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SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND COMPARATIVE STUDY OF METFORMIN HCl IN API AND SOLID DOSAGE FORM USING UV-SPECTROSCOPY

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ABSTRACT

An effort has been made to develop a simple, accurate method to estimate Metformin hydrochloride (MET) in bulk and pharmaceutical preparation and to validate the method, according to ICH guidelines. The absorbance maxima was recorded at a wavelength of 232 nm for 0.01N NaOH and 212nm for 0.01N HCl which is shown in Beers law range was confirmed by linear curve of metformin hydrochloride, shown Linearity for MET at concentration range of 2-10 µg/ml shown MET linearity for HCl 0.2-1.0 µg/ml. From the above studies the optical characteristics such as linearity range (2-10µg/ml), for 0.01N NaOH of correlation coefficient (0.999), slope (0.067) and intercept (+0.0074) where linearity range (0.2-1.0µg/ml), for 0.01NHCl Relation coefficient (0.973), slope (0.076) and intercept (+0.006) were calculated and results were found to be satisfactory. Quantitative data subjected to statistical analysis. The % RSD values < 2 indicate the precision of methodology. The accuracy was confirmed by recovery studies by adding a known amount of pure drug to the previously analyzed formulation and the mixture was analyzed by the proposed method was found to be 99.72 % - 100.81%. The values are given in recovery was confirmed and shown.

KEY WORDS

Metformin HCl, UV-Spectrophotometry, Validation.

INTRODUCTION

Metformin HCl is a antidiabeticdrug prescribed orally for the Treatment of non-insulin-dependent diabetes Mellitus.[1]

Chemically N, N-dimethylimidodicarbonimidic diamide hydrochloride (1, 1- dimethylbiguanide hydrochloride)[2].

MET is White crystalline powder, soluble in water, HCl, and NaOH. The literature survey revealed that there are different economical methods available which includes. *G. Mubeen and Khalikha Noor* have been developed simple and sensitive spectrophotometric method has been developed and validated for the estimation of metformin hydrochloride in bulk and in tablet formulation. The primary amino group of metformin hydrochloride reacts with ninhydrin in alkaline medium to form a violet color chromogen, which is determined spectrophotometrically at 570 nm. It obeyed Beer's law in the range of 8-18 µg/ml. Percentage recovery of the drug for the proposed method ranged from 97-100% indicating no interference of the tablet

excipients. The proposed method was found to be accurate and precise for routine estimation of metformin hydrochloride in bulk and from tablet dosage forms[3]. *Rashmi Ranjan et al*., develeoped A simple, accurate, validated and reproducible UV-Spectrophotometric method has been developed for the simultaneous estimation of Glipizide Hydrochloride and Metformin in both bulk and tablet formulation. Glipizide and Metformin in combined tablet formulation were estimated by using the multi component mode at 276 nm for Glipizide and 237 nm for Metformin in their solution in methanol. The Beer's law obeyed the concentration range of 2-20µg/ml for both Glipizide and Metformin. The mean recovery of 99.90% for Glipizide and 99.99% for Metformin respectively signifies the accuracy of the method. This method can be used for the routine simultaneous estimation of Glipizide and Metformin in industries and other analytical laboratories[4,5].

The objective of the present work is to develop and validate the proposed methods for MET as per I.P and ICH Guidelines for intended analytical application and to apply the proposed method for analysis in API and its application.

MATERIALS & METHODS

Materials

Metformin Hydrochloride obtained as a gift sample from, Aurobindo Pharma Ltd, HYD, all the chemicals used are of analytical grade.

Instrument

LAB INDIA –Double Beam UV-VIS Spectrophotometer3000+ with a pair of 1.0 cm matched quartz cells was used for the measurement of absorbance.

METHODOLOGY

SELECTION OF SOLVENT:

The solubility of Metformin Hydrochloride was determined in a variety of solvents as per Indian Pharmacopeia standards. Solubility test was carried out in different polar and nonpolar solvents from the solubility studies, 0.01N NaOH and 0.01N HCl were selected as suitable solvents for the proposed method.

PREPARATION OF STANDARD STOCK SOLUTION:

The standard stock solution was prepared by dissolving, accurately weighed 100 mg of METFORMIN Hydrochloride in 0.01N NaOH and the volume was made up to 100 ml with 0.01N NaOH in 100 ml volumetric flask (1° Stock solution, 1000 µg / ml).

PREPARATION OF STANDARD STOCK SOLUTION:

The standard stock solution was prepared by dissolving, accurately weighed 100 mg of METFORMIN Hydrochloride in 0.01N HCl and the volume was made up to 100 ml with 0.01N HCl in 100 ml volumetric flask (1° Stock solution, 1000 µg / ml).

DETERMINATION OF ABSORBANCE MAXIMA (λ_{max}):

10 ml of the primary stock solution was diluted to 100 ml with 0.01N NaOH (secondary Stock solution, 100 µg / ml). 1 ml of the secondary stock solution was taken in 10 ml standard volumetric flasks diluted to 10 ml with water to get the concentration of 10 µg/ml. The absorbance of resulting solution was measured against respective blank solution (0.01N NaOH) in the UV region of 200-400 nm, which shows maximum absorbance at 232 nm and was shown in Fig. 1.

DETERMINATION OF ABSORBANCE MAXIMA (λ_{max}):

10 ml of primary stock solution was diluted to 100 ml with 0.01N HCl (secondary Stock solution, 100 µg / ml). 1 ml of the secondary stock solution was taken in 10 ml standard volumetric flasks diluted to 10 ml with water to get the concentration of 10 µg/ml. The

absorbance of resulting solution was measured against respective blank solution(0.01N HCl) in the UV region of 200-400 nm, which shows maximum absorbance at 212 nm and was given in Fig 1.

ANALYSIS OF MARKETED FORMULATION IN 0.01N NaOH

10 tablets of METFORMIN Hydrochloride were weighed, pulverized and the powder equivalent to 0.01 gm of METFORMIN was weighed accurately and transferred into a 100 ml standard volumetric flask. The contents were dissolved in 0.01N NaOH. This solution was filtered through Whatmann filter paper number 40. 0.6 ml of the above was diluted to 10 ml with 0.01N NaOH to obtain a solution of 6 µg / ml. Same concentration was repeated six times were presented on Table No: 2.

ANALYSIS OF MARKETED FORMULATION IN 0.01 N HCl

10 tablets of METFORMIN Hydrochloride were weighed, pulverized and the powder equivalent to 0.01 gm of METFORMIN HYDROCHLORIDE was weighed accurately and transferred into a 100 ml standard volumetric flask. The contents were dissolved in 0.01N HCl. This solution was filtered through Whatmann filter paper number 40. 0.6 ml of the above was diluted to 10 ml with 0.01N HCl to obtain a solution of 0.6 µg / ml. Same concentration was repeated six times were presented on table Table No: 2.

METHOD VALIDATION

ACCURACY

Accuracy is the closeness to the true value. To study the accuracy, 10 tablets of Metformin HCl were taken, and the powder was used to carry out the analysis. Recovery studies were carried out by addition of standard drug solution (80%, 100%, 120% µg/ml) to the sample at 3 different concentration levels and results were presented in Table No: 3.

PRECISION

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogeneous samples shown in Table 5 & 6.

Intra and inter-day precision

A variation of results within the same day (intra-day), variation of results between days (inter-day) was analyzed and was shown in table no: 5. Intra-day precision was determined by analyzing Metformin Hydrochloride for three times in the same day at 232 nm. Inter-day precision was determined by analyzing the drug daily once for three days at 232 nm.

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Linearity in 0.01N NaOH

For preparation of different concentrations, aliquots of stock solution of suitable concentrations of Metformin Hydrochloride were transferred into a series of 10 ml standard flasks and volumes were made up to mark with0.01N NaOH. Five different concentrations were prepared in the range of 2-10µg/mL and the absorbances were measured at 232 nm against solvent(0.01N NaOH) blank and the absorbance values were shown in table 5. The obtained absorbance values are plotted against the concentration of Metformin Hydrochloride to get the calibration graph and were represented in Fig. no 3. The concentration of the unknown sample was determined from the calibration graph. The regression equation and

correlation coefficient were determined and are given in Table 1.

Linearity in 0.01N HCl

For preparation of different concentrations, aliquots of stock solution of suitable concentrations of Metformin Hydrochloride were transferred into a series of 10 ml standard flasks and volumes were made up to mark with 0.01N HCl. Five different concentrations were prepared in the range of 0.2-1.0µg/mL and the absorbances were measured at 212 nm against solvent(0.01N HCl) blank and the absorbance values were shown in table 5. The obtained absorbance values are plotted against the concentration of Metformin Hydrochloride to get the calibration graph and were represented as . The concentration of the unknown sample was determined from the calibration graph. The regression equation and correlation coefficient were determined and are given in Table 1.

LIMIT OF DETECTION (LOD):

Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

It is calculated from the formula,

$$LOD = 3.3\ \sigma / S$$

LIMIT OF QUANTITATION (LOQ):

Based on the LOD strength , the LOQ values were calculated by multiplication with three times. The quantitation limit (QL) is calculated from,

$$LOQ = 10\ \sigma / S$$

RESULTS

A simple difference spectrophotometric method was developed and optimized by Double Beam UV-VIS Spectrophotometer 3000+ as per ICH guidelines. Metformin is well known UV absorbing molecule. The developed method was found to be precise as % RSD values were found to be less than 2%. The absorbance maxima was recorded at wavelength of 232 nm for 0.01N NaOH and 212nm for 0.01N HCl which is shown in Beers law range was confirmed by linear curve of metformin hydrochloride, shown Linearity for metformin hydrochloride is shown at for concentration range of 2-10 µg/ml.shown Metformin HCl linearity for hcl 0.2-1.0 µg/ml from the above studies the optical characteristics such as linearity range(2-10µg/ml), for 0.01N NaOH of correlation coefficient (0.999), slope (0.067) and intercept (+0.0074) were linearity range (0.2-1.0µg/ml), for 0.01 NHCl correlation coefficient (0.973), slope (0.076) and intercept (+0.006) were calculated and results were found to be satisfactory. Quantitative data subjected to statistical analysis. The % RSD values < 2 indicate the precision of methodology.

The accuracy was confirmed by recovery studies by adding a known amount of pure drug to the previously analyzed formulation and the mixture was analyzed by the proposed method was found to be 99.72 % - 100.81%. The values are given in recovery was confirmed and shown.

Table 1: Summary of validation

Parameters	Values in 0.01N HCl	Values in 0.01N NaOH
Linearity Range µg/ml	0-1 µg/ml	0-10 µg/ml
λ_{max} (nm)	232 nm	212
Beer's law limits (µg/ml)	2-10	0.2-1.0
Regression equation (Y*)	Y = 0.067x-0.0074	Y = 0.076x-0.006
Slope (b)	0.067	0.0753
Intercept (a)	0.0074	+0.0667
Correlation coefficient(r^2)	0.999	0.973
% RSD**	< 2%	< 4%
Limit Of Detection (µg/ml)	0.0325	0.0318
Limit Of Quantitation (µg/ml)	0.0987	0.0963

Table no 2: Calibration curve of Metformin in 0.01N NaOH & 0.01N HCl

S.No	Concentration	Absorbance in 0.01 N NaOH	Concentration	Absorbance in 0.01 N HCl
1	0	0	0	0
2	2	0.127± 0.001	0.2	0.025± 0.001
3	4	0.249± 0.007	0.4	0.043± 0.001
4	6	0.382± 0.005	0.6	0.058± 0.002
5	8	0.538± 0.005	0.8	0.07± 0.002
6	10	0.673± 0.008	1	0.083± 0.006

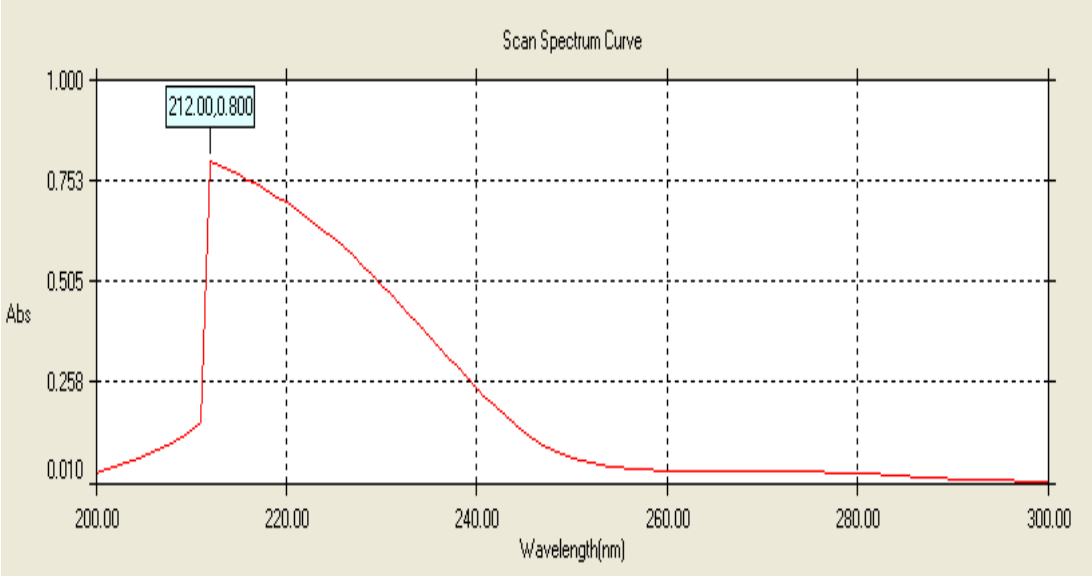

Fig: 1 λ –max of METFORMIN HCl

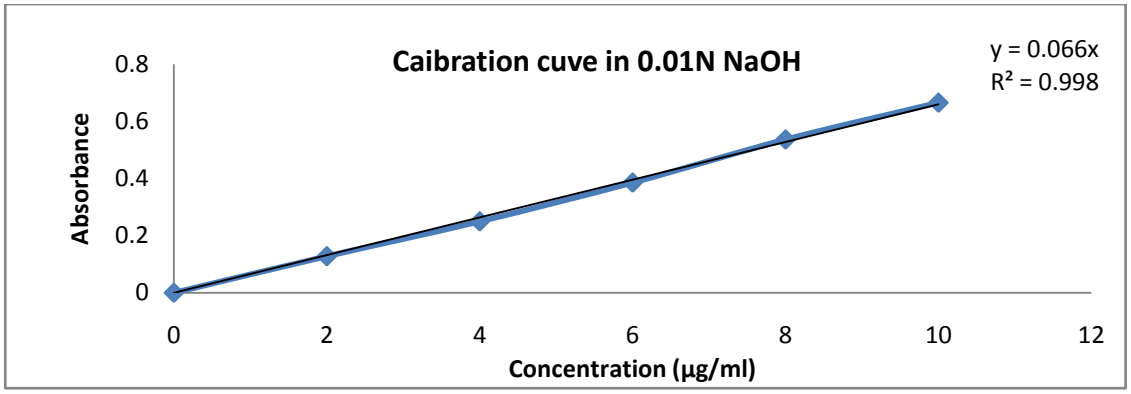

Fig: 2 Calibration curve of Metformin on 0.01N NaOH

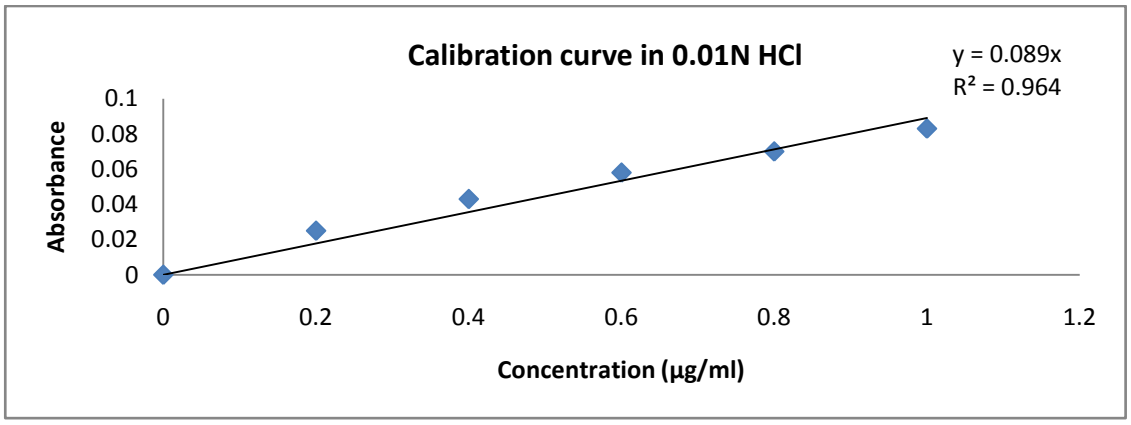

Fig: 3 Calibration curve of Metformin on 0.01 N HCl

Table no 2: Assay of Metformin HCl formulation in 0.01 N NaOH & 0.01N HCl

Solvent	Formulation	Label claim (mg/tab)	Amount found (mg) (n=3) Mean ± SD	Assay	%RSD
0.01 N NaOH	Glycomet	500mg	40.003±0.43	100.2%	0.797 ± 2 %
0.01NHCl	Glycomet	500mg	40.003±0.43	117.18%	4.841 ± 2 %

Table no 3: Determination of accuracy results for Metformin HCl in 0.01N NaOH at 232nm.

Brand name	Spiked level	Amount of sample (mcg/ml)	Amount of drug added (mcg/ml)	Amount Recovered	% Recovery ± SD**
Glycomet	80%	0.3	2.4	5.1	99.44 ± 0.026
Glycomet	100%	0.3	3	5.9	99.50 ± 0.061
Glycomet	120%	0.3	3.6	6.4	99.09 ± 0.080

Table no 4: Determination of accuracy results for Metformin HCl in 0.01N HCl at 212nm

Brand name	Spiked level	Amount of sample (mcg/ml)	Amount of drug added (mcg/ml)	Amount Recovered	% Recovery ± SD**
Glycomet	80%	0.03	0.24	0.51	105 ± 0.633
Glycomet	100%	0.03	0.3	0.59	112 ± 0.901
Glycomet	120%	0.03	0.36	0.6.4	114 ± 1.258

Table no 5: Determination of precision results Metformin HCl in 0.01N NaOH AT 232 nm

Concentration µg / ml	Inter-day Mean ± SD**	Absorbance % RSD	Intra-day Mean ± SD**	Absorbance % RSD
LQC (2µg/ml)	0.164 ± 0.00057735	0.352	0.167 ± 0.00057735	0.345

Table no 6: Determination of precision results Metformin HCl in 0.01N HCl at 212 nm

Concentration µg / ml	Inter-day Mean ± SD**	Absorbance % RSD	Intra-day Mean ± SD**	Absorbance % RSD
LQC (0.2µg/ml)	0.032 ± 0.001	4.77	0.033± 0.001	3.49

CONCLUSION

All the above factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, robust and cost effective and can be applied successfully for the estimation of silodosin in bulk and pharmaceutical dosage formulations.

REFERENCES:

1. P Umapathi, J.Ayyappan, S Darlin Quine, Quantitative Determination of Metformin Hydrochloride in Tablet Formulation Containing Croscarmellose Sodium as Disintegrant by HPLC and UV Spectrophotometry, Trop J Pharm Res, February2012;11 (1): 107.
2. Madhukar. A, A. Prince, vijay kumar. R, Sanjeeva. Y, Jagadeeshwar. K, D. Raghupratap. Simple and Sensitive Analytical Method Development and Validation of Metformin Hydrochloride by RP-HPLC. Int J Pharm Pharm Sci, Vol 3, Issue 3, 2011, 117-120.
3. Mubeen G, Noor K. Spectrophotometric method for analysis of metformin hydrochloride. Indian J Pharm Sci 2009;71:100-2.
4. Rashmi Ranjan Sarangi, Simultaneous uv-spectrophotometric estimation of glipizide and metformin in bulk and its dosage form, International Journal of Pharmaceutical & Biological Archives 2011; 2(4):1137-1145. 5.Badawy SS, Abdel-Gawad FM and Ibrahim MM. Spectrophotometric Studies on Determination of Cefadroxil with Copper(II) and Vanadium(V) in Sulphuric Acid Medium;vol. 26, issue 3, March 1993: p. 48.

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