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Original Article

Utilization of 4-Chloro-7-Nittobenzo-2-Oxa-1, 3-Diazol (NBD-CL) as Chromogenic Reagent for Determination of Metformin hydrochloride (MET) in Pharmaceutical Formulation

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ABSTRACT

A new, simple, selective, precise and accurate spectrophotometric method for the determination of metformin in pharmaceutical formulation was developed and validated. The method was based on the reaction between metformin and 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) at alkaline medium (pH 12.0) to form brown adduct. Beer's law was obeyed in the concentration range of 20-100 µg mL^{-1} of metformin at maximum wave length 465 nm . Under the optimized reaction conditions, the linear regression equation of the calibration curve was found to be y=0.003x +0.156 with a linear correlation coefficient (r=0.999). The limit of detection (LOD) and limit of quantification (LOQ) were found to be 5.8, 17.5 µg mL^{-1} respectively. The method is useful for routine analysis of metformin in quality control laboratories.

Keywords: Spectrophotometric, Metformin, 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) pharmaceutical formulation

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1. INTRODUCTION

Metformin hydrochloride (MET) (Fig.1), chemically is 1,1-Dimethylbiguanide hydrochloride with a molecular formula of $C_4H_{12}N_5$ Cl [1]. It is an oral [2] antidiabetic drug that has been used in the treatment of type 2 diabetes [3] (non-insulin dependent diabetes) which improves 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 . Recently metformin has also become available for the treatment of polycystic ovary syndrome [1] and has been found to improve vascular function, prevent pancreatic cancer and reverse fatty liver diseases [4]

Several types analytical procedures have been reported for the analysis of metformin hydrochloride in a bulk form, pharmaceutical form or biological fluids. These include high performance liquid chromatography [5,6], gas liquid chromatography [7], capillary electrophoresis [8], near infrared spectroscopy [9], uv spectrophotometry [10,11], conductometry [12], voltammetry [13], or by visual titration [14], NMR spectrometry [15], Chemiluminescence [16], and atomic spectrometry [17]. However, some of these methods are complicated and time consuming, involve the use of large volume organic solvents and specific reagent and requirement of expensive instruments.

NBD-CL has been proved to be useful and sensitive analytical derivatizing agent for spectrophotometric analysis of pharmaceutical bearing a primary or secondary amine group [18-35] . In depth review on the applications of NBD-CL for determination of pharmaceutical bearing amine group have been reviewed by Elbashir et al [36,37] .

The use of NBD-CL for spectrophotometric determination of metformin hydrochloride has not been reported yet, therefore in this work a sensitive and

simple spectrophotometric method for determination of metformin in pharmaceutical formulation has been developed.

2. EXPERIMENTAL

2.1 Apparatus

Absorbance was carried out by using UV-visible spectrophotometer model Shimadzu 1800 with quartz cells of 1 cm optical path length. pH meter model HI 255 (Hanna Instruments, Mumbai, India) was used for PH measurement, analytical balance, ultrasonic bath and shaker.

2.2 Reagents and solutions

Metformin hydrochloride was Kindly provided from general medicine company (Khartoum, Sudan) and used as received; its purity was 100%, A solution of 2-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-CL) was freshly prepared in methanol at 0.3% (w/v) concentration.

buffer solution of PH 12.0 was prepared by adding 23.4ML of O.2 M NaoH to 25 mL of 0.2M NaH_2PO_4 and dilute to 100 ML with distilled water and adjusted to pH 12.0 with 1M sodium hydroxide. Tablet containing a label claim 850, 500 mg per tabs were purchased from a local pharmacy. All other chemicals were of analytical grade.

2.3 Preparation of standard and sample solution

Stock standard solution of metformin (1000 µg/mL)

An accurately 0.1 mg of metformin hydrochloride standard was dissolved in distilled water into 100 ml volumetric flask, diluted to the mark with same solvent and mixed well. This stock solution was further diluted to obtain working solutions in the range of 20- 100 µg/mL.

2.4 Sample solution

Twenty tablets of metformin hydrochloride were accurately weighted and powdered. A quantity of the powder containing 100mg of metformin was transferred into 100 mL volumetric flask, about 70 mL of water was added, and the mixture was shaken for 10 min and the volume was made up with water to give a concentration of 1000 µg/ML , and the solution was filtered.

3. RESULTS AND DISCUSSION

3.1 Absorption spectra

The absorption spectra of metformin was recorded against water (Figure 1), it was found that metformin exhibits a maximum absorption peak (λ_{max}) at 233 nm . Because of highly blue shifted λ_{max} of metformin its determination in the dosage form based on the direct measurement of its absorption for ultraviolet is susceptible to potential interferences from the common excipients. Therefore, derivatization of metformin red – shifted light absorbing derivative was necessary. The reaction between metformin and NBD-CL was performed, and the absorption spectrum of the product was recorded against reagent blank (Figure 1). It was found that the product is brown colored exhibiting λ_{max} at 465 nm, and the λ_{max} of NBD-CL was 344nm. The λ_{max} of metformin –NBD-CL derivative was red-shifted, eliminating any potential interference. Therefore, the measurement was carried out at 465 nm.

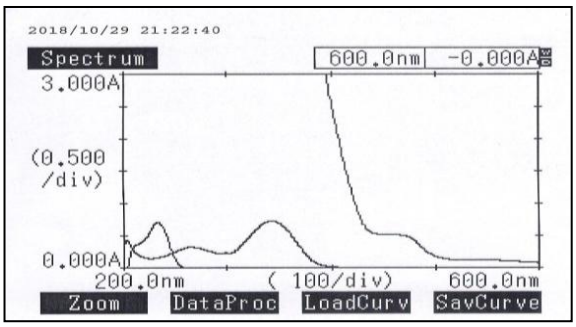

Figure 1. (1) Absorption spectra of metformin (10 µg/ml**)** against water blank, (2) NBD-CL (0.002%) against methanol blank, (3)the reaction product of metformin with NBD-CL against reagent blank.

3.2 Optimization of the reaction conditions

The optimum conditions for the development of method were established by varying the parameters one at a time while keeping the others fixed and observing the effect produced on the absorbance of the colored product. In order to establish experimental conditions, the effect of various parameters such as PH, time, buffer volume and concentration of NBD-CL were studied.

3.2.1 Effect of pH

The influence of pH on the absorbance of the result of reaction between Metformin and NBD-CL was investigated in the range of 7.0-14.0, the absorbance of the solution increases up to pH 12.0 and then decrease as shown Figure 2. At pH 12.0 the absorbance reaches its maximum; this was possibly due to the existence of amino group of metformin in the form of hydrochloride salt, which facilitate nucleophilic substitution capability. As the pH increased the reading increased dramatically, at pH values more than 12.0 , a decrease in the absorption occurred .This was attributed probably to the increase in the amount of hydroxide ion that increases the rate of the backward reaction of metformin with NBD-CL.

3.3 Procedure

A 1.0 mL of 1000 µg/mL of metformin was transferred into 10 ML volumetric flask,1.5 mL of phosphate buffer pH 12 was added and followed with 1.0 Ml of 0.3% of 2-chloro-7-nitrobenzo-2-oxa-1,3-diazole. The solution was allowed to stand for 25 min before the completion of volume to 10 Ml with distilled water.

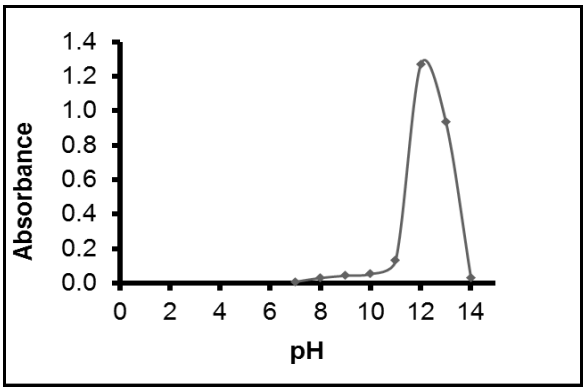

Figure 2. Effect of pH on the reaction of metformin with NBD-CL, 2.0 mL of metformin (1000 µg/mL), 1.0 mL NBD-CL (0.3% w/v), reaction time: 25 min

3.2.2 Effect of standing time

The absorbance of the reaction product was determined at different time (Figure 3). Keeping other conditions unchanged, the absorbance of the reaction product was measured after standing for different time periods at 25 ° C and the absorbance begin to increase instantly and becomes constant after 25 min . Furthermore, it is also observed that the absorbance remains constant for 30 min.

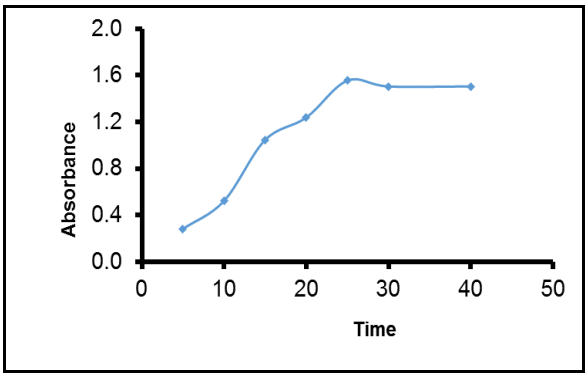

Figure 3. Effect of standing time on the reaction of metformin with NBD-CL, 2.0 mL of metformin (1000 µg/mL), 1.0 mL buffer solution (pH 12.0), .0 mL NBD-CL (0.3%).

3.2.3 Effect of NBD-CL concentration

The effect of NBD-CL concentration was studied over the range 0.1-0.6% (w/v) as shown in Figure 4. The highest absorption intensity was attained at NBD-CL concentration of 0.3% (w/v) , and higher concentration of NBD-CL leads to a decrease in the absorbance.

3.2.4 Effect of Amount of the Buffer

Keeping pH at 12.0, the effect of amount of buffer solution on the absorbance of product 3 was also studied. it shows that the absorbance of product 3 enhances rapidly with the rise of amount of buffer solution, and become maximal when the amount of buffer solution is 1.5 mL. Therefore the amount of 1.5 mL buffer solution was selected to ensure the highest absorbance of product 3 , as shown in Figure (5).

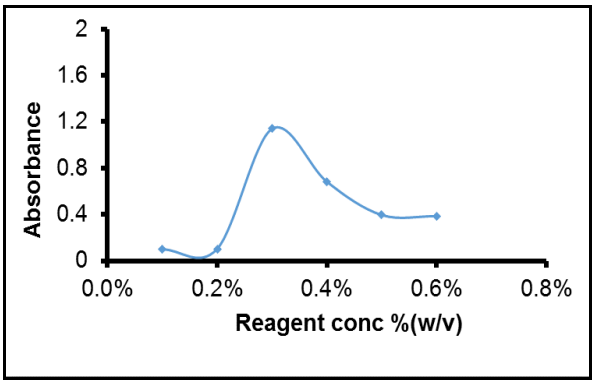

Figure 4. Effect of NBD-CL concentration on the reaction of metformin with NBD-CL, 2.0 mL of metformin (1000 µg/mL), 1.0 mL buffer solution (pH 12.0), reaction time : 25 min .

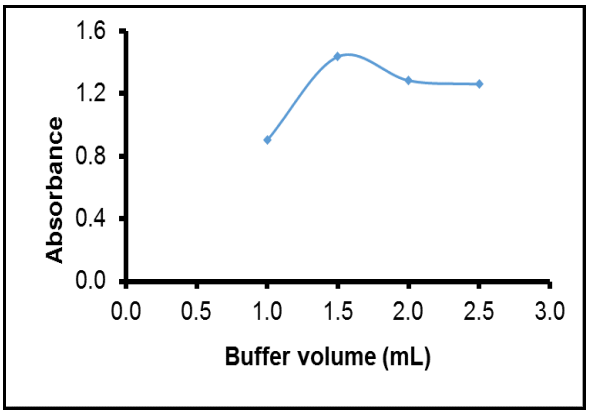

Figure 5. Effect of amount of the buffer on reaction of metformin with NBD-CL, 2.0 mL metformin (1000 µg/mL), buffer solution (pH 12.0), 1.0 mL NBD-CL (0.3%), reaction time : 25 min

The effect of temperature on the reaction was studied by carrying out the reaction at different temperature ( ) it was found that the reaction of metformin with NBD-CL was not affected by increasing the temperature ,and the reaction at room temperature (25^0C) went to completion in 25 minutes , and longer reaction time up to 40 minutes did not affect the reaction ,further experiments involving NBD-CL reagent were carried out at room temperature (25^0C) for 25 minutes.

From the above experiments, the optimized conditions used to be for the assay was: pH 12.0, volume of the buffer 1.5 mL, NBD-CL concentration 0.3 %(w/v), reaction time 25 min and temperature 25◦C.

Furthermore, the molar ratio of NBD-CL to metformin in the reaction mixture was studied according to Job's method of continuous variation [38]. Equimolar aqueous solution of metformin and NBD-CL ($1x10^{-3}$ M) were prepared in 10 mL volumetric flask containing complementary proportions of the two solutions (1:9…,9;1), inclusive) and 1.5 ml of buffer solution (pH 12.0). The job plot of absorption versus molar ratio was symmetrical and indicated that 1:2 ratio (drug/reagent) confirming that one molecule of metformin reacts with

two molecule of NBD-CL, Figure 6) . Based on the observation molar ratio the reaction pathway was postulated to proceed as shown in scheme I.

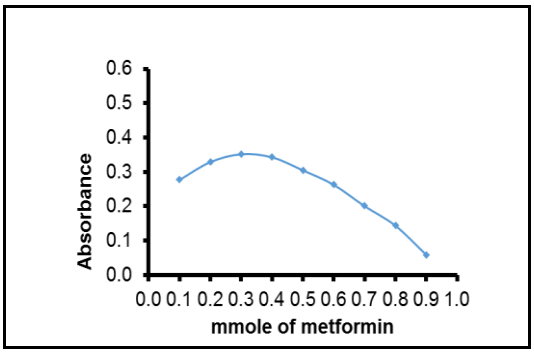

Figure 6. The continuous variation plot for the stoichiometry of the reaction of metformin with NBD-CL

3.3 Analytical method validation

The validity of the method was tested regarding to linearity, limit of detection(LOD), limit of quantification (LOQ), accuracy, precision and robustness according to the International Conference on Harmonization(ICH) guidelines [39] .

3.3.1 Linearity and Sensitivity

Under the describe experimental conditions, linear relationship was found between the absorbance at λ_{max}465nm and the concentration of the drug. The regression equation was found to be as y=0.003x +0.156 (r =0.999, n=8), where Y is the absorbance at 465 nm, C is the concentration of metformin in µg/mL in the range 20-100 µg/mL. The limit of detection (LOD) and limit of quantification (LOQ) were determined using the formula LOD= 3.3x 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 found to be 5.8 and 17.5 µg/ mL respectively, Table 1.

3.3.2 Accuracy and Precision

The accuracy of the proposed method was carried out by applying standard addition technique. A different amount of standard solution was added to known concentration of the drug sample. The average percent recoveries obtained in range 98.9-101.6 (Table 2), with corresponding intraday precision (RSD) 1.1% and intraday precision 1.2%.

3.3.3 Robustness

Robustness was examined by evaluating the influence of small variation in the method variable on its analytical performance. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that small variation in the method variables did not significantly affect the procedures; recovery values were recorded in (Table 3).

3.4. Application of the proposed method

Metformin hydrochloride tablets were subjected to the analysis by the proposed method as well as with the official spectrophotometric method (British Pharmacopeia) and the obtained results were statistically compared with each other. The label claim percentage was101.4 and 100.7 for metformin 500 and 850 mg respectively Table 4. With respect to t- and F-test, no significant difference was found between the calculated and theoretical values of both the proposed and the reported methods at 95% confidence level. This indicated similar accuracy and precision in the analysis of metformin in tablets. The proposed method has the advantage of being virtually free from interferences by excipients.

4. CONCLUSION

The present paper described the evaluation of NBD-CL as analytical reagent in the development of simple, sensitive and accurate spectrophotometric methods, for determination of metformin in bulk and pharmaceutical formulation. The proposed method is simple, reliable, specific, accurate, reproducible, and highly sensitive, for the determination of metformin in commercially available dosage forms. The procedure presented here dos not need necessitate any expensive apparatus; therefore, the proposed method can be used advantageously as a routine method for the determination of metformin in quality control and industry.

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