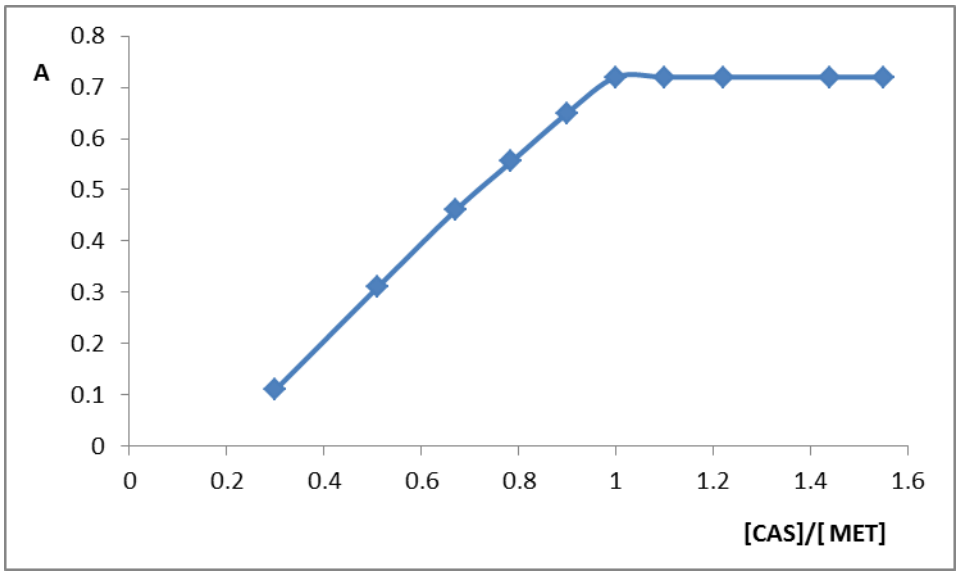

Figure (11): Determination of the correlation ratio of the MET-CAS complex in the molar ratio method.

We conclude from Figure (4) that

[CAS]/[MET]+[CAS]= 0.5 (1)

[MET]/[MET]+[CAS]= 0.5 (2)

5. Method validation

The proposed method was validated in terms of linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ). The accuracy was expressed in terms of percent recovery of the known amount of the standard drug added to the known amount of the pharmaceutical dosage forms. In order to determine the accuracy and precision of the method, solutions containing two different concentrations of the studied drug were prepared. Four replicates determinations were carried out for both pure form and the pharmaceutical preparations of Metformin The data obtained in this investigation, are summarized in (Table 1), (Table 2) and (Table 3). The low value of RSD% and high recovery percentage indicate good precision and reproducibility of the proposed method. The average percent recoveries obtained were quantitative, indicating good accuracy of the method.

Table (1): Spectral characteristics of MET-CAS complex.

Parameter	Value
λmax (max.)	570
Beer's law limit (µg/mL)	3.31–57.96
Molar absorptivity (L mole-1 cm-1)	0.354×10^{4}
Sand ell's sensitivity (µg/mL per0.001 A)	0.354
Slope (m)	0.2765
Intercept (c)	0.0014
Stoichiometric relationship	1:1
Limit of Detection (µg/mL)	2.485
Limit of quantitation (µg/ml)	8.281
Correlation coefficient R^2	0.9969
Relative Standard Deviation*	1.40

Y = mx + C

Where X is the concentration of analyte (µg/mL) and Y is absorbance unit.

* = Calculated from five determinations.

Table 2: Evaluation of precision and accuracy of the proposed methods for determination MET-CAS complex.

Relative Recovery (%) R	Detection limit (µg/ml) $\overline{X} \pm [t \times SD/(n)^{1/2}]$	analytical Error SD/(n)$^{1/2}$	RSD%	SD	Found* (µg.ml^{-1})	Drug samples (µg/ml) Amount taken (µg.ml^{-1})
101	0.0224±1.6726	0.0105	1.4002	0.02342	1.6726	1.656
100.04	0.0173±8.2833	0.0081	0.2190	0.01814	8.2833	8.28
100.8	0.2059±16.6925	0.0966	1.2295	0.215916	16.6925	16.56
99.93	0.0354±24.8234	0.0166	0.1492	0.0370	24.8234	24.84
100.22	0.1554±33.1929	0.0729	0.4109	0.1629	33.1929	33.12
99.944	0.0495±41.3768	0.0232	0.1253	0.05184	41.3768	41.40
100.21	0.2189±49.7827	0.1027	0.4612	0.2296	49.7827	49.68
99.98	0.0021±57.9501	0.0010	0.03834	0.0222	57.9501	57.96

Five independent analyses

Table 3: Determination of Metformin in Table dosage from proposed method.

Formulation	Quantity Metformin Mg	Concentration taken µg.ml-1)	Specific Focus a (µg.ml-1)	Specific quantity a Mg	SD	Relative Standard Deviation RSD %	Recovery% claim (mg)
Metformin	500	50 25	50.006 24.982	499.5 499.2	1.7275 1.286	0.15 0.2236	100.01 99.90
Metforal	500	50 25	49.98 24.95	499.3 498.7	2.5221 2.6251	0.2292 0.4568	99.96 99.84

*Five independent analyses.

6. CONCLUSIONS

The proposed method for determination of metformin is simple, selective, sensitive, cost-effective, and free from auxiliary reagents. Moreover, the proposed method is free from tedious experimental steps such as heating and extraction. The statistical parameters and the recovery data reveal good accuracy and precision of the method. The proposed method can be used as alternative method to reported ones for the routine determination of metformin in both pure form and in pharmaceutical formulations.

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