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METHOD DEVELOPMENT AND VALIDATION OF METFORMIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY USING SPECTROPHOTOMETRIC METHOD

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ABSTRACT

In the present research a simple, accurate, precise and cost effective UV-Vis spectrophotometric method for the estimation of Metformin, in bulk and pharmaceutical dosage form was illustrated. The absorption maxima of the drug was found to be 233 nm in 0.1 N HCl: Distilled water (27:75). A linear response was observed in the range of 5-10 µg/ml with a regression coefficient of 0.999. Validation parameters were carried out as per the guidelines of International Conference for Harmonization. This method can be used in the industries for determination of Metformin to analyze the quality of formulation without interference of the excipients.

Keywords: - Metformin, Biguanides, UV-Vis spectrophotometer, λmax.

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INTRODUCTION

Metformin (dimethylbiguanide*) is a blood glucose lowering agent used in the treatment of non-insulin dependent diabetes mellitus. Its therapeutic effect is achieved without raising insulin concentrations, and it appears to reduce insulin resistance. Unlike the other major blood glucose-lowering agents, namely suphonylurea and insulin, Metformin does not cause weight gain. Indeed, Metformin may produce a small decrease in body weight during the initial months of treatment. This cannot be attributed to a chronic decrease in food consumption or increased physical activity, suggesting that the drug increases metabolic energy expenditure. The drug is found official in Merck index [1]. There are various spectrophotometric methods developed for estimation of metformin[2, 3, 4]. Solvents used for the methods are comparatively of high cost. There is a need for a simple, rapid, cost effective and reproducible method for assay of Metformin in its dosage forms. Therefore, it was thought of interest to develop simple, accurate, fast and cost effective method for the analysis of Metformin in its tablet formulation. This paper describes development and validation of simple, specific, sensitive, accurate and precise Ultraviolet spectroscopic method for the estimation of Metformin in bulk and its formulation

MATERIALS AND METHODS

Zero order spectroscopic method

Water used for dilution was distilled in the laboratory. A double beam UV spectrophotometer (Shimadzu UV-1800) was used with 1 cm matched quartz cell. Tablet formulation [Melmet-500, Micro Labs Ltd., Baddi, Solan- Dist, Himachal pradesh] were procured from a local pharmacy with labeled amount 500 mg per tablet.

Solvent selection

Various solvents were selected for the solubility studies and it was found that Metformin was soluble in the following solvents; dimethyl sulfoxide, dimethyl formamide, methanol, 0.1 N HCl, chloroform, acetonitrile, *etc.* In the present investigation 0.1 N HCl: Distilled water (25:75) was selected as a solvent.

Preparation of stock solutions

Standard Metformin 100mg was weighed and transformed to a 100 ml volumetric flask and dissolved in 25 ml of 0.1 N HCl. The flask was shaken and volume was made up to the mark with Water to give a solution containing 1000 µg/ml (Stock solution A). From this stock solution A, pipette out 5 ml and place into 50 ml volumetric flask. The volume was made up to the mark

with 0.1 N HCl: Distilled water (25:75) to give a solution containing 100 µg/ml (Stock solution B).

Selection of analytical concentration range

From the standard stock solution B of Metformin, appropriate aliquots 0.5, 0.6, 0.7, 0.8, 0.9 and 1 ml were pipette out in 10 ml volumetric flasks and dilutions were made with 0.1 N HCl : Distilled water (25:75) to obtain working standard solutions of concentrations from 5-10µg/ml. Absorbance for these solutions were measured at 233 nm. For standard solution analytical concentration range was found to be 5-10 µg/ml and overlain spectra was obtained.

Sample preparation for determination of Metformin from Dosage form

Twenty tablets of formulation were weighed and finely powdered. The powder equivalent to 100 mg of Metformin was accurately weighed. It was then transferred to volumetric flask of 100 ml capacity containing 25 ml of 0.1 N HCl and sonicated for 30 min. The flask was shaken and the solution was filtered through Whatmann filter paper (No. 41) into 100 ml volumetric flask. Volume was made up to the mark with distilled water to give a solution of 1000 µg/ml (Stock solution A). From this solution 5 ml was taken and placed in 50 ml volumetric flask. The volume was made up to the mark using 0.1 N HCl: Distilled water (25:75) to give a solution of 100 µg/ml (Stock solution B). From the stock solution B, 2.0 ml was taken and diluted to 10 ml to give 20 µg/ml and it was further used for the estimation of Metformin.

METHOD VALIDATION

Validation parameters[5]

1) Linearity,

2) Precision,

3) Accuracy,

4) Ruggedness.

5) LOD

6) LOQ

RESULTS AND DISCUSSION

Selection of analytical wavelength and absorption maxima

Appropriate dilutions were prepared for drug from the standard stock solution and the solutions were scanned in the wavelength range of 200-400 nm. The absorption spectra thus obtained was derivatized for zero order spectroscopy. This zero order spectrum was selected for the analysis of

the drugs. The absorption maximum was found at 233 nm which can be further used for analysis (Figure 1).

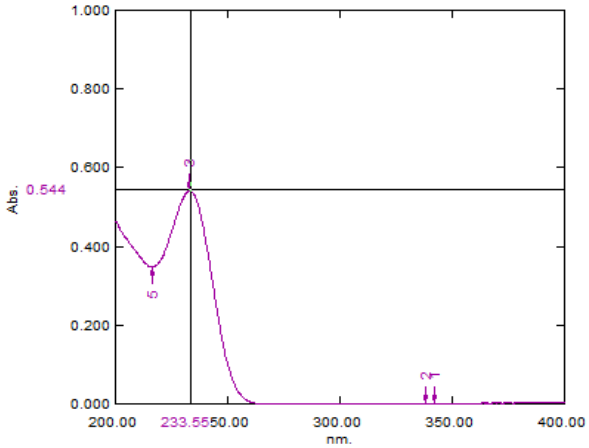

Figure 1.UV spectrum of Metformin standard by zero order method

Calibration curve for the Clobazam

Appropriate volumes of aliquots from standard Metformin stock solution B were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with 0.1 N HCl : Distilled water (25:75) to obtain concentrations of 5, 6, 7, 8, 9 and 10 µg/ml. Absorbance value of each solution against 0.1 N HCl : Distilled water (25:75) as a blank were measured at 233 nm. From that absorbance value, regression equation and correlation coefficient (r^2) are determined and presented (Figure.2).

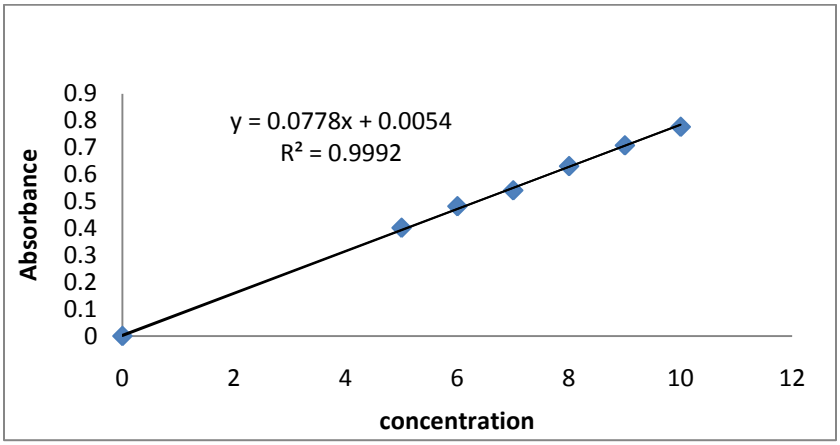

Figure 2.Calibration curve for Metformin at 233 nm.

METHOD VALIDATION

Validation parameters[5]

Linearity

The linear regression equation and the statistical evaluation of the calibration plots for the analysis of authentic samples are listed. Under the described experimental conditions, linear

correlations were obtained at the wavelength 233 nm over the concentration range of 5-10 µg/ml of Metformin. The calculated correlation coefficient (r) of least square linear regression was found to be 0.999 for the zero order (Table. 1).

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (50, 100, and 150%) of standard bulk sample of Metformin within the linearity range were taken and were added to the pre-analyzed formulation of concentration 6 µg/ml and percentage recovery values were calculated. They were found to be present within the range. The accuracy results were obtained for zero order spectroscopy (Table 2).

Table. 1. Absorbance values for calibration curve of Metformin at 233 nm by Zero order spectroscopy.

Concentration (µg/ml)	Absorbance*	±S.D*
0	0	±0
5	0.402	±0.002082
6	0.482	±0.003055
7	0.541	±0.001528
8	0.631	±0.000577
9	0.708	±0.004041
10	0.777	±0.0000
	Average of SD	**±0.001513**

Table.2. Recovery study data of Metformin by Zero order spectroscopy.

Level of recovery	Amount of sample (µg/ml)	Amount of drug added (µg/ml)**	Amount of drug recovered (µg/ml)**	% Recovery ± S.D**
50%	6	3	3.012	100.40±0.0.00050
100%	6	6	6.073	101.21±0.0.00057
150%	6	9	9.146	101.626±0.0.00057

** is average of three determinations

Precision

The precision of the proposed method was ascertained by determination of six replicates of same concentration of sample and standard for method precision and system precision. Both intraday precision and interday precision was carried out for zero order spectroscopy. The deviation between repeated readings was found to be present within the limit (Table 3).

Ruggedness

Ruggedness is a measurement of reproducibility of test results under the variation in condition normally expected from laboratory to laboratory and from analyst to analyst. In the current study

it was carried by two analysts for zero order spectroscopy. The results thus obtained by two analysts were not having considerable deviation (Table 4).

Table.3. Precision study data of Metformin by Zero order spectroscopy

Concentration (µg/ml)	Inter-day absorbance mean ± SD**	% RSD	Intra-day absorbance mean ± SD**	% RSD
5	0.457 ±0.00063	0.138	0.455 ±0.0007	0.165
6	0.530 ±0.00081	0.153	0.529 ±0.00051	0.097
7	0.615 ±0.00051	0.083	0.614 ±0.00103	0.168
8	0.716 ±0.00051	0.072	0.710 ±0.00030	0.433
9	0.794 ±0.00051	0.064	0.793 ±0.00109	0.138
10	0.946 ±0.00136	0.144	0.949 ±0.00098	0.103

** is average of six determinations.

Table.4. Ruggedness study data of Metformin by Zero order Spectroscopy.

Sample	Label claim (mg)	Analyst 1		Analyst 2	
		Amount found (mg)	%Recovery ±SD**	Amount found (mg)	% Recovery ±SD**
Melmet-500	500	494.55	98.91±0.00057	494.9187	98.98 ±0.001

** is average of six determinations.

Limit of detection

The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from 5-10 µg/ml. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected, but not necessarily quantitated as an exact value.

Limit of quantification

The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve.

The method was validated according to International Conference of Harmonization guidelines for validation of analytical procedures. The proposed method showed absorption maxima at 233 nm and obeyed Beer's law in the concentration range of 5-10 µg/ml. The limit of detection (LOD) was found to be 1.241 µg/ml and limit of quantification (LOQ) to be 3.762 µg/ml. The percentage recovery value indicates no interference from excipients used in formulation. The low value of percentage relative standard deviation shows that the developed method was precise. All statistical data prove validity of proposed method, which can be applied in industries for routine analysis of Metformin drug from tablet.

CONCLUSION:

For routine purposes it is always of interest to establish methods capable of analyzing a sample in a short period with due accuracy and precision. The main purpose of this study was to develop accurate, precise and economic method for the determination of Metformin. In UV-Visible technique namely Zero-order Spectroscopic method, was applied without using any prior chemical pretreatment. The proposed UV Spectrophotometric method is rapid, selective, simple, cost effective, fast and efficient. Finally, the proposed method could be useful and suitable for determination of Metformin in bulk and pharmaceutical formulations.

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REFERENCES:

1. Budavari S, in. The Merck Index., ed. Merck and Co Inc., 13thed., 1997; Whitehouse Station (NJ), 5792.
2. Arayne MS, Sultana N, Zuberi MH, Siddiqui FA. Spectrophotometric quantitation of Metformin in bulk drug and pharmaceutical formulations using multivariate technique. Indian J Pharm Sci 2009; 71 (3): 331-335.
3. Chaturvedi PK, Sharma R. Simultaneous spectrophotometric estimation and validation of three component tablet formulation containing pioglitazone hydrochloride, Metformin hydrochloride and Glibenclamide. Analytical Letters 2008; 41(12):2133-2142.
4. Rohokale BS, Jadhav VM, Kadam VJ. Studies in prospective process validation of Metformin HCl tablet Dosage formulation. Int J PharmaTech Res 2010; 2(3):1673-1678.
5. Validation of analytical procedures: text and methodology. International conference on harmonization of technical requirement for registration of pharmaceuticals for human use Q_2 (R1) 1996/Nov.