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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF METFORMIN HYDROCHLORIDE BY UV SPECTROSCOPY

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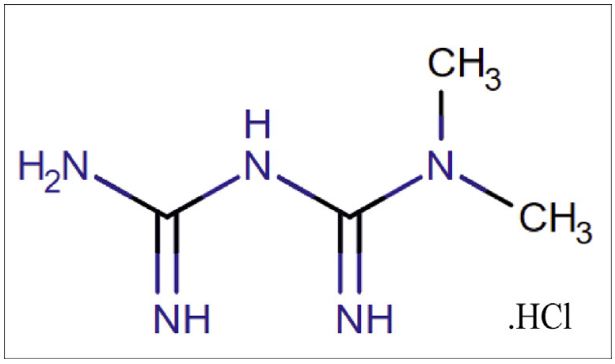
ABSTRACT

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 0.01N sodium hydroxide at 233nm. Calibration graph constructed at 233nm was linear in concentration range of 1-25µg/ml with correlation coefficient of 0.9998. The method was validated as per ICH guidelines in terms of linearity (within 1-25µg/ml), accuracy (% recovery), precision (inter-day and intraday), specificity and robustness. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.2226µg/ml and 0.6745µg/ml respectively. 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.

INTRODUCTION

Metformin Hydrochloride is an oral antidiabetic medication that belongs to the biguanide class. It is commonly used to treat type 2 diabetes mellitus, particularly in patients with insulin resistance. the indications are type 2 diabetes mellitus as monotherapy or in combination with other antidiabetic medications, polycystic ovary syndrome (PCOS)to improve insulin sensitivity and ovulation. The dosage recommended starting dose is 500-850 mg once daily, taken with breakfast. The maximum daily dose is 2550 mg. The half life is 2.5 to 4.5 hours. Brand name: AXPINET, DIAGMET, GLUCIENT, GLUCOPHAGE, METABET. The oral bioavailability is 50 to 60% the absorption half life is 0.5 to 1.5 hours the volume of distrubtion is 1.5 to 4.5 L/kg, metabolism is by hepatic, via glucuronidation and oxidative metabolism, the excretion via primary route of elimination.

STURCTURE



Chemicalname: 1, 1-dimethylbigunidehydrochlorid

Molecularformula: C4H11N5.HCL

Molecularweight: 129.164g/mol

Description

A medication used alongside diet and exercise to control blood sugar in patients with type 2 diabetes.

Solubility

Metformin is soluble in water and 95% alcohol, but practically insoluble in ether or chloroform.

Mechanism of action

Metformin primarily lowers blood glucose through non-insulin-dependent mechanisms, reducing hyperglycemia without causing hypoglycemia. It acts through multiple pathways:

- Inhibition of Hepatic Glucose Production:
  - This reduces ATP production and activates AMP-activated protein kinase (AMPK), a key regulator of energy metabolism.
- Increased Insulin Sensitivity:
  - Metformin improves peripheral glucose uptake in muscle and adipose tissue.
  - It enhances GLUT4 transporter expression, increasing glucose utilization.
- Reduced Intestinal Glucose Absorption:
  - Delays carbohydrate absorption from the intestines, contributing to lower postprandial glucose levels.
- Effects on Gut Microbiota:
  - Modulates gut microbiome composition, which may contribute to its metabolic effects.

ADVERSE EFFECTS

- **Common:**
- Gastrointestinal (GI) distress: nausea, diarrhea, bloating, abdominal discomfort.
- Metallic taste.
- **Serious (but rare):**
- Lactic Acidosis (especially in renal impairment, heart failure, or severe infections).

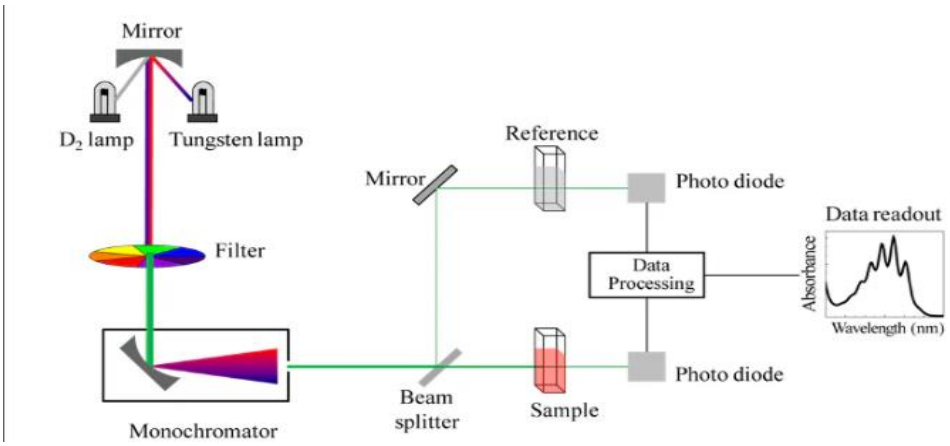
Vitamin B12 deficiency with long-term use.

EXPERIMENTAL WORK

MATERIALS AND METHODS

MATERIALS: All chemicals and reagents were of analytical grade unless stated otherwise. MET standard was a gift from Beximco Pharmaceuticals Ltd, Dhaka. Sodium hydroxide (NaOH) used was purchased from E. Merck, Darmstadt, Germany. Water was deionized and double distilled. Marketed tablet formulations containing 500mg of MET were purchased from local drug stores in Dhaka city after checking their manufacturing license numbers, batch numbers, production and expiry dates.

INSTRUMENTATION



A double beam Shimadzu (Kyoto, Japan) UV Visible spectrophotometer, Model UV mini 1700, equipped with 1 cm quartz cells, with a fixed slit width (1 nm), wavelength accuracy of ±0.5 nm (with automatic wavelength correction) was used. The drug analyses data were acquired and processed using UV Probe software (Version 2.0, Shimadzu, Japan) running under Windows XP on a Pentium PC. For scanning, the wavelength range selected was from 400 nm to 200 nm with medium scanning speed.

Preparation of Standard Solutions: Stock solution of MET was prepared by dissolving 10 mg drug in 100 ml 0.01N NaOH. Several aliquots of standard solutions of MET (100µg/ml) were diluted to get standard solutions across the range of 1-25µg/ml.

Preparation of Sample Solutions: Average weight of MET tablets of each brand was calculated. Then the tablets were grinded to fine powder with the help of mortar and pestle. Then, powder containing 10 mg MET was dissolved in 0.01 N NaOH, shaken for about 10 minutes and filtered through filter paper. The filtered solution was further diluted to make the final concentration of working sample equivalent to 100% of target concentration (10µg/ml).

Determination of λmax: Standard solution containing 10µg/ml of MET was scanned using 0.01N NaOH as blank in the range of 200-400 nm to determine the wavelength of maximum absorption (λmax) of the drugs. MET showed absorbance maxima at 233 nm.

Development of Equation for Assay: 10 ml of the stock solution was diluted to 100 ml with 0.01N NaOH to obtain a 10 µg/ml MET reference standard solution. The absorbance of the sample solutions and the reference standard solution was measured at 233 nm using 0.01N

NaOH as blank. The amount of MET per tablet and respective potency (%) in the marketed brands were determined using the following equations-

Amount of MET (mg) per tablet,

$$Z = \frac{A}{As} \times \frac{Ws}{100 \times 10} \times \frac{100 \times 10}{W} \times Wt \times \frac{P}{100}$$

Potency; (%) =

$$\frac{Z}{Wc} \times 100\% = \frac{A}{As} \times \frac{Ws}{100 \times 10} \times \frac{100 \times 10}{W} \times Wt \times \frac{P}{100} \times \frac{100}{Wc}\%$$

Where, A= absorbance of sample solution; As= absorbance of reference standard solution; Ws= weight of reference MET powder (mg); W = weight of generic powder sample (mg); Wt= average weight of tablet (mg); Wc= weight of drug claimed per tablet (mg), P = potency of reference MET powder.

Method Validation

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for spectroscopic determination of MET in solid dosage form. The method was validated for the parameters like linearity, accuracy, precision and robustness as per ICH guidelines.

Specificity: Specificity of the method was determined by comparing the spectrum of standard MET with that of market product.

Linearity Study: The linearity of an analytical method is its ability to elicit that test results are proportional to the concentration of analyte in samples within a given range. This was determined by means of calibration graph using increasing amounts of standard solutions (1-25µg/ml). Calibration curves were constructed and the proposed method was evaluated by its correlation coefficient and intercept value calculated in the corresponding statistical study. Characteristic parameters for regression equation of the method were obtained by least squares treatment and of the results and these parameters were used to confirm the good linearity of the method.

Intraday and Inter-day Precision Study: Intraday precision (reproducibility) was determined by performing three repeated analysis of the five standard MET solutions (1, 2, 10, 20, 25µg/ml) on the same day, under the same experimental conditions. Inter-day precision (ruggedness) of the method was assessed by carrying out the analysis of standard solutions on three different days in the same laboratory. Measurement of absorbance was in triplicate manner and the mean, standard deviation and relative standard deviation (% RSD) was determined in order to assess the precision of the method.

Accuracy Study: This study was carried out using preformulated placebo granules prepared according to a common formulation. 8 mg, 9 mg, 10 mg, 11 mg, and 12 mg pure MET powder were then transferred respectively in to five 100 ml volumetric flasks and fixed amount of placebo granules were added to each of the volumetric flasks. These were then dissolved and then diluted up to 100 ml with 0.01N NaOH. Same dilution pattern as sample solution was followed to obtain five concentrations, 80%, 90%, 100%, 110%, and 120% of sample solution respectively. The solutions were then analyzed for the content of MET using the proposed method with reference solution (10µg/ml of pure Metformin hydrochloride). All analyses were carried out in triplet.

Robustness Study: The robustness of the method was assessed by altering the solvent composition of the experiment.

Limit of detection (LOD) and Limit of quantification (LOQ): Limit of detection (LOD) and Limit of quantification (LOQ) for the assay were calculated using the following equations.

Limit of detection (LOD) and Limit of quantification (LOQ): Limit of detection (LOD) and Limit of quantification (LOQ) for the assay were calculated using the following equations.

LOD= 3.3xSo/b and LOQ= 10xSo/b

Where So and b are the standard deviation and the slope of the calibration line respectively.

Analysis of Marketed Products: This was carried out using the developed and validated method.

Statistical Analysis: Where applicable, results were expressed as mean ± SD and analyzed statistically.

RESULTS AND DISCUSSION

Specificity: UV spectroscopic method for MET analysis was found specific as spectrum of standard MET coincide with that of market product indicating that excipients has no noticeable effect on the effectiveness of the method.

Linearity: Linearity of the method was evaluated from the correlation coefficient of calibration curves that were constructed from average absorbance of drugs at different concentration level (1-25µg/ml). The linearity parameter (Table 1 and Figure 2) and the corresponding regression data indicated excellent linear relationship (R2= 0.9998) over the working concentration range (1-25 µg/ ml). Table 2 shows statistical data of the calibration curve.

TABLE 1: ABSORBANCE AND CORRESPONDING CONCENTRATION OF STANDARD MET

Concentration (µg/ml)	Absorbance
0	0.000
1	0.081
2	0.149
5	0.381
10	0.752
15	1.151
20	1.548
25	1.943

TABLE 2: STATISTICAL DATA OF THE CALIBRATION CURVE

Parameter	Criteria	Values
Slope	Mean ± SD	0.0776 ± 0.0004
	RSD (%)	0.5135
Intercept	Mean ± SD	-0.0059 ± 0.0052
R^2	Mean ± SD	0.9998 ± 0.0001
	RSD (%)	0.0057

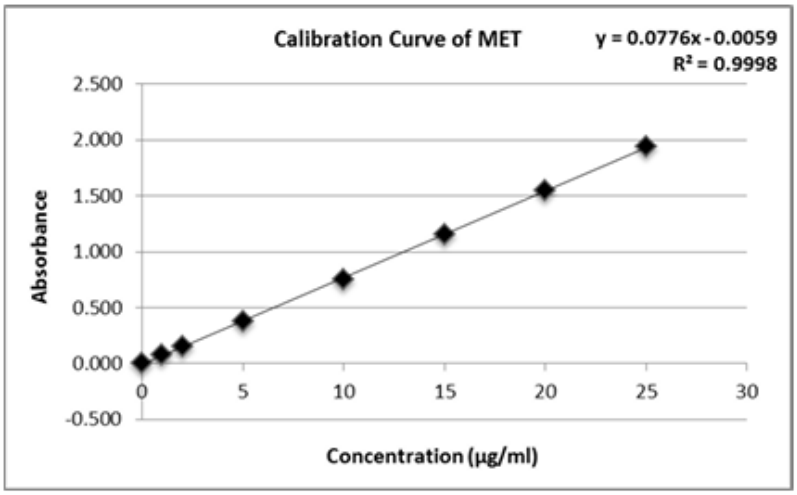


FIGURE 2: CALIBRATION CURVE FOR MET

Intraday and Inter-day Precision: Intra-day precision and accuracy of the proposed method were evaluated by replicate analysis (n= 3) of calibration standards at five concentration levels (1, 2, 10, 20, 25 µg/ml). Inter day precision and accuracy were determined by assaying the calibration standards at five concentration levels (1, 2, 10, 20, 25µg/ml) on three consecutive days. % RSD was calculated for various runs. The method is highly precise as % RSD for Intraday and Inter-day Precision study was less than 2% (table 3 & 4).

TABLE 3: INTRADAY PRECISION AND ACCURACY STUDY OF STANDARD MET

Declared Conc. (µg/ ml)	Calculated Conc. (µg/ml)			Mean ± SD (µg/ml)	Accuracy (%)	RSD (%)
	1	2	3			
1	1.0168	1.0425	1.0296	1.0296 ± 0.0129	102.9639	1.2516
2	1.9704	1.9961	1.9446	1.9704 ± 0.0258	98.5180	1.3080
10	9.7668	9.8441	9.9085	9.8398 ± 0.0710	98.3978	0.7213
20	19.9472	19.7925	19.7410	19.8269 ± 0.1073	99.1345	0.5412
25	25.0245	24.3028	25.0116	24.7796 ± 0.4130	99.1186	1.6666

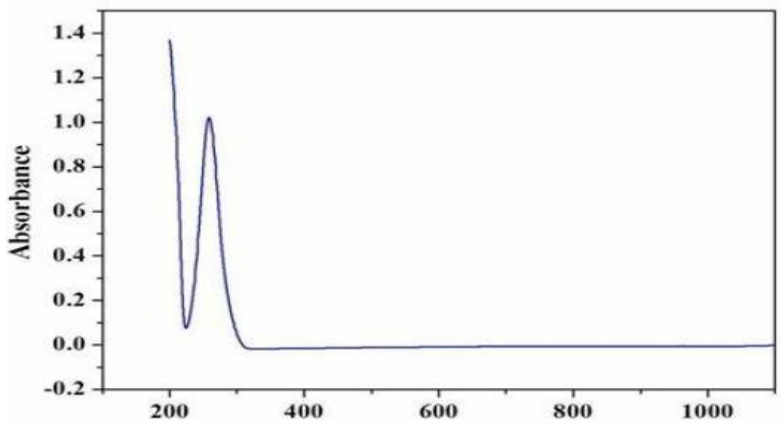
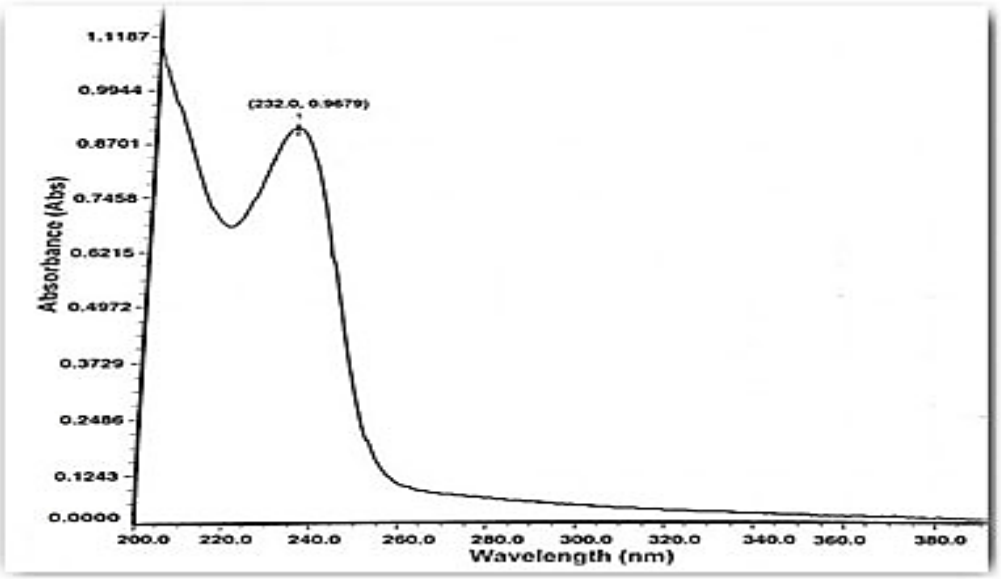


TABLE 4: INTER-DAY PRECISION AND ACCURACY STUDY OF STANDARD MET

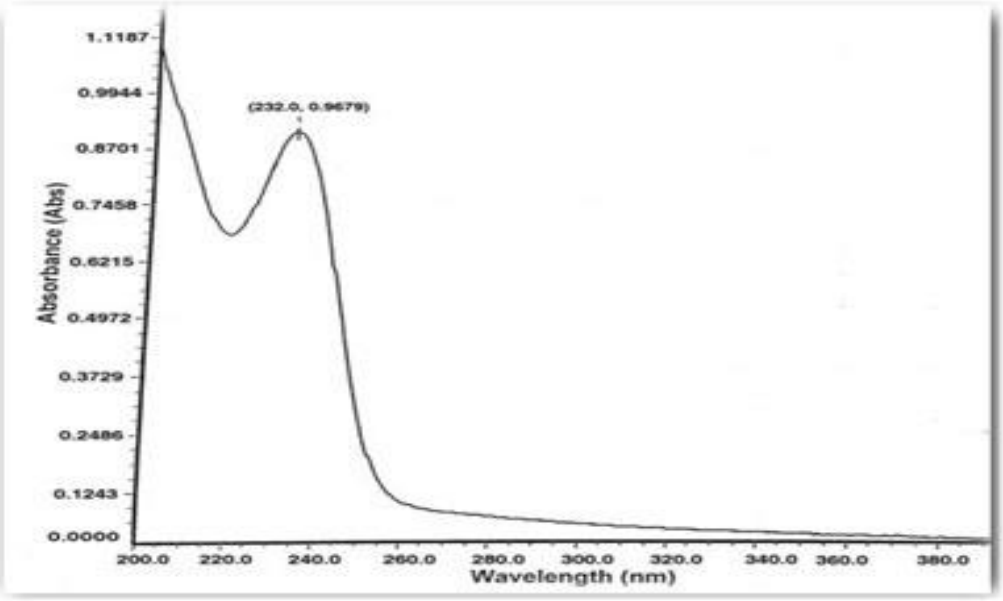
Declared Conc. (µg/ ml)	Calculated Conc. (µg/ml)			Mean ± SD (µg/ml)	Accuracy (%)	RSD (%)
	Day 1	Day 2	Day 3			
1	1.052	1.044	1.018	1.038 ± 0.018	103.788	1.724
2	2.028	2.006	2.058	2.031 ± 0.026	101.530	1.276
10	9.819	9.716	9.888	9.808 ± 0.087	98.076	0.882
20	20.468	20.043	19.901	20.137 ± 0.295	100.686	1.466
25	25.586	24.718	25.032	25.112 ± 0.440	100.449	1.750



Accuracy: Accuracy is generally assessed by analyzing samples with known concentration and comparing the measured value with the true value. The measured values were obtained by recovery test. % recovery was found satisfactory for MET with % RSD values below 2.0%. All the results indicate that the method is highly accurate (table 5).

TABLE 5: ACCURACY STUDY OF STANDARD MET

Declared Conc. (µg/ ml)	Calculated Conc. (µg/ml)			Mean ± SD (µg/ml)	Accuracy (%)	RSD (%)
	1	2	3			
8	8.145	7.998	8.123	8.089 ± 0.079	101.108	0.980
9	9.210	9.003	9.016	9.076 ± 0.116	100.848	1.277
10	9.819	9.716	9.888	9.808 ± 0.087	98.077	0.883
11	11.346	11.210	11.243	11.266 ± 0.071	102.421	0.630
12	11.989	11.875	12.011	11.958 ± 0.073	99.653	0.610



Robustness: Robustness study was performed by making slight variations in solvent composition. No significant effect was observed in the recovery of drugs. % recovery was 98% to 102%. So we can say that the method is robust.

Limit of Detection (LOD) and Limit of Quantification (LOQ): Limit of detection (LOD) and Limit of quantification (LOQ) were calculated as 0.2226µg/ml and 0.6745µg/ml respectively. Low limit of quantification and limit of detection makes this method suitable for use in quality-control testing.

Extinction Coefficient of MET: In addition, the reliability of the proposed method was also evaluated by means of the determination of the extinction co efficient of MET using Beer-Lambert's Law. Average value was found 12579.1 $M^{-1}cm^{-1}$ with standard deviation of 245.294 $M^{-1}cm^{-1}$, RSD was 1.95%.

Analysis of Marketed Products: After the validation of the UV Spectrophotometric method, the potency test of four marketed tablet products were performed by the proposed validated method. Among the different marketed brands supplied, the potency of all the brands was found to be within the limit of 98.597-101.790%.

TABLE 6: EXTINCTION COEFFICIENT OF MET

Conc. (µg/ml)	Molar Conc. (M)	Absorbance (λmax=233nm)	Extinction Coefficient, € ($M^{-1}cm^{-1}$)	Mean ± SD ($M^{-1}cm^{-1}$)	RSD (%)
1	6.04x10-6	0.075	12422.3		
2	1.21x10-5	0.149	12339.4		
10	6.04x10-5	0.752	12447.1	12579.1 ± 245.294	1.95%.
20	1.21x10-4	1.548	12815.6		
25	1.51x10-4	1.943	12871.1		

TABLE 7: POTENCY DETERMINATION OF THE MET MARKETED PRODUCTS

Serial no.	Code for brand name	Label claimed (mg)	Calculated amount (mg)	Potency (%)
1	P-a	500	508.95	101.790
2	P-b	500	492.99	98.597
3	P-c	500	499.07	99.814
4	P-d	500	499.93	99.986

CONCLUSION

The UV spectroscopic analysis of Metformin Hydrochloride successfully determined its absorbance characteristics and concentration in the given sample. The maximum absorbance (λmax) was observed at **~232 nm** in the selected solvent, confirming the presence of Metformin Hydrochloride. The method demonstrated good linearity, accuracy, and precision, making it suitable for routine quality control and pharmaceutical analysis. The results indicate that UV spectroscopy is a simple, cost-effective, and reliable analytical technique for the quantitative estimation of Metformin Hydrochloride in bulk and dosage forms.

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