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UV Spectrophotometric Method Development and Validation for Metformin Hydrochloride in Bulk and its Tablet Formulation

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Abstract
A simple, economic, sensitive, precise and accurate UV Spectrophotometric method was developed and validated for quantification of metformin hydrochloride in bulk and in tablet dosage form. Adequate drug solubility and maximum assay sensitivity was found in pH 7.4 buffer solution at 235nm. Calibration graph constructed at 235nm was linear in concentration range of 2-10µg/ml with correlation coefficient of 0.9995. The method was validated as per ICH guidelines in terms of linearity (within 2-10µg/ml), accuracy (% recovery), precision (inter-day and intraday), specificity and robustness. Therefore, the proposed method is suitable and can be adopted for the determination of Metformin hydrochloride from pharmaceutical dosage form in routine quality control analysis.
Keywords: UV Spectrophotometry, metformin hydrochloride, anti-diabetic, accuracy.

1. Introduction

Metformin hydrochloride is an anti-diabetic drug. Metformin hydrochloride, USP 2.0 g is soluble in 20 mL of water. The pKa value of metformin hydrochloride is 12.4. The pH of a 1% aqueous solution of metformin hydrochloride is 6.68. It is freely soluble in water; slightly soluble in alcohol; practically insoluble in acetone and in methylene chloride. The chemical formula is $C_4H_{11}N_5$. HCl, pKa value is 12.4. IUPAC name of metformin hydrochloride is 1-carbamimidamido-N,N-dimethyl methanimidamide. Molecular weight is 165.62g/mol [1-3].

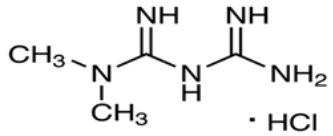

Figure 1: Structure of metformin hydrochloride

Metformin mechanism of action is unique from other classes of oral anti- hyperglycemic drugs. Metformin decreases blood glucose levels by decreasing hepatic glucose production (also called gluconeogenesis), decreasing the intestinal absorption of glucose, and increasing insulin sensitivity by increasing peripheral glucose uptake and utilization. Activated AMPK phosphorylates two isoforms of acetyl-CoA carboxylase enzyme,thereby inhibiting fat synthesis and leading to fat oxidation, reducing hepatic lipid stores and increasing liver sensitivity to insulin [4-7].

2. Materials and Methods

Instruments

The present work was carried out with Elico SL 164 UV-Visible spectrophotometer having double beam detector configuration. The absorption spectra of reference and test solution were carried in a 1cm quartz cuvette over the range of 200-800nm.

Chemicals

Metformin hydrochloride (API) Hetero, Metformin hydrochloride tablets Lifecare Neuro Products Limited brand name Glucophage and Distill water. All chemicals are analytical grade only.

Preparation of standard: Accurately weigh approximately 100 mg of metformin hydrochloride (API), transfer it into a 100 ml volumetric flask, and then sonicate it. Pipette 10 ml of the stock solution above, then transfer it into a 100 ml volumetric flask and use the same solvent to make up the volume. A drug's molecular weight, pKa value, solubility, and other characteristics all play a role in the appropriate technique selection. Using a UV-Visible spectrophotometer, the wavelength of maximum absorption (λmax) of a medication in a solvent solution at a concentration of 1 mg/ml was scanned between the 200-400 nm ranges. The λ max was observed at 235nm [8,9].

Preparation of formulation: Weighed precisely, twenty metformin hydrochloride tablets; transferred, crushed, and ground using a pestle and mortar. Weigh off the equivalent of 100 mg of powder from this, transfer it into a 100 ml volumetric flask, dissolve it with distill water, sonicate the solution for 15 min, filter it, and add water to make up the remaining volume. Next, the solution is run through the UV spectrophotometer, namely the 200-400 nm range. The λmax was observed at 235nm [10-12].

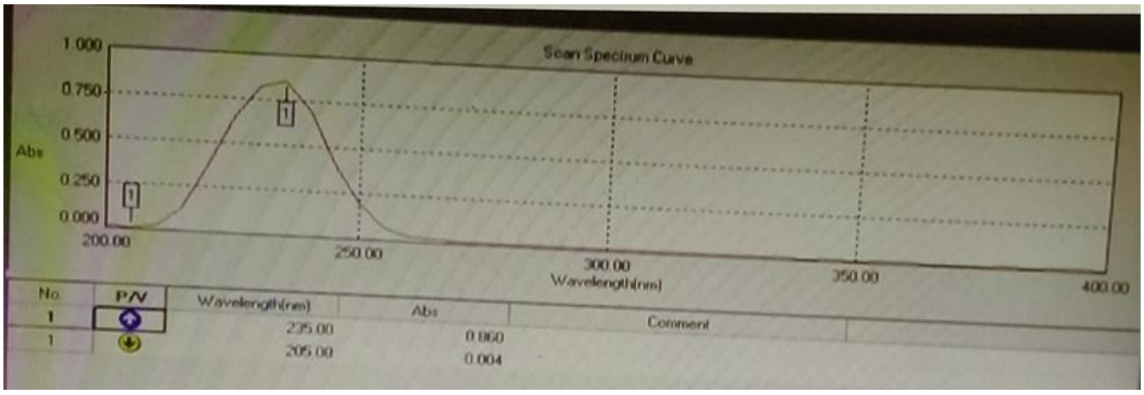

Figure 2: The λ_{max} of metformin hydrochloride

Assay

The maximum absorbance was observed at 235nm, Figure 2, the observances was measured for the Metformin hydrochloride and calculated the assay using following formula.

$$\% \text{ Assay} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \frac{\text{Wt.of Std.}}{\text{Dilution of Std.}} \times \frac{\text{Wt. of Sample}}{\text{Dilution of Sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Wt. of Tablet}}{\text{Lable claim}} \times 100$$

Table 1: Assay of metformin hydrochloride formulation

	Amount of tablet (mg)		% Label claim	%RSD
Metformin hydrochloride	**Labeled**	**Found**		
	500	501	100.20	0.95

Linearity

The method's linearity was performed across a concentration range of 2-10µg/ml of the intended concentration. A precisely weighed 100 mg pure drug was added to a 100 ml dry volumetric flask, which was then cleaned, dried, and filled with a little volume of water to make the volume reach 100 ml. As a result, the drug concentration (Stock solution-I) was 1000µg/ml. From here, 10 ml of the solution were pipette out into a 100 ml volumetric flask, and

distilled water (Stock solution-II, 100µg/ml) was added to bring the volume up to the mark. Concentrations 2, 4, 6, 8, and 10µg/ml were prepared from above prepared Stock solution-II, calibration curve was plotted and the correlation coefficient was calculated. The acquired absorbance readings are plotted against the metformin hydrochloride concentration to create the calibration graph. Correlation coefficient of the linearity was found for method and reported in Table 2.

Limit of detection (LOD)

LOD for Metformin hydrochloride by the proposed method was determined on the response and slope of the regression coefficient.

Limit of quantization (LOQ)

Limit of quantization for Metformin hydrochloride by the proposed method was determined on the response and slope of the regression coefficient.

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 homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation.

Intra and inter-day precision

A variation of results within the same day (intraday), variation of results between days (inter day) was analyzed. Intra-day precision was determined by analyzing Metformin hydrochloride for five times in the same day at 235 nm. Inter day precision was determined by analyzing drug daily once for five days at 235 nm.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. The recovery technique was performed to judge the accuracy of the proposed method. For this, known quantities of the Metformin hydrochloride solution were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed. The total amount of metformin hydrochloride was determined by using the proposed method and the amount of added drug was calculated by the difference.

Ruggedness and robustness

The solutions were prepared and analyzed with change in the analytical conditions like different laboratory conditions and different analysts [12-14].

3. Results and Discussion

Linearity

The linearity was done across a concentration range of 2-10µg/ml of the intended concentration. A precisely weighed 100 mg pure drug was added to a 100 ml volumetric. Concentrations 2, 4, 6, 8, and 10µg/ml were prepared from above prepared Stock solution calibration curve was plotted and the correlation coefficient was calculated. The acquired absorbance readings are plotted against the Metformin hydrochloride concentration to create the calibration graph**.** Correlation coefficient is less than 2 and the linearity was found for method reported in Table 2.

Table 2: Linearity of metformin hydrochloride at 245 nm & statistical data of the regression equation

S. No.	Concentration (µg/ml)	Absorbance	Metformin hydrochloride	
1	2	0.177	Parameters	235 nm
2	4	0.229	Std. dev.	0.154
3	6	0.282	Correlation	0.999
4	8	0.337	Slope	0.027
5	10	0.395	Y- intercept	0.120

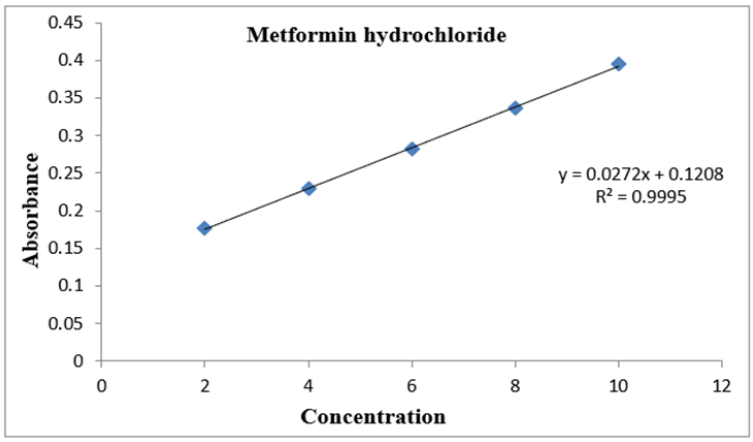

Figure 3: Calibration curve of metformin hydrochloride

Limit of detection

LOD for Metformin Hydrochloride by the proposed method was determined on the response and slope of the regression coefficient.

$$\mathbf{LOD = 3.3 \times \sigma / S}$$

Where, σ = Standard deviation,
S = Linearity curve slope.

Limit of quantization

Limit of quantization for Metformin Hydrochloride by the proposed method was determined on the response and slope of the regression coefficient.

$$\mathbf{LOQ = 10 \times \sigma / S}$$

Where, σ = Standard deviation,
S = Linearity curve slope.

Table 3: LOD & LOQ values of metformin hydrochloride

LOD (µg/ml)	0.74
LOQ (µg/ml)	2.25

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 homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation.

Intra and inter-day precision

A variation of results within the same day (intraday), variation of results between days (inter day) was analyzed. Intra-day precision was determined by analyzing Metformin Hydrochloride for five times in the same day at 235 nm. Inter day precision was determined by analyzing drug daily once for five days at 235 nm.

Table 4: Precision of metformin hydrochloride

Conc. (µg/ml)	**Inter day**			**Intra day**		
	Absorbance Mean	**SD**	**%Assay**	**Absorbance Mean**	**SD**	**%Assay**
2	0.085	1.39	98.79	0.085	1.30	99.99
4	0.160	1.30	101.98	0.166	1.35	100.98
6	0.238	1.35	101.59	0.230	1.40	101.59
8	0.327	1.45	101.00	0.317	1.35	101.59
10	0.399	1.30	101.05	0.390	1.40	101.13

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. The recovery technique was performed to judge the accuracy of the proposed method. For this, known quantities of the Metformin hydrochloride solution were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed. The total amount of Metformin hydrochloride was determined by using the proposed method and the amount of added drug was calculated by the difference.

Table 5: Accuracy of metformin hydrochloride

Sample (%level)	Amount Taken (µg/ml) (Sample)	Amount Added (µg/ml) (API)	Amount Recovered (µg/ml)	%Recovery	Mean
80	5	4	5.75	98.99	
80	5	4	5.72	99.58	99.45%
80	5	4	5.73	99.80	
100	5	5	7.12	102.00	
100	5	5	7.15	102.50	102%
100	5	5	7.09	101.50	
120	5	6	8.33	101.80	
120	5	6	8.36	102.20	102.21%
120	5	6	8.39	102.63	

The solutions were prepared and analyzed with change in the analytical conditions like different laboratory conditions and different analysts.

Table 6: Ruggedness of metformin hydrochloride

S. No.	Assay (% of claim) metformin hydrochloride	
	Analyst 1	**Analyst 2**
1	98.79	99.99
2	101.98	100.98
3	101.59	101.59
4	101.00	101.59
5	101.05	101.13
Mean	**101.05**	**101.05**
SD	**1.397**	**1.316**
RSD	**0.96**	**0.91**

The optimum conditions for UV-spectroscopy method have been established by varying the parameters one at a time and keeping the other parameters fixed and observing the effects of products on the absorbance of the sample and colored species. Beer's law limits, molar Absorptivity, Sandal's sensitivity, %range of error and %relative standard deviation is summarized in Table 2. The regression analysis using the method of least squares was made for the slope (b), intercept (a) and correlation coefficient (r^2=0.999) obtained from different concentrations are given in Table 2. The results showed that the method have reasonable precision. To evaluate the validity and reproducibility of the methods, known amounts of pure drug were added to the previously analyzed pharmaceutical dosage forms and the mixtures were analyzed by the proposed methods. The percentage recoveries are given in Table.5. The interference studies veiled that the common excipients and other additives that are usually present in the injection dosage forms did not interfere at their regularly added levels.

4. Conclusions

Based on the aforementioned findings, the procedure outlined in this work for determining the amount of metformin hydrochloride in tablet formulation is simple, precise, repeatable, and sensitive. The suggested method might be used in quality control labs for routine analysis. According to ICH guidelines, the developed method was validated.

Expert analysis and scholarly investigation in daily life. The test content result for pharmaceutical formulation closely matches the drug label claim.

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