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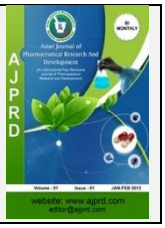

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Research Article

UV Spectroscopic Method Development and Validation for the Estimation of Metformin HCL in Pure and Its Marketed Tablets

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ABSTRACT

The present research study was to Ultra Violet spectroscopy (UV) method develop and validatefor the quantification of model anti-diabetic drug Metformin HCl (MET) in pure formand marketed tablets. Different solvent blends were screened blend comprising of Acetonitrile: Methanol: DW at 1:1:1was selected and same was used for study. The validation is done as per ICH guidelines, validated for their accuracy, precision and robustness of the chosen solvent blend. The results show the linearity in the concentration range (1-50 µg/mL) with correlation coefficient R^2 = 0.9989 with percent recovery (100 – 101.5 %) with RSD was less than 2%, LOD and LOQ 0.0433, 0.132µg/mL respectively. This method can show high quality and there is no significant interference with the excipients and in pharmaceutical forms. None of the studies showed the development of this method before.

Key words: Diabetes mellitus; Metformin HCl;UV spectroscopy; ICH; Validation

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INTRODUCTION

Analytical methods are systematic approaches for examining and interpreting data, aiding researchers and professionals in making informed decisions. These methods work by breaking down complex information into smaller, more manageable components, allowing for a deeper understanding of various phenomena. Various analytical methods viz., UV, HPLC, LC-MC, Visible, Potentiometric, Electrophoresis etc., were used for systemic analysis of active pharmaceutical ingredient in bulk and its formulations.UV spectroscopy is an analytical technique used to measure the absorption of ultraviolet light by a substance to reveal its chemical composition. As UV light passes through sample, specific wavelengths are absorbed based on the molecular structure of the substance. This absorption produces a unique spectrum, aiding in the identification of compounds and concentration analysis. Widely applied in chemistry, biology, and pharmaceuticals, UV spectroscopy is vital for quality control, molecular analysis, and impurity detection. Its precision and non-destructive nature makes it invaluable for both research and industrial purposes. Keeping these points into consideration, the present research work was planned to develop and validate a simple, economic UV method for model antidiabetic drug Linagliptin.

Diabetes mellitus (DM) is a chronic disease affecting millions worldwide, with projections indicating a continued rise in prevalence, particularly in low- and middle-income countries. The disease occurs when the pancreas doesn't produce enough insulin or the body can't effectively use the insulin it produces, leading to elevated blood sugar levels. This can result in serious health complications including heart attacks, stroke, kidney failure, blindness, and limb amputation. The International Diabetes Federation (IDF) estimates that 537 million adults (20-79 years) currently live with diabetes, with a prevalence rate of 10.5%. Projections show this number rising to 783 million by 2045, according to the IDF.Type 2 diabetes, often associated with lifestyle factors, is the most common form, accounting for 90-95% of all cases.India is estimated to have 77 million adults with diabetes and 25 million prediabetics. The disease is a leading cause of death and disability in India. Diabetes mellitus

treatment focuses on managing blood sugar levels to prevent complications. This often involves a combination of lifestyle changes, medication, and potentially insulin therapy, depending on the type and severity of diabetes. Type 1 Diabetes was treated with insulin therapy, there are different types of insulin, including rapid-acting, short-acting, intermediate-acting, and long-acting.Type 2 Diabetes was treated withvarious oral medications Biguanide viz., Metformin, SGLT-2 inhibitors, DPP-4 Inhibitors viz., Sitagliptin, Linagliptin, these medications help lowering blood sugar levels.

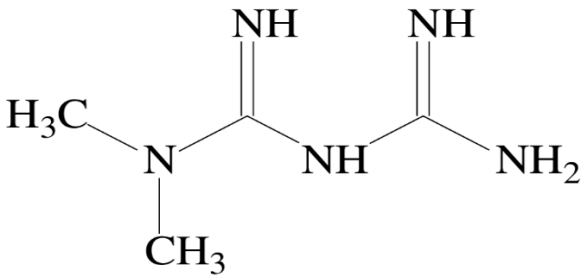

Figure 1: Chemical structure of MET

MET is a white to off-white crystalline powder, has melting point of 223-226 °C, soluble in water, Methanol, Acetonitrile and varied buffer pH, insoluble DMSO. Chemically it is 1-carbamimidamido-N, N-dimethylmethanimidamide hydrochloride (figure1).

MET is a biguanide antihyperglycemic agent and first-line pharmacotherapy used in the management of type II diabetes, which 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[1].It is well established that metformin inhibits mitochondrial complex I activity, and it has since been generally postulated that its potent antidiabetic effects occur through this mechanism[2-3].MET isn't available as a specific method for analyzing in British pharmacopeia (BP) or the United States Pharmacopeia (USP). The review of research literature has shown several articles for the determination of MET in pharmaceutical forms viz., LC[4], HPLC[5-8], TLC[9], RP-HPLC[10-15], UV/VIS[16], UPLC[17-18], Spectrophotometric[19]. In the present study a simple, precise, economic UV method comprising solvent blend for the development, validation and estimation of MET in bulk and marketed formulations.

MATERIALS AND METHODS

Materials

Instruments: Shimadzu UV-1900 Spectrophotometer connected to compatible computer with UV probe software, Anamed digital balance, Bath sonicater

Chemicals: Metformin HCl gift sample provided by Magnus Pharma Ltd, Nepal, Acetonitrile, Methanol were procured from SD Fine Chemicals Mumbai, Distilled water. All chemicals used were of analytical grade.

Marketed tablets: Glycomet 500mg; Glyciphage 500mg

Methods

Screening of solvents: The ideal solvent blend was selected based on the solubility and stability of the model drug MET. The solvents comprising Acetonitrile, Methanol, DMSO, DMF, Distilled water, pH 1.2, pH 2, Phosphate buffer pH 6.8, Phosphate buffer pH 7.2 alone and in combination at different ratios were studied. The ideal solvent blend comprising Acetonitrile: Methanol: Distilled water at 1:1:1 was selected and used throughout the study.

Preparation of Stock solution (1000µg/ml): Accurately weighed 50 mg of MET was transferred into 50 ml volumetric flask, which is previously containing 40 ml of solvent blend under the study. The mixture was sonicated for 30 min and cyclomixing for 10 min to get clear solution, finally volume was adjusted to 50 ml with solvent blend.

Preparation of working standard solution (100 µg/ml):5 ml of stock solution was accurately measured with calibrated pipette and transferred in 50 ml volumetric flask and adjust the volume with solvent blend.

Preparation of standard solution for the determination of Absorption maxima: 1 ml of working standard solution was transferred into 10 ml volumetric flask; volume was adjusted to 10 ml with solvent blend to get 10 µg/ml.

Preparation of standard solutions for the determination of linearity range: The working standard solution was appropriately diluted with solvent blend in a series of 10 ml volumetric flask to obtain series of concentrations viz., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 24, 28, 30, 35, 40, 45 and 50µg/ml.

Preparation of standard solutions for the preparation of calibration curve: The working standard solution was appropriately diluted with solvent blend in a series of 10 ml volumetric flask to obtain series of concentrations viz., 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µg/ml.

Preparation of sample solutions from marketed tablets: Two brands viz., Glycomet 500mg; Glyciphage 500mg., tablets were selected for the study. In each case 10 tablets were weighed and crushed into fine powder, powder equivalent to 25 mg of MET was extracted with solvent blend under the study for 2 hr, sonicate for 30 min. The contents were filtered through Whatmann no 45 filter paper, the filtrate was appropriately diluted with solvent blend to obtain a series of sample concentrations under the study. These solutions were further used for assay and % recovery studies.

Method development

Absorption maxima (λ max): The standard solution i.e., 10 µg/ml was scanned in the range of 200 to 400 nm using UV spectrophotometer and note the characteristic peak at specific wave length and consider as absorption maxima.

Linearity range: The absorbance of standard solutions under linearity range study viz., concentration ranges1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 24, 28, 30, 35, 40, 45 and 50 µg/ml at obtained λ max. The studies were carried out in triplicate and appropriate curve was plotted viz., concentrations vs absorbance.

Calibration curve: The absorbance of standard solutions under linearity range study viz., concentration ranges1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µg/ml at obtained λ max. The studies were carried out in triplicate and appropriate curve was plotted i.e., concentrations vs absorbance and relevant data viz., Molar absorptivity, Sandells constant, Slope, Correlation coefficient, Regression coefficient and best fit data were generated using Graph Pad Prism V8 software.

Limit of detection (LOD) and Limit of quantitation (LOQ): The working standard solution was appropriately diluted with solvent blend in a series of 10 ml volumetric flask to obtain series of lowest concentrations, and measure the absorbance. The LOD and LOQ were determined based on standard deviation (SD) of response and slope (S) by using the following equations

LOD=3.3xσ/S ; LOQ=10xσ/S

Where, σ is standard deviation; S is slope

Method Validation: The proposed method was validated as per ICH Q2 guideline, the parameters viz., Precision, Accuracy, Robustness, Ruggedness, Method suitability and System suitability.

Precision: Precision was studied in terms of repeatability, reproducibility, Interday and Intraday analysis of solvent blend under the study. In each case dilute the primary stock solution appropriately in a series of 10 ml volumetric flask to obtain 10 and 16µg/ml. For the system suitability six replicates of same concentrations were measured and determine the % recovery. For intermittent precision, the solutions were measured in the same day at different intervals of time and subsequent days for 4 days, determined the % recovery in each case. All the results were interpreted in terms of % RSD.

Method suitability: For these studied the solvent blend under the study was checked for their suitability at various storing temperature. In each case dilute the primary stock solution appropriately in a series of 10 ml volumetric flask to obtain 10 and 16µg/ml. The solutions were stored at different temperature viz., at 25°C, 40°C and 2-4°C.Measure the absorbance of the solutions and determined the % recovery in each case, interpreted the results in terms of % RSD.

Robustness: The solvent blend under the study was test for suitability under varying conditions. In each case the primary stock solution was appropriately diluted with solvent blend in a series of 10 ml volumetric flask to obtain 10 and 16µg/ml. At changed nm viz., ± 2 nm, ±5 nm, the absorbance was measured and determined the % recovery and interpreted the results in terms % RSD.

Ruggedness: The ruggedness of solvent blend under the study was determined by varying the conditions viz., different instruments and different analyst. Express the lack of influence on the results of operational conditions.In each case the primary stock solution was appropriately diluted with solvent blend in a series of 10 ml volumetric flask to obtain 10 and 16µg/ml. The absorbance of these solutions were measured by two analysts and two instruments. In each case % recovery was determined and results were interpreted in terms % RSD.

Assay: The marketed sample solutions were appropriately diluted with solvent blend under the study. The labelled claim of the MET in marketed tablets were determined in terms of % recovery and % RSD.

Accuracy: Accuracy was studied in terms of % recovery by standard addition method. In each case known amount of LIN (50 %, 100 % and 150 %) spiked on fixed concentrations of MET marketed sample solutions in a series of 10 ml volumetric flask. Measure the absorbance of the solutions and determined the % recovery in each case, interpret the results in terms of % RSD.

RESULTS AND DISCUSSION

The analysis of the results was discussed on three levels viz., the absorption spectra, Validation of the developed method and estimation in bulk and formulations.

The absorption spectra of LNG (10 µg/ml) was recorded on a UV-vis spectrophotometer in the wavelength region of 200-400 nm, which shows the absorption maxima with characteristic peak at 298 nm (figure 2).

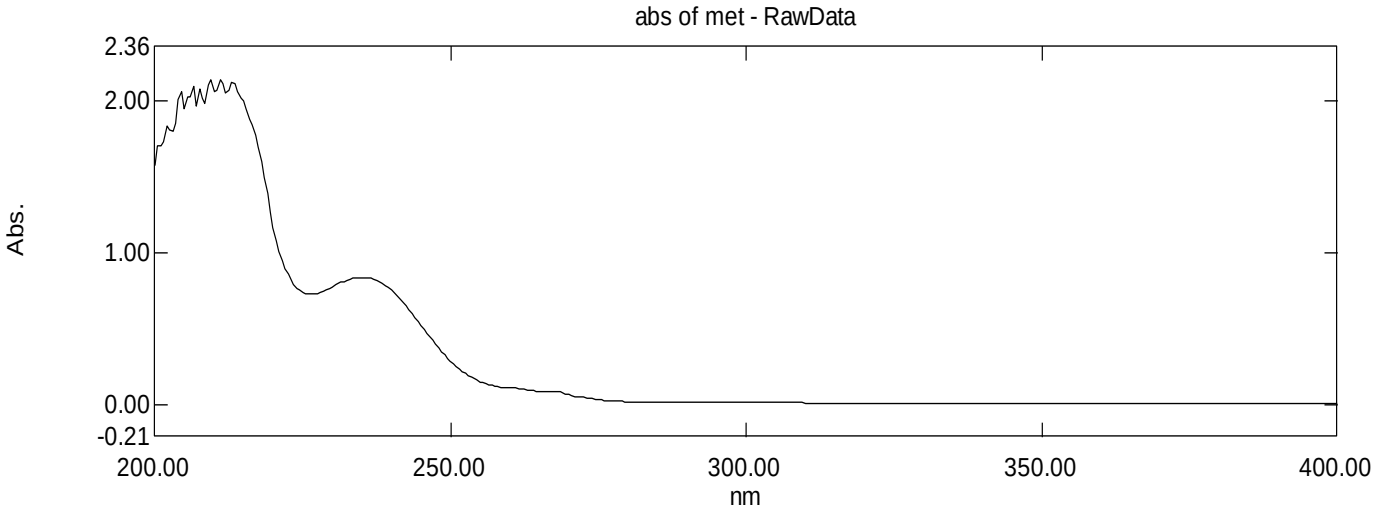

Figure 2: Absorption maxima of MET

Table 2: Linearity range data of MET in solvent blend

Concentration in (µg/ml)	Mean ± SD n=6
1	0.044±0.00058
2	0.088±0.00086
3	0.132±0.00086
4	0.177±0.00050
5	0.220±0.00050
6	0.264±0.00086
7	0.308±0.00050
8	0.352±0.00086
9	0.397±0.00087
10	0.440±0.00086
12	0.528±0.00086
14	0.616±0.00087
16	0.704±0.00088
18	0.792±0.00057
20	0.880±0.00086
24	1.056±0.00086
28	1.232±0.00057
30	1.320±0.00086
35	1.555±0.00282
40	1.760±0.00021
45	2.016±0.00144
50	2.201±0.00057

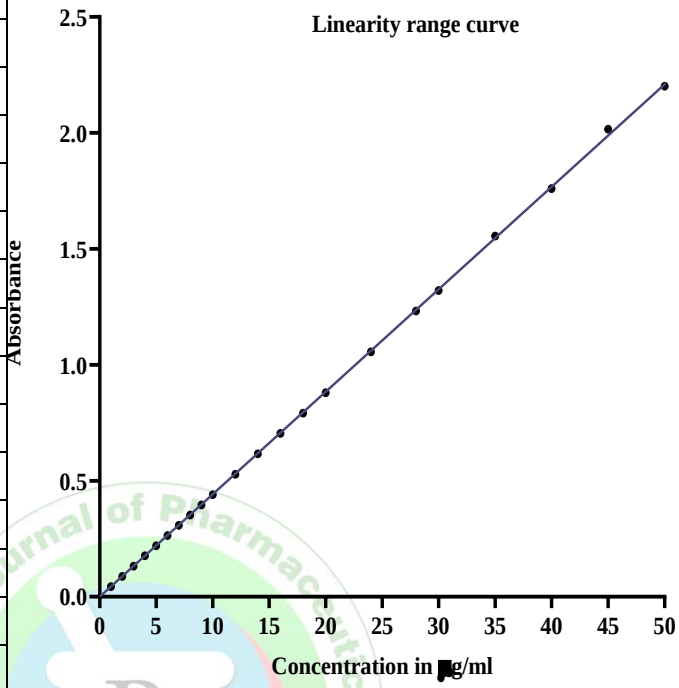

Figure 3: Linearity range curve of MET in solvent blend

The linearity range and calibration curve of the MET was studied in solvent blend at 235 nm and same were given table 1,2 and figures 3,4. The range was found in the region of 1-50 µg/ml and calibration curve is prepared in concentrations from 2-20 µg/ml. The response of the LIN in calibration curve was linear in the investigation range, showed regression equation Y = 0.04400*X- 0.0004with a goodness of fit correlation coefficient, R^2=0.9989, where y represents absorbance and x represents concentration (µg/ml)and validated with intercept response < 2%. which was calculated by least square method indicate functional linearity between the concentration of analyte and the absorbance.The LOD and LOQ values was calculated based on standard deviation of the response and the slope of the solutions, it was found to be 0.0433 ± 0.019 µg/ml; 0.13131± 0.019µg/ml for LOD and LOQ with % RSD values less than 2.

Table 3: Calibration curve data of MET in solvent blend

Concentration in (µg/ml)	Mean ± SD n=6
2	0.088±0.0005000
4	0.177±0.0005000
6	0.264±0.0008165
8	0.352±0.0008165
10	0.528±0.0005000
12	0.704±0.001258
16	0.88±0.0005000
20	0.088±0.0005000

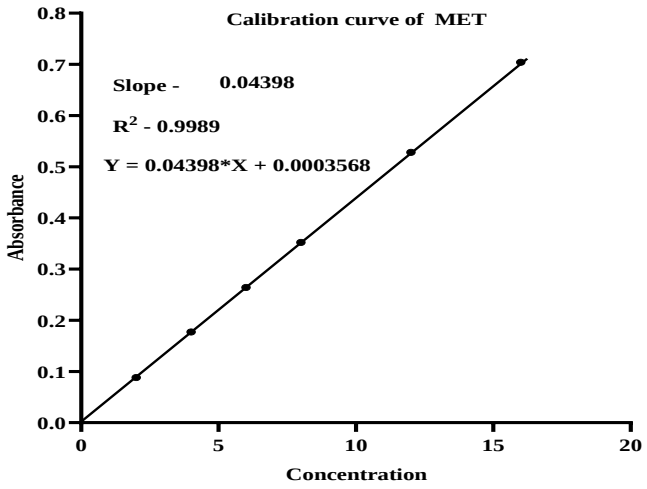

Figure 4: Calibration curve of MET in solvent blend

The precision study in terms of repeatability, Intra-day and Inter-day precision were carried out and was given in table 4,5. The precision (measurement of inter-day, intra-day repeatability) results showed good reproducibility with the % RSD below 2 indicated that the method was highly precise.

The stability in terms of method suitability was conducted to check the stability of solvent blend under different storage conditions, the data was given table 6. The results indicated the solvent blend was stable over the period of 4 days at specified storage condition.

The robustness and ruggedness study was studied to check the influence of small variations in experimental conditions, instrument to instrument and analyst to analyst. The data was given in table 7, the results indicated the solvent blend under the study was highly robust and can be successfully rugged at varied conditions

Table 4: Repeatability data of MET in solvent blend

Labelled claim µg/ml	Absorbance	Amount Recovery	% Recovery	Mean ± SD	% RSD
10	0.441	10.02	100.2	100.1±0.1033	0.1032
10	0.44	10	100		
10	0.44	10	100		
10	0.441	10.02	100.2		
10	0.44	10	100		
10	0.44	10	100		
16	0.704	16	100	100.0±0.08165	0.08162
16	0.704	16	100		
16	0.704	16	100		
16	0.704	16	100		
16	0.705	16.02	100.2		
16	0.704	16	100		

Table 5: Intraday and Interday data of MET in solvent blend

Period	Labelled claim µg/ml(n=6)	Amount Recovery	% Recovery Mean ± SD	% RSD
Intra day	10	10.06	100.6±0.3512	0.3492
	16	16.02	100.1±0.1250	0.1249
Inter day	10	10.02	100.2±0.3464	0.3457
	16	16.30	100.2±0.0751	0.0749

Table 6: Method stability data of MET in solvent blend

Stability conditions	Labelled claim µg/ml (n=6)	Amount Recovery	% Recovery Mean ± SD	% RSD
Room temperature	10	10.04	100.4±0.2000	0.1992
2-8°C	10	10.05	100.5±0.1155	0.1149
40°C	10	10.02	100.2±0.2000	0.1997
Room temperature	16	16.02	100.1±0.1250	0.1249
2-8°C	16	16.06	100.3±0.0693	0.0690
40°C	16	16.04	100.2±0.0693	0.0691

Table7: Robustness and Ruggedness data of MET in solvent blend

Conditions	Labelled claim µg/ml (n=6)	Amount Recovery	% Recovery Mean ± SD	% RSD
Robustness				
+ 2 nm	10	10.15	101.5±0.6937	0.6789
At 235 nm	10	10.03	100.1±0.1033	0.1032
- 2nm	10	9.78	97.70±0.8185	0.8378
+ 5 nm	10	10.23	101.2±0.1859	0.1464
At 235 nm	10	10.01	100.0±0.0816	0.0815
- 5 nm	10	9.829	101.2±0.1859	0.1464
Ruggedness				
Analyst -1	10	10.15	101.5±0.2517	0.2479
Analyst-2	10	10.02	100.2±0.1250	0.1249
Instrument-1	10	10.04	100.4±0.4041	0.4024
Instrument-2	10	9.95	99.50±0.1850	0.1860

Table 8: Assay and % recovery data of MET

Brand Name	Labelled Claim µg/ml	Amount Recovery µg/ml	% recovery	Mean ± SD	% C.V
Glyciphage 500mg	10	10.2	102	100.4±1.365	1.359
	16	15.84	99	99.16±0.2987	0.3028
Glycomet 500mg	10	9.95	99.5	99.450±0.1931	0.1949
	16	15.77	98.56	98.70±1.281	1.285

Table 9:% Recovery data of MET

Brand Name	Prequantified sample(µg)	% of pure drug added	Pure drug added(µg)	Amount recovery µg/ml	% recovery	Mean ± SD	% C.V
Glyciphage500mg	10	50	5	4.97	99.4	99.27±0.2309	0.2326
	10	50	5	4.95	99		
	10	50	5	4.97	99.4		
	10	100	10	10	100	99.67±0.2887	0.2896
	10	100	10	9.95	99.5		

CONCLUSIONS

The proposed method comprising Acetonitrile: Methanol: Distilled water at 1:1:1 was developed and validated. The experimental results conclude that the accuracy and precision results suggest the method was found to be reproducible, recovery data reveal high precision and accuracy of the methods besides being robust and rugged. The proposed method can be conveniently applied to estimate the Metformin HCl in bulk and marketed tablets There fore, the validated method could be useful for routine quality control assay of the studied drug in pure form and pharmaceutical formulations.

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AUTHORS CONTRIBUTION:

The collaborative efforts of all authors have successfully led to the development of a UV spectroscopic method for estimating Metformin HCl in both bulk and commercially available formulations. Rishika Vaishnav andVasavi Yadav, under the mentorship of N. Srinivasulu, conducted the development and validation of the UV spectroscopic technique for Metformin. Y. Anand Kumar provided guidance in crafting the manuscript and structuring the research paper in accordance with the journal's guidelines.

Conflict of interests: No conflict of interest

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