Method Development and Validation of Metformin Hydrochloride in Tablet Dosage Form

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Abstract: A simple, reproducible and efficient method for the determination of metformin hydrochloride (MET) was developed and validated. The analysis complied with Beer's law in the concentration range of 8-13 µg/mL at 233 nm for MET. In our study the validation of analytical method for determination of MET by UV in tablets formulation was performed in accordance the parameters including-system suitability, specificity, limit of quantification, limit of detection, linearity of response, accuracy, precision (reproducibility & repeatability), robustness (change of wave length ± 2 nm).

Keywords: Validation, Metformin HCl (MET), Robustness, LOQ, LOD

Introduction

A new fixed dose containing metformin hydrochloride (MET) is available in the market in tablet dosage form for treatment of type-2 diabetes. Type-2 diabetes is a long-term metabolic disorder wherein the body becomes resistant to the effects of insulin, a hormone that regulates sugar absorption¹. Several methods have been reported for the estimation of MET, such as high performance liquid chromatography (HPLC) with ultraviolet (UV) detection²⁻⁶ or fluorescence detection⁷ and capillary electrophoresis (CE) with ultraviolet (UV) detection^{8,9}. Most of these methods are tedious and time-consuming, involving complex sample preparation. So, there is a need for the development of simple sensitive effective and economic methods and hence the present work was planned to validate the UV spectroscopic method for MET in tablet formulations by using following parameter like accuracy, precision, linearity and range, limit of detection, limit of quantification, specificity, robustness, ruggedness and system suitability as per ICH guidelines¹⁰.

Experimental

MET was received as gift sample in tablet form from Aristo Pharmaceutical (Bhopal, India). Each film coated tablet contains metformin HCl I.P. (1000 mg). Methanol A.R grade was procured from Qualigens fine chemical, Mumbai, India, Potassium dihydrogen orthophosphate AR grade, sodium hydroxide AR grade. UV spectroscopy (Shimadzu UV-1700) UV Vis spectrophotometer with 1 cm matched quartz cells was used for the measurement of the absorbances. Shimadzu -AX -200 electronic balance was used for weighing the samples.

Method development

Selection of analytical wavelength was done by diluting the stock solution (5 mg in 50 mL methanol) of MET to obtain 100 $\mu\text{g/mL}$ concentration. They scanned in the wavelength range of 400-200 nm from the overlain spectra and wavelength 233 nm was selected for the construction of calibration curve. The series of different concentration range of (8-13 $\mu\text{g/mL}$) for MET were prepared from the working standard solutions. The absorptivities ($^{1\%}_{1\text{cm}}$) of the drug at the wavelength 233 nm were determined. The calculated values were the mean of 6 independent determinations. The validity was checked by accordance the parameters including-system suitability, specificity, limit of quantification, limit of detection, linearity of response, accuracy, precision (reproducibility & repeatability), robustness (change of wave length ± 2 nm).

Sample preparation

20 Tablets were weighed and finely powdered. About 50 mg equivalent of MET was transferred in to 250 mL volumetric flask added with about 50 mL of methanol and sonicated it for 10 min and the volume was made up with methanol. The solution was filtered and diluted 5 mL of filtrate to 100 mL with methanol and subjected for UV detection. Absorbance of sample and standard preparation were measured at 233 ± 2 nm using methanol as blank.

Results and Discussion

The proposed UV-spectrophotometric method requires fewer reagents and materials and it is simple and less time saving. This method could be used in quality control tests in pharmaceutical industries. The calibration curve of standard MET is shown in Figure 1 and their assay data is given in Table 1. The values demonstrated the suitability of the system for the analysis of the MET (Figure 2). Optical parameters were within standard range during routine performance of the method. The summary of the validation parameters results are shown in the Table 2. The response for the detector was determined to be linear over the range of 8-13 $\mu\text{g/mL}$. The proposed method was evaluated for its correlation coefficient and intercept value calculated in the statistical study. They were represented by the linear regression equations;

Figure 1. Linernity

Figure 2. System suitability for metformin HCl

Figure 3. Specificity for metformin HCl

Table 1. Assay of metformin HCl

S.No	Weight of tablet powder, mg	Sample absorbance	Assay %
1	72.9	0.276	98.68
2	72.8	0.277	99.17
3	70.9	0.276	101.46
4	71.3	0.276	100.89
5	73.4	0.278	98.72
6	71.8	0.277	100.55
Mean			99.91
Standard deviation			1.21
RSD			1.207

Table 2. Analytical method validation

Validation Parameter	MET
Recovery, %	99.91
Intraday precision, %RSD	1.312
Interday precision, %RSD	0.093
Linearity (r^2)	0.99996
Robustness, %RSD*	1.55
LOD, $\mu\text{g/mL}$ *	1.0 μg
LOQ, $\mu\text{g/mL}$ *	3.0 μg
Specificity	Specific

*LOD is the limit of detection while *LOQ is limit of quantification, *RSD is relative standard deviation and r^2 is correlation coefficient

Where 'r' value=0.99996, Slopes and intercepts were obtained by using regression equation ($y=mx + c$) and least square treatment of the results were used to confirm linearity of the method developed. The accuracy of method was determined by recovery experiments. The recovery studies were carried out 9 times and the percentage recovery and percentage relative standard deviation were calculated. From the data obtained, recoveries of standard drugs were found to be accurate (Table 3 & Figure 3).

Table 3. Accuracy for metformin HCl

S.No	%Recovery/ concentration	Placebo weight, mg	Sample weight, mg	Sample absorbance	% Recover
1	80	22.2	40.6	0.722	98.92
2	80	22.1	40.2	0.721	99.77
3	80	22.3	40.1	0.721	100.01
4	100	22.1	50.6	0.904	99.38
5	100	21.9	50.8	0.905	99.10
6	100	22.1	50.4	0.904	99.77
7	120	22.4	60.1	1.080	99.96
8	120	22.3	59.7	1.082	100.82
9	120	22.4	59.9	1.083	100.57
Mean					0.99810
Standard deviation					0.00627
RSD					0.62823

(Standard weight = 50.5 mg. & Standard absorbance = 0.904)

The intra-day and inter-day precision for MET were 1.312 and 0.093. From the data obtained, the developed UV-spectrophotometric method was found to be precise and accurate. Recovery studies were satisfactory, which showed that there is no interference of excipients. The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined for a series of progressive concentration of standard solution. The LOD is the lowest concentration of the analyte that can be detected with signal to noise ratio (1:3) and LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy with signal to noise ratio (1:10). The LOD of MET was 1.0 $\mu\text{g/mL}$ & LOQ of MET was 3.0 $\mu\text{g/mL}$. The robustness of the method was studied by deliberate changes in the method, like percentage organic content, alteration in pH of the mobile phase and changes in the wavelength. It was observed that there were no marked changes in the absorbance, which

demonstrated that the UV-spectrophotometric method developed is robust¹⁰. This method is simple, specific and easy to perform and requires short time to analyze the samples. Low limit of quantification and limit of detection makes this method suitable for use in quality control testing.

Conclusion

In present study, we found that the developed method for metformin HCl found to be accurate, precise, linear, robust and rugged.

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