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Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Metformin HCl and Repaglinide in Pharmaceutical Formulation

Sukanya Kharbade*, Alpana Asnani, Kumar Pratyush.

Priyadarshini J. L. College of Pharmacy, Electronic Building, Electronic Zone, Hingna Road, Nagpur-440016

ABSTRACT

Objective: A new, simple, rapid, accurate and economical method have been developed for the simultaneous estimation of Metformin HCl and Repaglinide in formulation.

Method: The absorbances of both the drugs were determined at 238 nm and at 294 nm. The linearity was observed in the concentration range of 2-100 µg/ml and 1-35 µg/ml for Metformin HCl and Repaglinide respectively. The method was validated as per ICH guidelines.

Result: The recovery of Metformin and Repaglinide was found in the range of 98.24 ± 0.325 to 100.25 ± 0.756.

Conclusion: The proposed method was accurate, reproducible and economical and can be used successfully for quantitative estimation of Metformin HCl and Repaglinide in bulk and tablet dosage form.

Keyword:- Metformin, Repaglinide, UV- spectrophotometric method, simultaneous estimation

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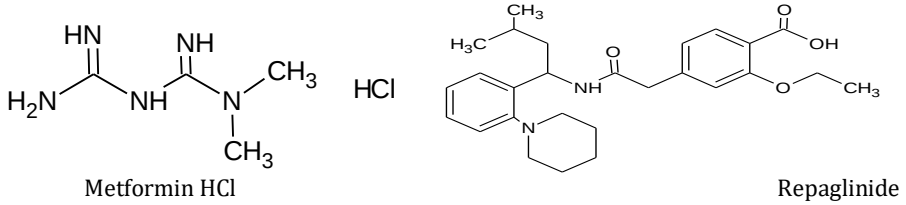
***Address for Correspondence:**

Sukanya Kharbade, Priyadarshini J. L. College of Pharmacy, Electronic Building, Electronic Zone, Hingna Road, Nagpur-440016

1. INTRODUCTION

Diabetes mellitus a chronic diseases across the world is a key disease for the exploring the therapeutic value of the drug [1]. The combined use of metformin and Repaglinide for type 2 diabetes mellitus was shown improved patient compliance by controlling the post prandial glucose levels and reaches normal glycemic levels. Metformin Hydrochloride (MET) (Figure 1) is a biguanide class of antidiabetic drug; chemically is N, N-dimethylimido dicarbonimidicdiamide hydrochloride. It is an oralanti-antidiabetic drug from the biguanide class.[2-3] It is the first-line drug for the treatment of type 2 diabetes, particularly in overweight and obese people and those with normal kidney function and evidence suggests it may be the best choice for people with heart failure. It is also used in the treatment of polycystic ovary syndrome. [4-9] Repaglinide (REPA) is the (Figure 1) Hypoglycaemic agents; Meglitinides, chemically it is (S)-2-Ethoxy-4-[2-[[methyl-1-[2-(1-piperidinyl)-phenyl] butyl] amino]-2-oxoethyl]-benzoic acid. For treatment of diabetes combinations with other hypoglycemic agents are commonly prescribed [10] In that 47.05% are two drug combination compares to single drug treatment (14.11%). There are various UV methods are available for estimation of this two drugs either individually or in combination with other drug and for both drug in combination two UV methods are available. Present work describes rapid, simple, sensitive, accurate and reproducible spectrophotometric methods [11]



Metformin HCl Repaglinide

Fig 1- Chemical structure of Metformin and Repaglinide

2. EXPERIMENTAL

MATERIAL AND METHODS

UV spectrophotometric method was carried out using Shimadzu 1800 double beam UV - Visible spectrophotometer with UV probe 2.33 software with a synchronized pair of 100 mm quartz cells were used for experimental reason.

Preparation of standard stock solution

An accurately weighed standard powder of 10 mg of MET and 10 mg of REPA were transferred in 10 ml volumetric flask separately, dissolved and diluted up to the mark with methanol AR grade, to get concentration 100 µg/ml of MET and 100 µg/ml of REPA. This solutions was used as a working standard solution (WSS). The working standard solution was further diluted by methanol to get require concentration of MET and REPA.

The standard solution of MET and REPA were scanned in the range of 200-400nm against methanol as blank. Maximum absorbance was obtained at 238 nm and 294 nm MET and REPA respectively.

Preparation of calibration curve

The standard solution of MET were diluted with methanol to get series of standard solution having concentrations 2, 5, 10, 20, 30, 40, 50,100 and REPA were diluted to get concentrations 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35 µg/ml. The absorbance of each solution was then measured at 238nm and 294 nm.

Calibration curves were prepared by plotting the absorbance *versus* concentration of drug. The concentration of the unknown was read from the respective calibration curve or computed from the regression equation derived using the Beer's law.

Determination of $E^{1\%}1cm$ of individual drug at selected wavelength

Absorbance of individual drug was divided by concentration in g/100 ml to get the absorptivity coefficients of these drug which were determined at selected wavelength

A(1%, 1cm)= Absorbance/Concentration (g/ml)

Table 1- A(1%1 cm) of MET and REPA

Drugs	Absorptivity of Drugs	
	238 nm	294 nm
MET	184.4	4.28
REPA	204.8	70.4

Simultaneous Equation Method

Simultaneous equations were formed using these absorptivity coefficients values as follows. Quantitative estimation of MET and REPA were carried out by solving following simultaneous equations

$$C_X = A_2 \times 0.01691 - A_1 \times 0.00581$$

$$C_Y = A_1 \times 0.000353 - A_2 \times 0.01523$$

Method Validation

Linearity & Range:

The linearity of proposed methods were evaluated by linear regression analysis, which was calculated by least square method. Calibration standards were prepared by spiking required volume of working standard solution100 µg/ml of MET and 10µg/ml of REPA into different 10ml volumetric flasks and volume made methanol to yield concentrations of 2, 5 10, 20, 30, 40, 50 and 100µg/ml of MET and 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, and 35 µg/ml for REPA. Absorbance of the drugs was measured.

Accuracy:

Accuracy of the methods was determined at three different concentration levels i.e.80%, 100% and 120% in triplicate for each drug as per ICH guidelines.

Precision:

Precision was studied to find out intra and inter-day variations in the test method of MET and REPA. In this spike method is used in which pure drug is added. Intra-day precision was determined by analyzing three concentration in three replicate measurements of within linearity range of drugs on three different times in the same day. Inter-day precision was conducted during routine operation of the system over a period of 3 consecutive days.

Limit of detection (LOD) and Limit of quantification (LOQ):

LOD is the lowest amount of analyte in a sample that can be detected but not necessarily quantify under the stated experimental conditions. LOQ is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions

The LOD and LOQ for MET and REPA were determined according to ICH guideline

$$LOD = \frac{3.3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

Where, σ = Standard deviation of the y intercept of calibration curves

S = Slope of the calibration curve

RESULTS AND DISCUSSION

Spectral characteristics

By appropriate dilution of standard stock solution, solution containing 10 µg/ml of MET and 10 µg/ml of REPA was prepared in methanol. These diluted solutions were scanned in range 200-400 nm separately. Maximum absorbance was obtained 238 nm and 294 nm for MET and REPA, respectively.

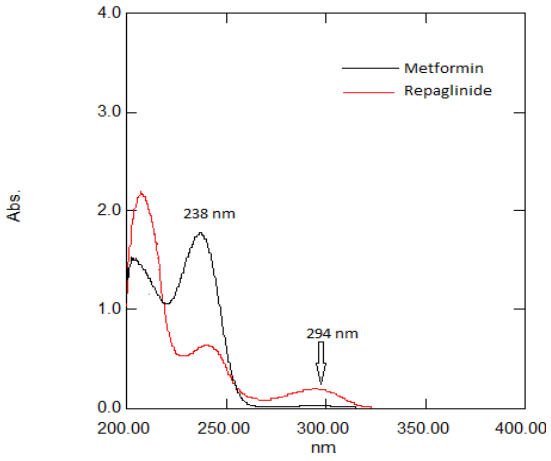


Fig 2- UV absorption overlay spectra of MET and REPA in methanol

Method validation

Linearity Calibration plot was linear over2, 5, 10, 20, 30, 40, 50,100 µg/ml for MET and 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35 µg/ml for REPA

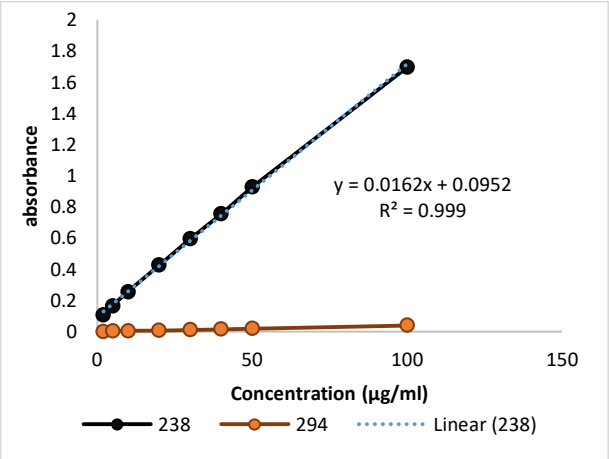


Fig 3a- Calibration of Metformin

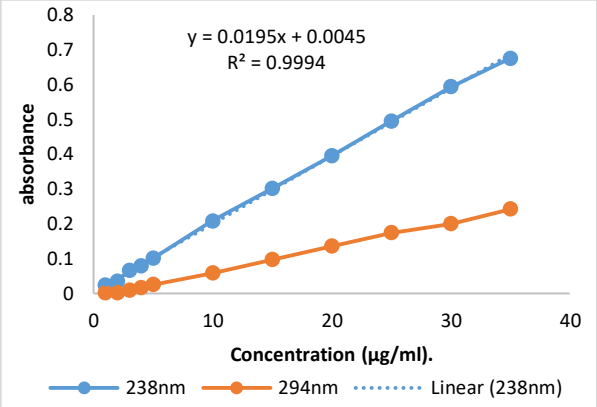


Fig 3b-Calibration of Repaglinide

Accuracy

Accuracy of the methods was determined at three different concentration levels i.e.80%, 100% and 120% in triplicate for each drug as per ICH guidelines. From the total amount of drug found, the percentage recovery was fond in range of 98.24 ± 0.325 to 100.25 ± 0.756.

Table 2- Accuracy of MET and REPA

Sr. No	Amount of pure drug added (µg/ml)		Amount of drug recovered (µg/ml)		% Drug recovered	
80% recovery						
	MET	**REPA**	**MET**	**REPA**	**MET**	**REPA**
1	40	4	39.85	4.02	99.6	100.5
2	40	4	40.02	3.96	100.06	99.09
3	40	4	39.67	3.97	99.17	99.25
100% recovery						
	MET	**REPA**	**MET**	**REPA**	**MET**	**REPA**
1	50	5	49.95	5.01	99.9	100.04
2	50	5	49.89	5.05	99.78	101.8
3	50	5	50.50	5.05	101.05	101.8
120% recovery						
	MET	**REPA**	**MET**	**REPA**	**MET**	**REPA**
1	60	6	59.14	5.91	98.56	98.5
2	60	6	58.95	5.90	98.25	98.33
3	60	6	58.75	5.93	97.91	98.83

Statistics

Drug	Mean	±SD	RSD
80% Recovery			
MET	99.61	0.445	0.446
REPA	99.61	0.772	0.775
100% Recovery			
MET	100.24	0.701	0.7003
REPA	100.25	0.756	0.758
120% Recovery			
MET	98.24	0.325	0.330
REPA	98.55	0.254	0.257

Precision:

Precision was studied to find out intra and inter-day variations in the test method of MET and REPA. Intra-day precision was determined by analyzing three concentration in three replicate measurements of within linearity range of drugs on three different times in the same day. Inter-day precision was conducted during routine operation of the system over a period of 3 consecutive days. The precision of an analytical method is expressed as %RSD of a series of measurements which should be less than 2 %.

Table 3 – Intraday study of MET and REPA

Time	Amount of tablet powder taken (g)	Amount of drug estimated (g/tablet)		% drug estimated	
		MET	REPA	MET	REPA
0 hrs	0.6424	0.4986	0.05010	99.78	100.02
3 hrs	0.6423	0.5005	0.04981	100.10	99.61
6 hrs	0.6426	0.4974	0.04975	99.44	99.45

Statistics

Drugs	Mean	±SD	R.S.D
MET	99.77	0.382	0.382
REPA	99.75	0.428	0.429

Table 4- Interday study of MET and REPA

Days	Amount of tablet powder taken (g)	Amount of drug estimated (g/tablet)		% drug estimated	
		MET	REPA	MET	REPA
Day 1	0.6425	0.5005	0.0496	100.1	99.21
Day 2	0.6426	0.4991	0.0499	99.80	99.83
Day 3	0.6424	0.4985	0.0497	99.65	99.45

Statistics

Drugs	Mean	±SD	R.S.D
MET	99.83	0.199	0.200
REPA	99.49	0.255	0.256

Table 11. LOD and LOQ of MET and REPA

Sr. No	Drug	LOD (µg/ml)	LOQ (µg/ml)
1.	MET	0.0203	0.0617
2.	REPA	0.0169	0.0512

CONCLUSION

The developed method was accurate, precise and reproducible. The statistical parameters showed good results. The method was found to be simple, economical and time saving. This method can be successfully applied for routine quantitative estimation of metformin hydrochloride and repaglinide in bulk and solid dosage form.

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