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DEVELOPMENT AND VALIDATION OF UV-SPECTROSCOPIC METHOD FOR THE ESTIMATION OF METFORMIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract:

A new simple, accurate, rapid, precise, reproducible and cost effective spectrophotometric method for the quantitative estimation of Metformin in bulk and pharmaceutical dosage form. The developed visible spectrophotometric method for the quantitative estimation of Metformin is based on measurement of absorption at maximum wavelength 242 nm using Methanol as a solvent. The stock solution of Metformin was prepared, and subsequent suitable dilution was prepared with methanol to obtained standard curve. The standard solution of Metformin shows absorption maxima at 242 nm. The drug obeyed beer lambert's law in the concentration range of 0 - 60 µg/ml with regression 0.999 at 242 nm. The overall % recovery was found to be 100.04% which reflects that the method was free from the interference of the impurities and other excipients used in the bulk and marketed dosage form. The low value of % RSD was indicative of accuracy and reproducibility of the method. The % RSD for inter-day and intra-day precision was found to be 0.53 and 0.51respectively which is<2% hence proved that method is precise. The results of analysis have been validated as per International Conference on Harmonization (ICH) guidelines. The developed method can be adopted in routine analysis of Metformin in bulk and tablet dosage form.

Keywords: *Metformin, UV Visible Spectrophotometry, Method development, Validation, , Methanol , Accuracy, Precision.*

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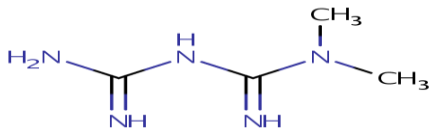
INTRODUCTION:

This metformin drug belongs to the class of organic compounds known as biguanides. These are organic compounds containing two N-linked guanines. i.e. That structure of metformin contains two amine groups in its structure.



Structure of metformin

Metformin (1-carbamimid amido-N, N-di methyl methanimidamide)

Metformin is not pharmacologically related to any other class of oral antidiabetic agents. Metformin does not affect insulin secretion [1-3].

Mechanism of action of Metformin is different from the other classes of oral anti diabetic agents. It is used to decreases the blood glucose levels by decreasing the intestinal absorption of glucose levels it leads to the decreasing the hepatic production of glucose and improving the insulin sensitivity by increasing the peripheral uptake and glucose utilization. These effects are performed by the initial activation by metformin of AMP-activated protein kinase (AMPK) enzyme (liver enzyme that plays an important role in insulin signalling) [4-8]. Why because whole energy balance of body, and the glucose and fats metabolism. Activation of AMP-activated protein kinase (AMPK) is essential for the inhibitory effect metformin on the glucose production by hepatic cells. Increased utilization of glucose in peripheral may be due to improved insulin binding to insulin receptors. AMPK is known to cause GLUT4 deployment to the plasma membrane, resulting in the insulin-independent uptake of glucose [9,10].

Metformin is inconsequentially bound to the proteins of plasma.

Metformin is not metabolized by the liver. It is metabolized by the kidney.

Half-life of Metformin is about 6.2 hrs and the duration of action is 8-12 hrs.

Brand Names: Kazano (125mg/500mg)- Dose is based on patient's condition. It comes as a tablet to take by mouth, with food two times per day.

MATERIALS AND METHODS:

Chemicals and Reagents: Methanol, Ethanol, Acetone, Dimethylsulphoxide, Sodium citrate with water.

Instruments:

SHIMADZU UV-1601 UV – Vis spectrophotometer, Electronic Balance **(CITIZEN BALANCE BL-220H),** Ultra Sonicator **(ANALYTICAL),** and P^H Analyzer **(ELICO),** Distillation unit **(BOROSIL),** Vaccum filteration unit**(BOROSIL).**

Reagents and Solutions

Diluent preparation:

Take 500 ml of methanol were degassed in an ultra-sonic water bath for 10min and then filtered

Preparation of standard stock solution of Metformin:

Accurately weighed 10mg of standard Metformin was transferred to a clean & dry 100 ml volumetric flask and dissolved in solvent system containing methanol and finally make up volume up to the mark with the same. The final solution contained 100 g per ml of Metformin solution. Standard Metformin solution (3ml) was transferred to separate 10 ml volumetric flask. The volume was adjusted to 10 ml with same solvent mixture.

Sample preparation:

Accurately weigh 25 mg of Metformin and transfer in to 25ml volumetric flask. Add about 10ml of solvent mixture sonicate to dissolve. Cool the solution to room temperature and dilute to volume with solvent mixture. Transfer 3ml of above solution in to a 10ml volumetric flask and make up the volume with diluent.

Determination of wavelength of maximum absorbance for metformin The absorbance of the final solution scanned in the UV spectrum in the range of 200 to 400nm against solvent mixture as blank.

Optimization of selection of Solvent

It is well known that the solvents do exerts a profound effect on the quality and the shape of the peak. The choices of solvents for UV method development are: Methanol, Ethanol, Acetonitrile, Dimethyl Sulphoxide, Sodium citrate etc. First optimize the different solvents. From that solvents methanol satisfied the all the optimized conditions.

Wavelength Selection [11-15]

The standard solutions are prepared by transferring the standard drug in a selected solvent and finally diluting with the same solvent or Diluent. That prepared solution is scanned in the UV visible wavelength range of 200-400nm.This has been performed to know the maxima of metformin. While scanning the metformin solution we observed the maxima at 242 nm. The visible spectrum has been

recorded on (SHIMADZU UV-1601 make UV – Vis spectrophotometer model UV-1601.The scanned visible spectrum is attached in the following page.The λ_{max} of the metformin was found to be 242 nm in diluents as solvent system.

METHOD VALIDATION [16-20]

1. Accuracy: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (50%, 100%, and 150%) of pure drug of Metformin were taken and added to the pre-analyzed formulation of concentration 30g/ml. From that percentage recovery values were calculated.

2. Precision:

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Metformin (API) the percent relative standard deviations were calculated for Metformin.

Intermediate Precision:

Intra-assay & inter-assay:

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Metformin revealed that the proposed method is precise. The results were shown in Table-3.

3. Linearity & Range:

The calibration curve showed good linearity in the range of 10-60µg/ml, for Metformin (API) with correlation coefficient (r^2) of 0.999 (Fig-2). A typical calibration curve has the regression equation of y = 0.1334x + 0.0019 for Metformin.

Standard solutions of Metformin in the concentration range of 10 g/ml to 60 g/ml were obtained by transferring (10, 20,30,40,50 & 60 ml) of Metformin stock solution (100ppm) to the series of clean & dry 10 ml volumetric flasks. The volumes in each volumetric flask were made up with the solvent system and mixed.

The absorbances of the solutions were measured at 242 nm against the solvent system as blank and calibration curve is plotted. The Lambert-Beer's Law is linear in concentration range of 10 to 60 g/ml at 242 nm for Metformin .

4. Method Robustness:

Robustness of the method was determined by carrying out the analysis under different Wavelength i.e. at 240nm,242nm and 244nm.. The respective absorbances of 30µg/ml were noted SD < 2%) the developed UV-Spectroscopic method for the analysis of Metformin (API).

5. LOD & LOQ:

The LOD and LOQ were calculated by the use of the equations LOD = 3.3 × σ / S and LOQ = 10 × σ / S where σ is the standard deviation of intercept of Calibration plot and S is the average of the slope of the corresponding Calibration plot.

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 2.97 & 9 µg/ml respectively.

6. ASSAY OF METFORMIN IN DOSAGE FORM [21-25]:

METFORMIN 10mg

Assay of marketed tablet formulation Brands: Metformin was procured from the local market as tablets of strength having 10mg, marketed with brand names of KAZANO. These marketed formulations were manufactured by the takeda Pharmaceuticals, respectively.

Weighed accurately about twenty tablets and calculate the weights of individual tablets and finally calculate the average weight. They were triturated to fine powder by using a mortar and pestle. The powdered tablet equivalent to 10mg of Metformin was dissolved in 15ml of diluent with the help of sonication process and the final volume was made upto the mark with the diluent in 100 ml volumetric flask. The resulted solution was filtered using whatman filter paper (0.45µm). This final solution was further diluted to obtain 30µg/ml concentration of the solution by using diluents used as a solvent and observed by UV analysis. This procedure was repeated in triplicate. The data are shown in Table-6.

Amount Present

RESULTS AND DISCUSSION:

The standard solutions of Metformin in methanol (30µg/ml) subjected to a scan individually at the series of wavelengths of 200 nm to 400 nm. Absorption maximum of Metformin was found to be at 242nm. Therefore, 242 nm was selected as λ_{max} of Metformin for the present study. The calibration curve of Metformin was found to be linear in the range of 10-60 µg/ml at 242 nm. Therefore, it was clear that Metformin can be determined without interference of any irrelevant substance in single

component pharmaceutical products. The used technique was initially attempted on bulk drugs in their synthetic sample and concentrations were estimated.

The % recovery was carried out at 3 levels, 50%, 100% and 150% of Metformin standard concentration. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were found to be satisfactory within the acceptable limits as per the content of the label claim for marketed tablet dosage form. The newly developed method was validated according to the ICH guidelines and the method validation parameters.

The developed method was subjected to do the various method validation parameters such as specificity, accuracy, precision, linearity and range, limit of detection and limit of quantification, robustness and ruggedness etc.

Table-1: Results of accuracy:

Concentration level	Amount added (mg)	Amount found(mg)	%recovery	Average % recovery
50%	15mg 15mg 15mg	15mg 15mg 15mg	100.5% 101.5% 100.9%	100.9%
100%	30mg 30mg 30mg	30mg 30mg 30mg	100.8% 100.4% 99.2%	100.1%
150%	45 45 45	45mg 45mg 45mg	100.0% 100.5% 100.3%	100.2%

Acceptance criteria: correlation coefficient should not be less than 0.990.

2. Precision:

Repeatability:

Table-2: Results of Repeatability

No. of samples	Sample Absorbance	Results obtained	
	Mean	Amount of drug present in µg	% of drug
1	0.520	30.27	100.9
2	0.521	30.12	100.4
3	0.523	30.0	100.0
4	0.524	30.15	100.5
5	0.525	30.03	100.1
6	0.523	30.09	100.3
		Mean	100.1
		Standard deviation	0.24
		% RSD	0.23

Table-3: Results of intra-Day & inter-Day

Con. taken (µg/mL)	Observed Conc. Of Metformin (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Absorbance	**Statistical Analysis**	Con. found (µg/mL)	**Statistical Analysis**
30	0.527	Mean = 0.516	0.517	Mean = 0.514
30	0.525	SD = 0.00816	0.514	SD = 0.0018
30	0.526	%RSD = 0.158	0.515	%RSD = 0.3636

Table-4: Results of Linearity

Concentration (µg/ml)	**Absorbance (n=6)**
10	0.490
20	0.50
30	0.51
40	0.51
50	0.52
60	0.52

Acceptance criteria: correlation coefficient should not be less than 0.990

Table-5: Result of Method Robustness Test

Wavelength (-2) (240nm)

Concentration(µg/ml)	**Absorbance**	**Statistical Analysis**
30	0.50	Correlation coefficient = 0.999, Intercept =0.133x+0.001 Slop = 0.001
30	0.49	
30	0.51	
30	0.52	
30	0.51	
30	0.50	

Wavelength(242nm)

Concentration(µg/ml)	**Absorbance**	**Statistical Analysis**
30	0.51	Correlation coefficient = 0.999, Intercept =0.133x+0.001 Slop = 0.001
30	0.51	
30	0.49	
30	0.52	
30	0.51	
30	0.52	

Wavelength(244nm)

Concentration(µg/ml)	**Absorbance**	**Statistical Analysis**
30	0.52	Correlation coefficient = 0.999, Intercept =0.133x+0.001 Slop = 0.001
30	0.52	
30	0.51	
30	0.49	
30	0.51	
30	0.52	

Table-6: Assay Results of Marketed Formulations

Formulations	Actual concentration of Metformin [Label Claim] (mg/ml)	Amount obtained of Metformin (mg/ml)	% Metformin
KAZANO	10	9.908	99.08

CONCLUSION:

Literature survey reveals that several spectrophotometric methods were reported for estimation of Metformin. In view of the need for a suitable UV-Visible spectroscopic method for routine analysis of Metformin in formulations, attempts were made to develop simple, precise and accurate analytical method for estimation of Metformin and extended it for their determination in bulk and dosage formulation.

We hope the developed method is accurate, precise and this method is not suffered from any interference such as degradants/impurities due to common excipients.

The method validation to be done by the help of method validation parameters as per ICH guidelines/ and recommendations. We hope the developed method is accurate, precise and simple.

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