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Estimation and validation of metformin by colorimetry method

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ABSTRACT

A simple highly sensitive spectrophotometric method was developed for the quantification of metformin hydrochloride. The method involves the reaction of the target compound with ninhydrin reagent at specific pH 5.6 to produce a wine red color chromogen. The derivative chromogen exhibits absorption maxima at 567nm. At the specific pH of the reaction where no degradation may occur with that medium the proposed method can be utilized as a stability indicating assay. The different experimental parameters affecting the derivatization reaction were carefully studied and incorporated into the procedure. Under the described conditions the proposed method is linear over the concentration range of 10-70mcg/ml and the coefficient of determination were >0.999(n=6) with a relative standard deviation of 0.083% (n=6). The average recovery of the target compound is 99.47% with a limit of quantification (LOQ) of 2.637mcg/ml and the limit of detection (LOD) 0.870mcg/ml. The mechanism of the derivatization reaction is proposed and advantages of the proposed method are discussed.

KEYWORDS

Metformin hydrochloride;
Ninhydrin.

INTRODUCTION

The quality control of active pharmaceutical ingredients (APIs) in the formulation is always a thrust area for the pharmaceutical industries. So the development of reproducible, sensitive, simple and extremely inexpensive methods for the determination of APIs in the formulation is always challenging. Metformin (*N*, *N*-dimethylimidodicarbonimidic diamide) is an oral anti-diabetic drug. It is the first-line drug of choice for the treatment of type 2 diabetes, particularly in overweight and obese people and those with normal kidney function[1-3].

Evidence is also mounting for its efficacy in gestational diabetes, although safety concerns still preclude its widespread use in this setting. It is also used in the treatment of polycystic ovary syndrome and has been investigated for other diseases where insulin resistance may be an important factor. When prescribed appropriately, metformin causes few adverse effects—the most common is gastrointestinal upset—and, unlike many other anti-diabetic drugs, does not cause hypoglycemia if used alone. Lactic acidosis (a buildup of lactate in the blood) can be a serious concern in overdose and when it is prescribed to people with contraindications, but otherwise, there is no significant risk. Metformin helps reduce LDL cholesterol and triglyceride levels and is not associated with weight gain,

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and is the only anti-diabetic drug that has been conclusively shown to prevent the cardiovascular complications of diabetes. As of 2009, metformin is one of only two oral anti-diabetics in the World Health Organization Model List of Essential Medicines (the other being glibenclamide)[4]. First synthesized and found to reduce blood sugar in the 1920s, metformin was forgotten for the next two decades as research shifted to insulin and other anti-diabetic drugs. Interest in metformin was rekindled in the late 1940s after several reports that it could reduce blood sugar levels in people, and in 1957, French physician Jean Sterne published the first clinical trial of metformin as a treatment for diabetes. It was introduced to the United Kingdom in 1958, Canada in 1972, and the United States in 1995. Metformin is now believed to be the most widely prescribed anti-diabetic drug in the world; in the United States alone, more than 40 million prescriptions were filled in 2008 for its generic formulations[5,6]. Number of analytical methods have been reported for measuring metformin in biological fluids and tissue extracts. These methods involved colorimetry techniques[7-9].

Aim of this work was to develop a sensitive and simple spectrophotometric method for the quantification of metformin is finding new dimensions of clinical importance. To support its investigation an appropriate analytical method (sensitive, selective, reproducible and simple) for quantification of metformin is essential. For the drugs that obey the beer Lambert's law, spectrophotometric methods of analysis of single component in solution are usually rapid, sensitive and economical.

EXPERIMENTAL

Apparatus

A model Shimadzu UV-1601 double beam spectrophotometer with a fixed slit width of 2nm using a pair of 1cm matched quartz cells was used for spectrophotometric analysis.

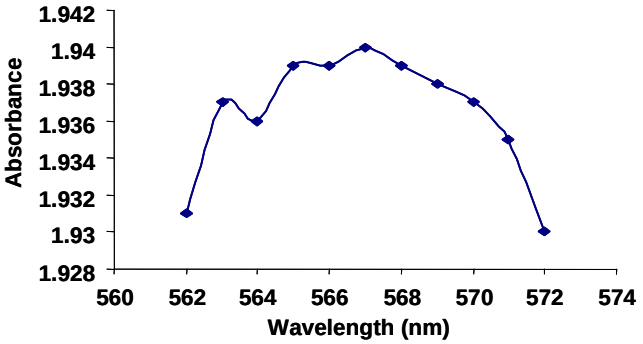
Materials

All the chemicals were of analytical reagent grade, and the solvents were of spectroscopic grades. Metformin hydrochloride (Wintac Limited, Bangalore, Karnataka State, India), Phthalate buffer solution (pH-5.6), 0.2M sodium hydroxide (NaOH) solution, 01% w/v Ninhydrin solution, distills water.

Spectrophometric method

Determination of absorption maxima of metformin hydrochloride

1.5 ml aliquot of standard Metformin hydrochloride solution of 500.0 mcg/ml was pipetted into a 25 ml volumetric flask. To this 10.0 ml of Phthalate buffer (pH=5.6) and 5.0 ml of 01% w/v reagent solution of Ninhydrin were added and heated on a water bath for 1 hour at 90º C. After one hour, solution was cooled to room temperature. The final volume was made up to 25.0 ml with distill water and solution was then scanned in the range of 400.0 to 800.0 nm against the reagent blank. On scanning the absorption maxima of Metformin hydrochloride 30.0 mcg /ml were found out to be 567.0 nm and graphically represented in Figure 1.



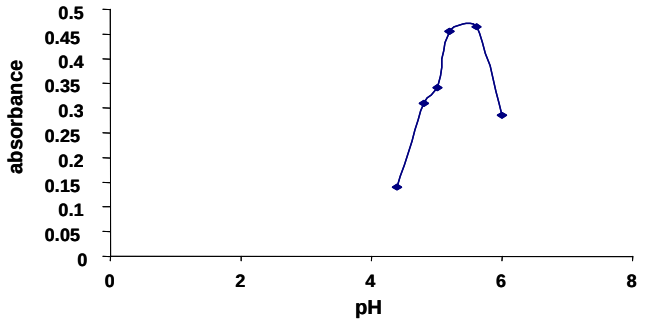
Metformin : 30.0 mcg/ml, Acid phthalate buffer solution (pH 5.6): 10.0 ml;
Ninhydrin : (01%w/v) 5 ml; reaction time: 60 min.

Figure 1

Effect of optimum pH of buffer solution required producing stable chromogen having maximum absorbance

2.0 ml aliquots of Metformin hydrochloride solution of 500 mcg/ml were pipetted into each of eight 25 ml volumetric flasks. To this 10 ml of phthalate buffer solution of various pH 4.4, 4.8, 5.0, 5.2, 5.6, and 6.0 was added to each volumetric flask respectively. Then followed by 5.0 ml of 01% w/v solution of Ninhydrin to all volumetric flasks and heated on a water bath at 90 °C for 1 hour and cooled to room temperature .The final volume of each volumetric flask was made up to 25.0 ml with distilled water. The absorbance was measured at 567.0 nm against reagent blank. The Opti-

mum pH of Potassium hydrogen phthalate buffer required to produce stable chromogen having maximum absorbance was found to be 5.6 and represented graphically in Figure 2.

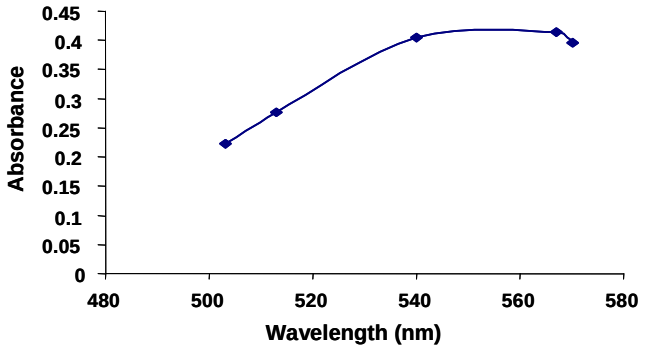


Metformin : 40.0 mcg/ml, Acid phthalate buffer solution (pH 5.6): 10.0 ml;
Ninhydrin : (01%w/v) 5 ml; reaction time: 60 min.

Figure 2

Determination of optimum volume of buffer (pH 5.6) required to produce chromogen having maximum absorbance

An aliquot of 2.0 ml Metformin hydrochloride solution of 500 mcg/ml was pipetted into each of two series of five 25 ml volumetric flasks. To this various volumes of phthalate buffer solutions of pH-5.6 viz., 4.0 ml, 6.0 ml, 8.0 ml, 10.0 ml, and 12.0 ml were added to each series of volumetric flasks respectively. Then followed by 5.0 ml of 01% w/v solution of Ninhydrin to all volumetric flasks and heated on a water bath for 1 hour at 90.0 °C and cooled to room temperature .The final volume was made up to 25.0 ml with distilled water. The absorbance was measured at 567.0 nm against reagent blank. The optimum volume of buffer of pH 5.6 required to produce stable chromogen having maximum absorbance was found to be 10.0 ml and represented graphically in Figure 3.

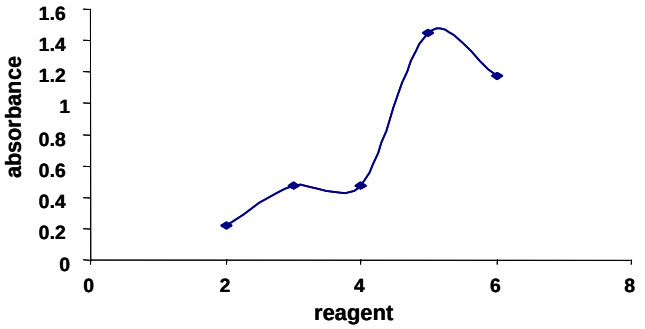


Metformin : 40.0 mcg/ml, Acid phthalate buffer solution (pH 5.6): 10.0 ml;
Ninhydrin : (01%w/v) 3.5 ml; reaction time: 60 min.

Figure 3

Determination of optimum strength of reagent (Ninhydrin) required producing optimum chromogens having maximum absorbance

An aliquot of 2.0 ml Metformin hydrochloride solution of 500 mcg/ml was pipetted into five 25 ml volumetric flasks. 10.0 ml of phthalate buffer solution (pH-5.6) was added to each volumetric flask respectively, and followed by 2.0 ml, 3.0ml, 4.0 ml, 5.0 ml, and 6.0 ml of 1.0% w/v reagent solution of Ninhydrin to two series of volumetric flasks respectively. The volumetric flasks were heated on a water bath for 1 hour at 90 °C and cooled to room temperature .The final volume was made up to 25.0 ml with distilled water. The absorbance was measured at λmax (nm) against reagent blank. The optimum strength of reagent 5 ml required to produce stable chromogen having maximum absorbance was found to be 10.0 ml and represented graphically in Figure 4.



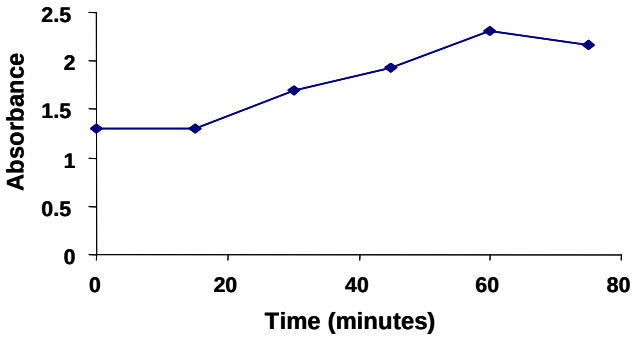
Metformin : 40.0 mcg/ml, Acid phthalate buffer solution (pH 5.6):10.0 ml;
Ninhydrin : (01%w/v) 5.0 ml; reaction time: 60 min.

Figure 4

Determination of optimum temperature required producing chromogen with maximum absorbance

An aliquot of 2.0 ml ml of 500 mcg / ml of Metformin hydrochloride solution was pipetted into six 25 ml volumetric flasks. 10.0 ml phthalate buffer (pH=5.6) and 5.0 ml of 01% w/v solution of reagent Ninhydrin were added to volumetric flasks respectively and heated on a water bath at different times i.e., 15.0 minutes, 30.0 minutes, 45.0 60.0 minutes and 75 minutes at 90 °C. The volumetric flasks were cooled to room temperature and the volume was made up to 25.0 ml with distilled water. The absorbance of each solution was mea-

sured at 567.0 against blank.The optimum strength of temperature 60 minutes required to produce stable chromogen having maximum absorbance was found to be 10.0 ml and represented graphically in Figure 5.

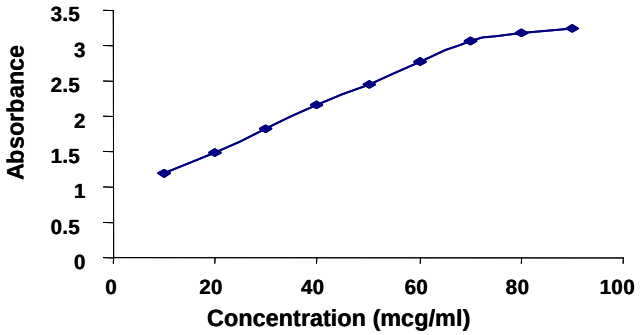


Metformin : 40.0 mcg/ml, Acid phthalate buffer solution (pH 5.6): 10.0 ml;
Ninhydrin : (01%w/v) 5.0 ml; reaction time: 60 min.

Figure 5

Determination of concentration range of metformin hydrochloride

Aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, 3.0 ml, 3.5 ml, 4 ml and 4.5 ml of 500 mcg/ml of Metformin hydrochloride solution was pipetted into each of nine 25 ml volumetric flasks. To this 10.0 ml of phthalate buffer solution (pH=5.6) and 3.5 ml of 01% w/v solution of reagent 1, 2- naphthoquinone-4-sulfonic acid sodium salt were added to each volumetric flasks respectively and heated on a water bath for 1 hour at 90 °C. The solutions were cooled to room temperature and the volume was made up to 25.0 ml with distilled water. The absorbance of solutions was measured at 567.0 nm against blank. The abs. Vs conc. curve was found to be linear in the concentration range 10.0-70.0 mcg/ml of Metformin hydrochloride and represented graphically in Figure 6.

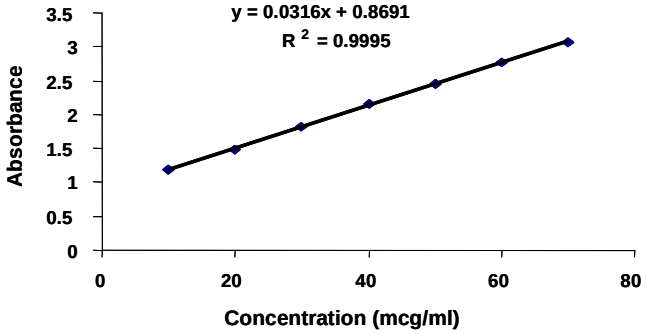


Metformin : 10.0-90.0 mcg/ml, Acid phthalate buffer solution (pH5.6): 10.0 ml;
Ninhydrin : (01%w/v) 5 ml; reaction time: 60 min.

Figure 6

Preparation of standard curve for the drug metformin hydrochloride

Aliquots of 0.5 ml, 1.0 ml, 1.5ml, 2.0 ml, 2.5ml, 3.0 ml and 3.5 ml of 500.0 mcg/ml solution of Metformin was pipetted into seven 25 ml volumetric flasks. 10.0 ml of phthalate buffer solution (pH=5.6) and 5 ml of 01% w/v reagent solution of Ninhydrin were added and heated on a water bath for 1hour at 90 °C. The volumetric flasks were cooled to room temperature and volume was made up to 25.0 ml with distill water. The absorbance of solutions was measured at 567.0 nm against blank. The calibration curve shows that Beer's law was obeyed in the concentration range 10.0-70.0 mcg/ml of Metformin hydrochloride and represented graphically in Figure 7.



Metformin : 10.0-70.0 mcg/ml, Acid phthalate buffer solution (pH 5.6): 10.0 ml;
Ninhydrin : (01%w/v) 5 ml; reaction time: 60 min.

Figure 7

Optical characteristics

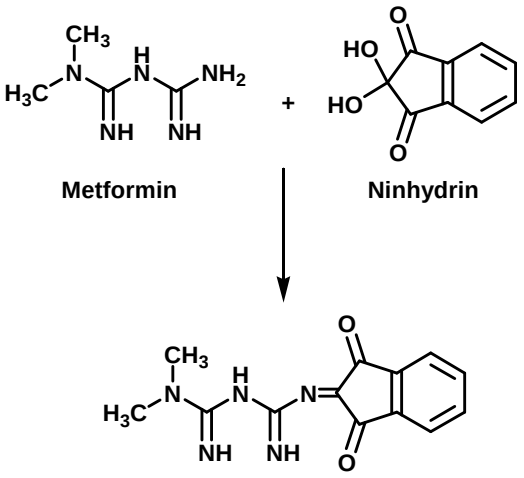
The optical characteristics of the proposed method have been calculated. The values are given in TABLE 1.

TABLE 1

Sl. No.	Parameters	Results
1.	Absorption maxima (nm)	567
2.	Beer's law limits (mcg/ml)	10-70
3.	Molar extinction coefficient (mole^{-1} cm^{-1})	133×10^{-3}
4.	Sandal's sensitivity (mcg/cm^2/0.001 absorbance units)	170×10^{-3}
	Regression equation (y)	0.9995
5.	Slope (b)	0.8691
	Intercept (a)	0.0316
6.	Coefficient of variance	4.3137
7.	Standard deviation	0.08336
8.	Limit of detection (mcg/ml)	0.8705
9.	Limit of quantitation (mcg/ml)	2.6379

Discussion of reaction mechanism

It was reported that Ninhydrin could react with the amino group of primary amino derivative. Alpha amino group of Metformin displays nucleophilicity due to the fact that its lone pairs of electrons of nitrogen can attack the electron deficient center.



Reaction mechanism

Validation parameters

Recovery studies

An aliquot of 2.0 ml of sample drug solution 500.0 mcg/ml was pipetted into each of three 25 ml volumetric flasks. To this 1.0 ml, 1.5 ml and 2.0 ml of standard drug solution of 100.0 mcg/ml was added respectively. 10.0 ml of phthalate buffer solution (pH-5.6) and 3.5 ml of 01% w/v reagent solution of Ninhydrin were added to all volumetric flasks respectively. Then heated on a water bath for 1 hour at 90 °C and cooled to room temperature. The final volume was made up to 25.0 ml with distilled water. The absorbance was measured at 567.0 nm against reagent blank. The percentage recovery by the proposed method was ranging from 98.75 % to 100.20 % indicating no interference of excipients present in the formulation.

Precision

Repeatability

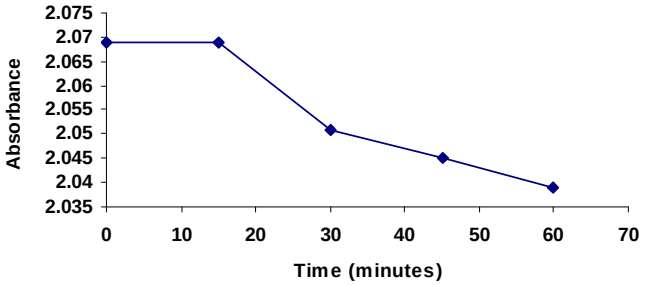
An aliquot of 2.5 ml of the above solution was pipetted into three 25 ml volumetric flasks. To this 10.0 ml of phthalate buffer solution of pH-5.6 and 3.5 ml of 01% w/v reagent solution of Ninhydrin were added to all volumetric flasks respectively. Then heated on a water bath for 1 hour at 90 °C and cooled to room temperature. The final volume was made up to 25.0 ml with distilled water. The absorbance was measured at 567.0 nm against reagent blank. The average percentage recovery by the proposed method was ranging from 100.18 and standard standard 0.0270 % indicating good repeatability.

Intermediate precision

An aliquot of 3.0 ml of above solution was pipetted into three 25 ml volumetric flasks respectively. To this solution 10.0 ml of phthalate buffer solution of pH-5.6 was added and then followed by 3.5 ml of 01% w/v reagent solution of Ninhydrin to all volumetric flasks respectively. Then heated on a water bath for 1 hour at 90 °C and cooled to room temperature. The final volume was made up to 25.0 ml with distilled water .The absorbance was measured at 567.0 nm against reagent blank. The average percentage recovery by the proposed method was ranging from 100.21 and standard standard 0.0321 % indicating good repeatability.

Stability

An aliquot of 2.0 ml of standard Metformin hydrochloride solution of 500.0 mcg/ml was pipetted into 25 ml volumetric flask. To this 10 ml of phthalate buffer solution of pH 5.6, and 5 ml of 01% w/v reagent solution of Ninhydrin solution was added respectively. The volumetric flask was heated on a water bath for 1 hour at 90 ºC and cooled the solution at room temperature. The final volume was made up to 25.0 ml with distilled water. The absorbance was measured at 567.0 nm against blank at different time intervals. The results are represents in Figure 8. The color of the derivative chromogen of Metformin hydrochloride was found to be



Metformin : 40.0 mcg/ml, Acid phthalate buffer solution (pH 5.6): 10.0 ml;
Ninhydrin : (01%w/v) 3.5 ml; reaction time: 60 min.

Figure 8

stable for 15.0 minutes after which the absorbance decreases slowly.

RESULT AND DISCUSSIONS

The analytical applications of Ninhydrin in the quantification, assay and characterization of primary amines have been established by Sullivan[31]. The use of Ninhydrin for the detection of primary aliphatic or aromatic amines initiated the present study. The reagent had been used to quantitate primary aliphatic and aromatic amines. The Ninhydrin derivative chromogenic reagent reacts with primary aliphatic or aromatic amines in medium to form wine red colored product.

The experimental conditions affecting the development and stability of the colored chromogens produced were carefully studied. It was found that stable colored chromogen was obtained at a definite pH. The colored chromogens are stable for at least 15 min which permits the convenient application of the proposed method.

Different experimental conditions, specially pH and Ninhydrin concentration were carefully selected as they could greatly affect the quantification of the target compound.

The primary aliphatic amine of Metformin hydrochloride was allowed to undergo coupling reaction with Reagent Ninhydrin (1%w/v) and during one hour heating to form pale wine red color chromophore, which was determined spectrophotometrically. Absorption maxima of pale wine red derivative chromophore were found to be at 567.0 nm. The linearity was obtained in the concentration range 10.0-70.0 mcg/ml. The color was stable for 30 minutes after which the absorbance decreases slowly, with Sandall's sensitivity of 0.0170095 (mcg/cm^2/0.001) absorbance units and Molar absorptivity of 0.13325 (mol^{-1}cm^{-1}). The Regression, slope and intercept was found to be 0.9995, 0.8691 and 0.0316.Coefficient of variance was found to be 4.3137. The standard deviation of 0.08336, LOD and LOQ was found to be 0.8705 mcg/ml and 2.6379 mcg/ml, indicated accuracy and reproducibility in color development. The method was extended for the determination in formulation i.e. bulk drug. It was observed that the results obtained were comparable to that of label claim. The recovery studies of the standard drug when performed in the preanalysed formulation gave Percentage recovery of 98.91% to 100.21% indicating practically no interference of formulation excipients with the proposed method.

CONCLUSION

It was found that no spectrophotometric methods were available for the estimation of metformin hydrochloride in bulk and in formulations.

The new method developed was New Simple, Accurate, Sensitivity, Economical and Reproducible which could provide satisfactory results. The methods can be used for routine analysis of metformin hydrochloride in bulk and in formulation. The methods are practical and valuable.

The described method has many advantages: it does not need expensive apparatus; it is simple and quick; its linear range is relatively wide, it has good selectivity. Furthermore, the proposed method may be successfully used to determine metformin in bulk and in pharmaceutical formulations. Accordingly, the method is practical and valuable.

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