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## Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Metformin HCL and Repaglinide in Bilayer Tablet

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### ABSTRACT:

A new, simple, accurate and sensitive UV-spectrophotometric absorption correction method has been developed for simultaneous determination of Metformin HCL and Repaglinide in bilayer tablet dosage form utilizing concept of standard addition. The method is based upon determination of Repaglinide at 299 nm where Metformin HCL shows zero absorbance & Metformin HCL at 208 nm in 0.1N HCL as a solvent & in phosphate buffer pH 6.8 Repaglinide at 283 nm where Metformin HCL shows zero absorbance & Metformin HCL at 232.4 nm. Linearity was observed in range of 10-60 µg/ml and 1-6 µg/ml for Metformin HCL and Repaglinide respectively. The correlation coefficient value was found to be 0.989-0.9998. All methods were statistically validated as per ICH guidelines and can be successively applied for analysis of tablets formulation.

**KEYWORDS:** Metformin HCL, Repaglinide, Absorbance correction method, UV-spectrophotometric, Bilayer Tablet, Simultaneous estimation

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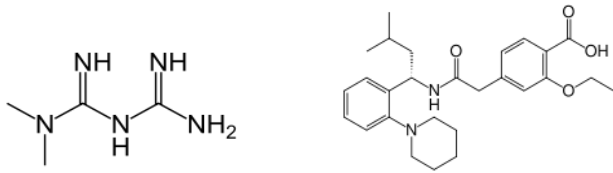
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### INTRODUCTION:

Metformin Hydrochloride (MET) (Figure 1) is a biguanide class of antidiabetic drug, chemically is N,N-dimethylimidodicarbonimidicdiamide hydrochloride 2-7. It is an oral anti-diabetic drug from the biguanide class.<sup>1-3</sup> 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. 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.<sup>4-6</sup> For treatment of diabetes combinations with other hypoglycemic agents are commonly prescribed. In that 47.05% are two drug combination compares to single drug treatment (14.11%).<sup>7</sup> There are various UV methods available for estimation of this two drugs either individually<sup>8-13</sup> or in combination with other drug<sup>14-17</sup> and for both drug in combination two UV methods<sup>19-20</sup> are available. Present work describes rapid, simple, sensitive, accurate and reproducible spectrophotometric methods.

### MATERIAL AND METHODS

UV spectrophotometric method was carried out using Shimadzu 1800 double beam UV - Visible spectrophotometer with UV probe 2.33 software, spectral band width of 2 nm, wavelength accuracy ±0.5 nm and 1 cm matched pair quartz cells. Standard of



Metformin Hydrochloride (C<sub>4</sub>H<sub>11</sub>N<sub>5</sub>.HCl)

Repaglinide (C<sub>27</sub>H<sub>36</sub>N<sub>2</sub>O<sub>4</sub>)

Figure 1 structure of Metformin HCL and Repaglinide

MET and REPA were obtained from Torrent Research Centre, Ahmedabad. AR grade methanol used for UV method and 0.1N HCl and pH 6.8 phosphate buffer were prepared in double distilled water. Bilayer Tablet dosage form having MET 500 mg and REPA 2 mg was prepared in house using Eudragit® and HPMC as release controlling polymer.

**Preparation of standard stock solution**

An accurately weighed standard powder of 100 mg of MET and 10 mg of REPA were transferred in 100 ml volumetric flask separately, dissolved and diluted up to the mark with methanol AR grade, to get final concentration 1000 µg/ml of MET and 100 µg/ml of REPA. From this standard stock solutions, 1 ml was transferred into 2 different 10 ml volumetric flask and volume was made up to the mark with 0.1N HCl and phosphate buffer pH 6.8 respectively to get final concentration 100µg/ml of MET and 10µg/ml of REPA. This solutions was used as a working standard solution (WSS).

**Selection of analytical wavelength**

By appropriate dilution of standard stock solution, solution containing 10 µg/ml of MET and 1µg/ml of REPA was prepared in 0.1N HCl and phosphate buffer pH 6.8. These diluted solutions were scanned in range 200-400 nm separately. MET shows λ<sub>max</sub> at 208 nm (figure 2) and REPA showed λ<sub>max</sub> at 299 nm in 0.1N HCl (figure 3)and in phosphate buffer MET shows λ<sub>max</sub> at 232.4 nm (figure 4)and REPA showed λ<sub>max</sub> at 283 nm (figure 5).

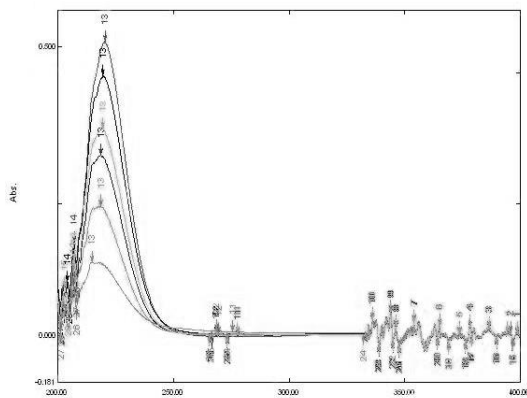


Figure 2 Spectra of MET in 0.1 N HCL

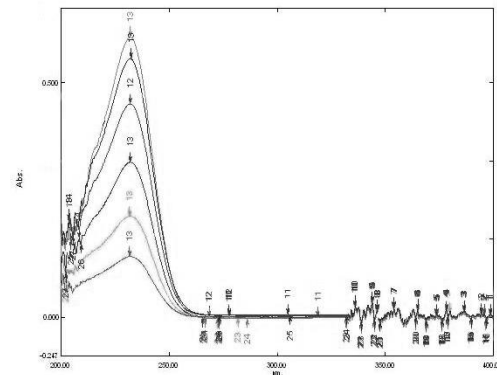


Figure 3 Spectra of MET in phosphate buffer pH6.8

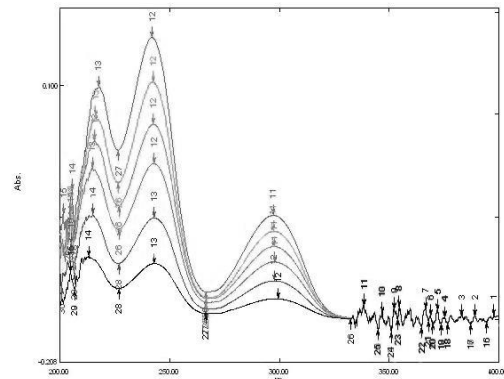


Figure 4 Spectra of REPA in 0.1 N HCL

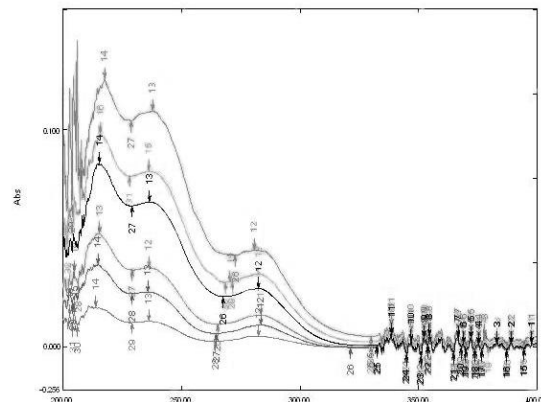


Figure 5 Spectra of REPA in Phosphate buffer pH 6.8

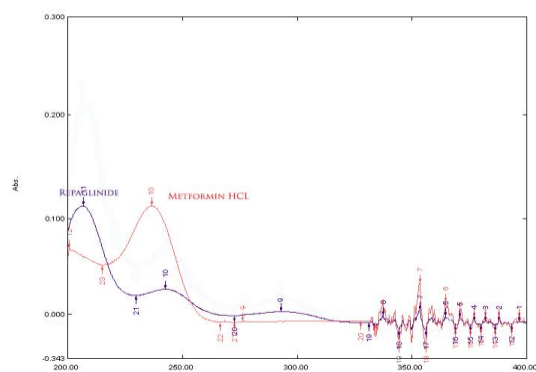


Figure 6: Overlay spectra of MET (10 µg/ml) and REPA (1 µg/ml)

## Preparation of calibration curve

For construction of calibration curve, two series of different concentration in range of 10-60 µg/ml for MET and 1-6 µg/ml for REPA were prepared in 0.1N HCl and in phosphate buffer pH 6.8 from WSS. These solutions were scanned in range of 200-400 nm and absorbance were measured at selective wavelength and calibration curve were plotted for absorbance vs. concentration.

## Absorbance correction method

From the overlay spectra of MET and REPA, two wavelengths were selected, one at 299 nm and 283 nm ( $\lambda_2$ ) for REPA at which MET shows zero absorbance and the other at 208 nm and 232.4 nm ( $\lambda_1$ ) for MET. The absorbance of the sample solutions were measured for both the drugs at selected wavelengths. The concentrations of drugs in sample solution were determined by using the following formula<sup>21-22</sup>:

$$C_y = \frac{A_{299/283 \text{ nm}}}{A(1\%, 1\text{cm})_{299/283 \text{ nm}} \text{ of REPA}}$$

$$C_x = \frac{C_{Ax} \text{ }_{208/232.4 \text{ nm}}}{A(1\%, 1\text{cm})_{208/232.4 \text{ nm}} \text{ of MET}}$$

Where,

$$C_{Ax} \text{ }_{208/232.4 \text{ nm}} = A_{208/232.4 \text{ nm}} - A_{y208/232.4 \text{ nm}}$$

$$A_{y208/232.4 \text{ nm}} = C_y \times A(1\%, 1\text{cm})_{208/232.4 \text{ nm}} \text{ of REPA}$$

Cx = Concentration of MET (gm/100 ml)

Cy = Concentration of REPA (gm/100 ml)

A = Absorbance

A (1%,1cm) = Specific Absorptivity

## Procedure for Analysis of Tablet Formulation

Twenty tablets were weighed and powdered. An accurately weighed tablet powder equivalent to 500 mg of MET and 2 mg of REPA was transferred in to 100 ml volumetric flask. For validation purpose, to this 48 mg of standard REPA standard powder was added to achieve ratio of MET to REPA 10: 1. Assay of drugs was done by addition of 8 mg of standard REPA powder. To this 20 ml of methanol was added and sonicated for 15 min. Volume was made up to the mark with methanol then filtered the solution through whatman filter paper no. 41. From this stock solution, necessary dilutions were made with 0.1N HCl and in phosphate buffer pH 6.8 to get final concentration 20 µg/ml and 2µg/ml of MET and REPA respectively. The above solution was then analyzed for the content of MET and REPA using the methods described above.

## METHOD VALIDATION<sup>23,24</sup>

The proposed methods were validated accordance to ICHQ2 (R1) guidelines for linearity, precision, accuracy, limit of detection, limit of quantification. The results are shown in Table 1, 2, 3 & 4.

## RESULTS

### 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 solution 100 µg/ml of MET and 10µg/ml of REPA into different 10ml volumetric flasks and volume made with 0.1N HCl and in phosphate buffer to yield concentrations of 10, 20, 30, 40, 50 and 60 µg/ml of MET and 1,2,3,4,5 and 6 µg/ml for REPA. Absorbance of the drugs was measured. Calibration curve was plotted between absorbance of drug against concentration of the drug. These results shown there was an excellent correlation between absorbance and analyte concentration (Table 1). The drugs were linear in the concentration range of 10-60 µg/ml for MET and 1-6 µg/ml for REPA.

### 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 found in range of 97.92 – 101.35 %.

### 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 %.

### 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

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

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

Where,  $\sigma$  = Standard deviation of the y intercept of calibration curves

S = Slope of the calibration curve

The results of LOD and LOQ were shown in table 1.

## DISCUSSION

The proposed methods for simultaneous estimation of MET and REPA in bilayer tablet dosage forms were found to be simple, accurate, economical and rapid. The method was validated as per the ICH Q2 (R1) guidelines. Standard calibration curves for MET and REPA were linear with correlation coefficients ( $r^2$ ) values in the range of 0.9970-0.9998 at all the selected wavelengths and the values were average of three readings. The values of %RSD are within the prescribed limit of 2 %, showing high precision of methods and recovery was close to 100% for both the drugs. Results of the analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their simultaneous determination with virtually no interference of usual additive present in pharmaceutical formulations. Further the UV method is compared with other published uv methods (table 3). Hence, the above methods can be applied successfully for simultaneous estimation of MET and REPA in formulations.

**Table 1: Summary of Linear regression analysis and optical characteristics of MET and REPA**

Parameter	0.1 N HCl		Phosphate buffer pH 6.8	
	MET	REPA	MET	REPA
Analytical wavelength(nm)	208	299	232.4	283
Beer's law limit ( $\mu\text{g/ml}$ )	10-60	1-6	10-60	1-6
Coefficient of Correlation( $r^2$ )	0.9978	0.9887	0.9997	0.9939
Slope	0.0083	0.0097	0.0112	0.0090
y intercept	0.0149	0.0032	0.004	0.002
Molar absorptivity (lit/mole/cm)	1514.57	3596.80	1891.25	3609.37
Sandell's sensitivity (mcg/cm <sup>2</sup> /0.001AU)	0.091743	0.2	0.087719	0.142857
LOD( $\mu\text{g/ml}$ )	1.30	0.19	1.98	0.21
LOQ( $\mu\text{g/ml}$ )	3.94	0.58	6	0.64

**Table 2: Analysis of formulation by proposed method**

Drug	Amount	0.1 N HCl	Phosphate buffer
MET	500 mg	98.04	98.47
REPA	2 mg	103.32	101.56

**Table 3: Comparison with other methods**

Parameter	Method 1 <sup>19</sup>		Method 2 <sup>20</sup>		Developed method			
	MET	REPA	MET	REPA	MET		REPA	
Method	Difference spectroscopy		Second derivative spectroscopy		Absorption correction spectroscopy			
Solvent	0.1 N HCl & 0.1 N NaOH		Methanol		0.1 N HCl		Phosphate Buffer pH 6.8	
Wavelength (nm)	234.5	269 & 304	234 or 252	287	208 & 232.4		299 & 283	
Linearity( $\mu\text{g/ml}$ )	2-12	10-50	2-12	5- 40	10-60		1-6	
LOD ( $\mu\text{g/ml}$ )	0.14	0.58 & 1.21	0.32 & 0.39	1.01	1.30	0.19	1.98	0.21
LOQ ( $\mu\text{g/ml}$ )	0.44	1.77 3.68	1.06 1.24	3.13	3.94	0.58	6	0.64

The proposed method utilize two medium ie 0.1 N HCL and pH 6.8 phosphate buffer. The comparison of method with already published two methods shows that the developed method is more accurate and economic as compared to other two method further the method complies with detection of drugs as per their label claim also no further derivetization or modification in spectra is required so the proposed method can be said as simple accurate and economic as compared to other published method.

## CONCLUSION

The developed UV methods were found to be more accurate, precise and reproducible. The analysis of tablets containing two drugs gave the satisfactory results. The statistical parameter of these methods showed good results. The recovery studies revealed excellent accuracy and high precision of the method. The methods were found to be simple & time saving. All three proposed methods could be applied for routine analysis in quality control laboratories.

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