Spectrophotometric Determination of an Antidiabetic Drug Metformin by $KMnO_4$ and Sulphanilic Acid Method

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Abstract

For the determination of Metformin, two simple and sensitive spectrophotometric methods are developed. The first method A is indirect; after allowing the reaction between $KMnO_4$ and drug, the excess amount of $KMnO_4$ is determined spectrophotometrically. The excess of $KMnO_4$ was made to react with a fixed amount of methyl orange dye and the absorbance is measured at 510nm. For this method, Beer's law is obeyed in the concentration range of 0.5- 5 µg/ml. Molar absorptivity and Sandell's sensitivity are found to be 0.12916 x 10^5 L mol^{-1}cm^{-1} and 0.01 µg/cm^2 respectively.

The second method B is based on the principle of formation of orange color as a result of reaction between drug and diazotised sulfanilic acid. The resulting solution is estimated spectrophotometrically by taking absorbance at 510 nm. Conditions of the methods are optimized by studying all the parameters that affect the stability and colour of the complex. For this method, Beer's law is obeyed in the concentration range 1.0 - 8.0 µg /ml. Molar absorptivity and Sandell's sensitivity are found to be 0.2583 x 10^4 L mol^{-1}cm^{-1} and 0.05 µg/cm^2 respectively. The proposed methods are appropriate for determination of metformin in pharmaceutical formulations.

Keywords: Metformin, Diazotised Sulphanilic Acid, Spectrophotometry, Methyl Orange, Oxidation.

Introduction

Metformin is an oral antidiabetic drug from the biguanide class[1] which is widely used in the management of the type - II diabetes[2] which is a common disease that combines defects of both insulin secretion and insulin action. IUPAC name of Metformin is 1,1-dimethylbiguanide and its molecular formula is $C_4H_{11}N_5$.

Metformin is considered an antihyperglycemic drug[3] because it lowers blood glucose concentrations in type II diabetes without causing hypoglycemia. Metformin improves glycemic control by improving insulin sensitivity and decreasing intestinal absorption of glucose and increasing insulin sensitivity by increasing peripheral glucose uptake and utilization.

It is used to treat heart disorders and polycystic ovary syndrome[4]. Metformin was first approved in Canada in 1972[5] followed in 1995 in the USA. This drug is available in regular and extended-release forms. Currently, metformin is the first drug of choice for the management of type II diabetes and is prescribed to at least 120 million people worldwide[6]. It is freely soluble in water and 50% alcohol but is insoluble in chloroform, acetone and ether. Metformin has a pKa value of 12.4[7]. The structure of metformin is shown in figure 1.

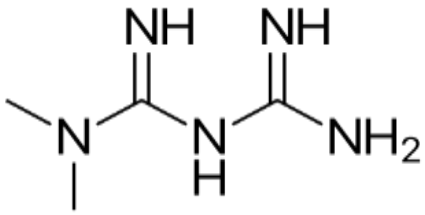

Figure 1: Structure of Metformin

Literature survey reveals that only few methods like HPLC, GC[8-10] have been reported for estimation of the metformin in pharmaceutical formulations. There is a need for simple spectrophotometric method for the analysis of metformin in pharmaceutical formulations. The present investigation describes two spectrophotometric methods for the determination of metformin by using $KMnO_4$ as an oxidizing agent and sulphanilic Acid as a coloring reagent respectively. The proposed methods are comparable with reported method with respect to sensitivity, moreover the methods neither require extraction nor prior separation of the drug.

Principle of developed method

Method A: The proposed spectrophotometric method is indirect; after allowing the reaction between $KMnO_4$ and drug, the excess amount of $KMnO_4$ is determined spectrophotometrically.[11,12] The excess of $KMnO_4$ was made to react with a fixed amount of methyl orange dye. $KMnO_4$ bleaches the dye by causing oxidative destruction of the dye. Drug when added in increasing concentrations to a fixed concentration of $KMnO_4$ consumes the $KMnO_4$ proportionally and there occurs a fall in the concentration of $KMnO_4$. When a fixed concentration of dye is added to decreasing concentrations of $KMnO_4$, there is increase in the concentration of dye. Thus, a proportional increase in the absorbance at the respective λ max is observed with increasing concentration of drug.

Method B: Coloring reagent was prepared by diazotization reaction with sulphanilic acid. Sulfanilic acid is dissolved in dilute aqueous acid. Sodium nitrite is added to produce a diazonium salt[12,13] (Figure 2). This reaction is called diazotization and it is extremely useful in organic synthesis.

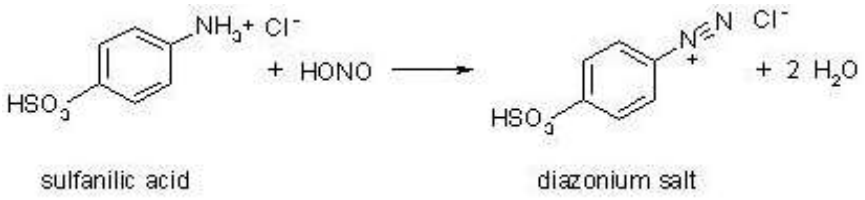

Figure 2: Conversion of sulfanilic acid to diazonium salt

The nitrous acid provides NO^+ which replaces a hydrogen on the $-NH_3^+$ group to produce $-NH_2NO^+$ and water; water is eliminated to produce the $-N_2^+$ group. This reagent is then made to react with metformin to form metformin orange colored complex. The concentration of complex formation increases with the increase in concentration of drug. Thus in this method reaction proceeds with increasing absorbance.

Material and Methods

Instrumentation: All measurements of absorption spectra were made on model LABMAN UV-VIS Spectrophotometer using quartz cells of 1cm path length and wavelength range 320-1000nm for absorbance measurement. All chemicals employed in the present study were of analytical grade and purchased from Loba Chemie. Double distilled water was used for preparation of standard solution as well as for all experimental work.

Preparation of standard drug solution: About 58 mg of metformin is exactly weighed and is dissolved in 50 ml of ethyl alcohol. The final dilution is made with ethyl alcohol. This is 100 ppm drug solution.

Experimental procedure

Method A: Different portions (0.5-5.0 mL, 100µg/mL) of standard Metformin solution are delivered into a series of 10 mL calibrated standard flask. Then to each flask, 1ml of 2M H_2SO_4 was added followed by 1ml of $KMnO_4$ solution (4 $x10^{-4}$M). The contents are mixed and the flasks are kept aside for 15 min under occasional shaking. Finally, 1ml of methyl orange solution (2.4 $x10^{-3}$M) is added to each flask, diluted to the mark with water and the absorbance of solution is measured at 510 nm against a reagent blank.

Method B: Different portions (1.0- 8.0 mL, 100µg/mL) of standard metformin solution are delivered into a series of 25 mL calibrated standard flask. To this 5 ml of coloring reagent is added. The final volume is made to 25 ml with NaOH (1M). The absorbance is measured at 510 nm after 10 minutes after dilution.

Results and Discussion

For Method A: The absorbance of the colored complex solution is measured against a reagent blank prepared under identical conditions from 360 to 600 nm. Absorption spectra of metformin show intense peak at 510 nm (Fig. 3). The calibration curve is found to be linear indicating that Beer's law is obeyed in the concentration range of (0.5-5.0 µg/mL,100µg/ml) for the developed method (Fig. 4).

Effect of Heating Time on Absorbance of Developed System: The color development for developed method was studied by varing time (Fig. 5). From the variation graph (absorbance against varing time), it has been concluded that 15 minutes are sufficient for full color development, hence 15 minutes time is selected for color development for further studies.

Optimization of Parameters

For Method A: The experiment was conducted many times in order to determine maximum concentration of methyl orange and $KMnO_4$ spectrophotometrically and it is found that $KMnO_4$ concentration of 4 $x10^{-4}$M was found to be sufficient to bleach the color of methyl orange upto concentration of 2.4 $x10^{-3}$M methyl orange. Sulphuric acid was found to be a convenient medium for these methods. For a quantitative reaction between drug and $KMnO_4$, a contact time of 15min was found sufficient.

For Method B: The absorbance of the colored complex solution is measured against a reagent blank prepared under identical conditions from 360 to 600 nm. Absorption spectra of metformin show intense peak at 510 nm (Fig. 6). The calibration curve is found to be linear indicating that Beer's law is obeyed in the concentration range of (1.0 – 8.0 µg/mL, 100µg/ml) for the developed method (Fig. 7).

Effect of Heating Time on Absorbance of Developed System: The color development for developed method was studied over a varying time (Fig. 8). From the variation graph (absorbance against varying time), it was found that after 15 minutes, absorbance decrease and it remains constant for first ten minutes, hence the absorbance was measured between first 10 minutes.

Optimization of Parameters for Method B: The optimum concentration of sulphanilic acid and sodium nitrite was found to be 500 mg and 200 mg respectively. Optimum volume of coloring reagent was 5 ml. The absorbance was measured between first 10 minutes. It was found that after 15 minutes, absorbance decreases and it remains constant for first ten minutes, hence the absorbance is measured between first 10 minutes.

Regression parameters, optical characteristics precision and accuracy of methods A and B are shown in table 1. Determination of pharmaceutical formulations of metformin by our proposed method and reference method is shown in table 2.

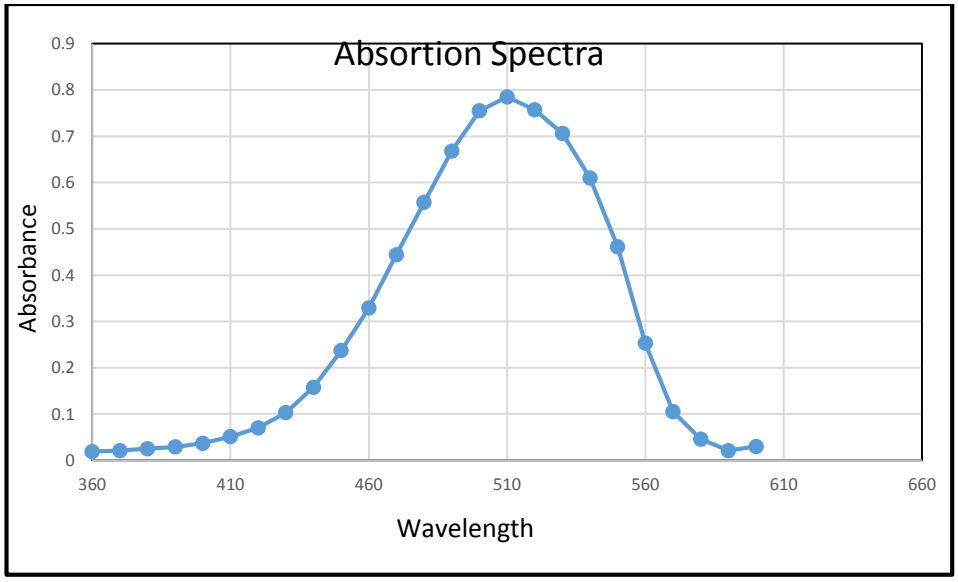

Figure 3: Absorption spectra showing λ max 510nm for Method A

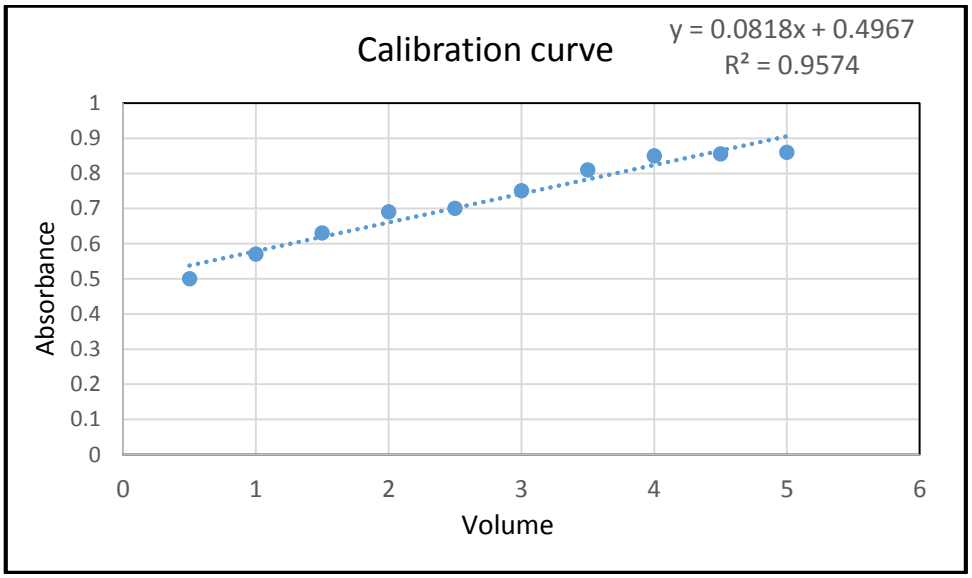

Figure 4: Calibration curve for method A

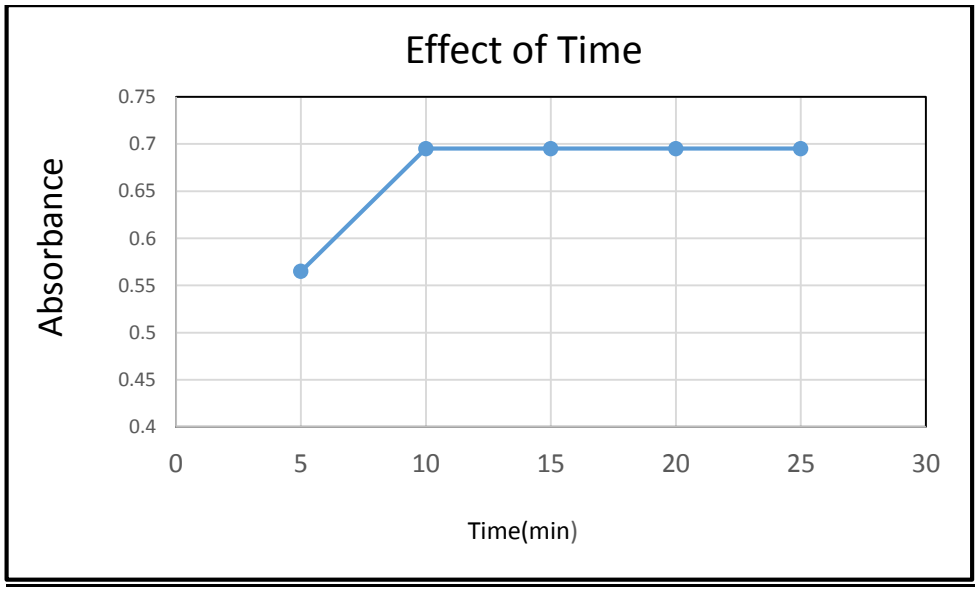

Figure 5: Effect of heating time on absorbance of developed system

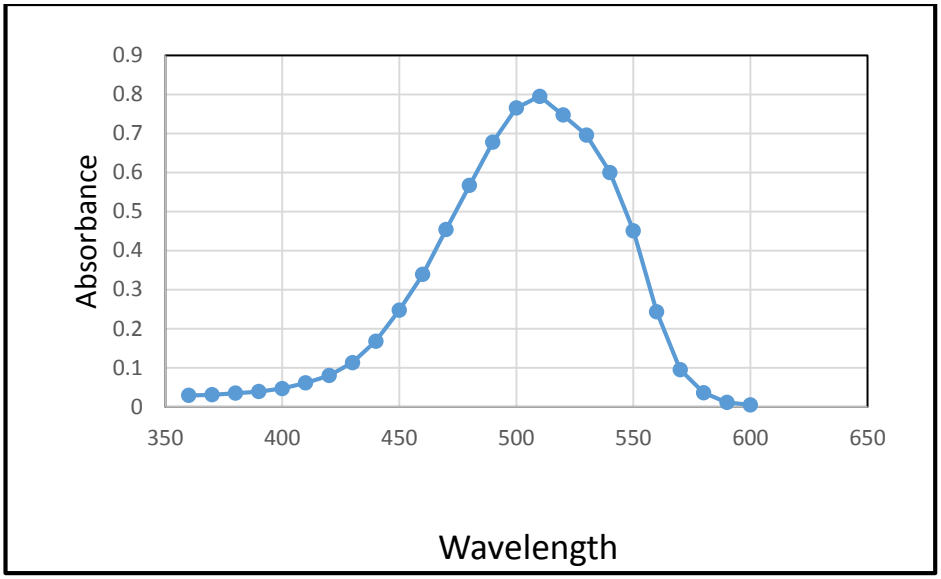

Figure 6: Absorption spectra showing λ max 510nm for Method B

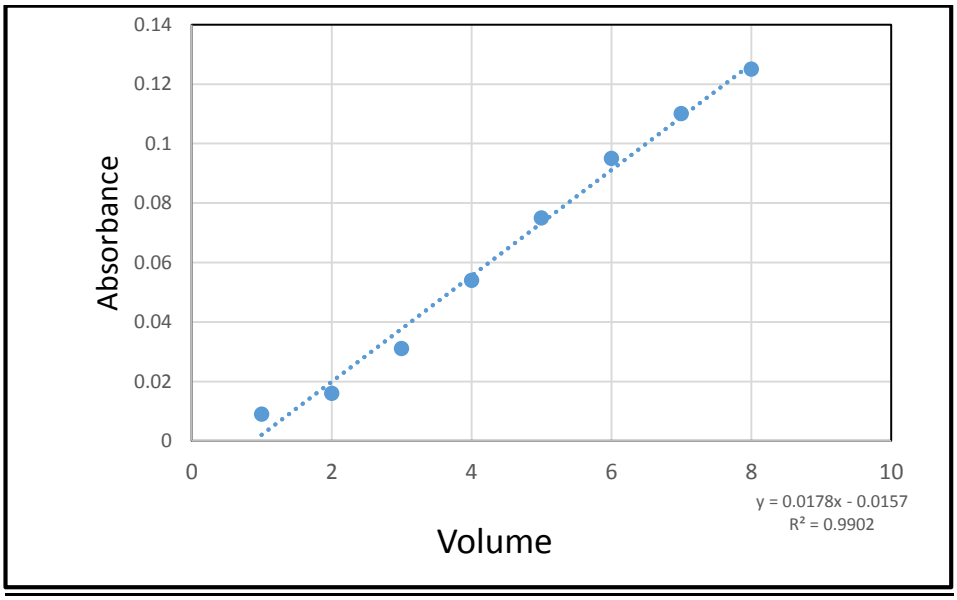

Figure 7: Calibration curve for Method B

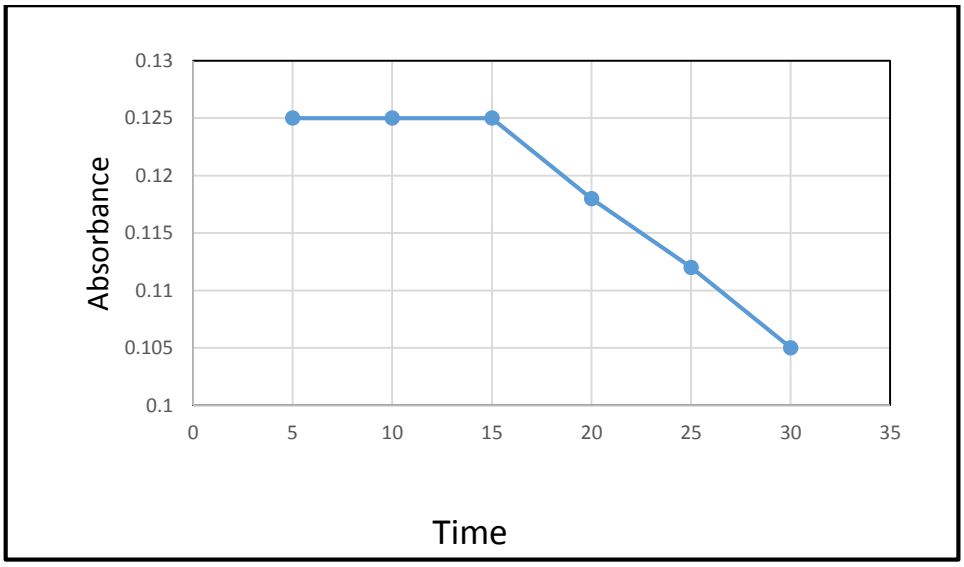

Figure 8: Effect of heating time on absorbance of developed system

Table 1
Regression parameters, optical characteristics precision and accuracy of method A and B.

Parameter	Method A	Method B
Maximum Wavelength λ_{max}	520 nm	520 nm
Beer's Law Limits µg/mL	0.5-5.0	1.0-8.0
Sandell's Sensitivity (µg/cm^2 /0.0001 Absorbance)	0.01	0.05
Molar Absorptivity Lt/mole/cm	0.12916 x 10^5	0.2583 x 10^4
Slope(b)[a]	0.0818	0.0178
Intercept(a)[a]	0.4967	0.0157
Standard Deviation on intercept(S_a)	0.0598	0.0103
Correlation Coefficient (r)	0.978	0.995
Standard Deviation (S)	0.369	0.586
Variation from mean at 95% level confidence limit	±0.264	±0.195
Limit of Detection (LOD)µg/mL	2.4123	2.160
Limit of Quantification (LOQ)µg/mL	7.31	6.560

[a]Regression equation Y=a+bC, Where Y stands for absorbance and C is concentration in µg/mL b is % Relative standard deviation which is calculated for ten determination

Table 2
Determination of pharmaceutical formulations of metformin

Drug	Manufacturing company	Labelled amount(mg)	*Amount found by Proposed Method A	*Amount found by Proposed Method B	*Amount found by Reference Method
Metformin tablet	Abbott Healthcare Pvt. Ltd.	500	499.69	499.75	499.87

Conclusion

Two simple, rapid, fairly accurate, precise and sensitive spectrophotometric methods were developed for the determination of metformin in bulk drug and in tablets. The low detection and quantification limits, simplicity and selectivity make the method suitable for quality control in pharmaceutical industry for routine analysis.

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