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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF REMOGLIFLOZINETABONATE AND METFORMIN HCL IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT:

A simple, robust, precise, UV spectroscopic method has been developed for the simultaneous estimation of Remogliflozin and metformin in bulk and tablet dosage forms. In this paper the estimation of those drugs was carried out by simultaneous equation method. This method is based on measurement of absorption at 234nm and 258nm i.e, λ_{max} of metformin and Remogliflozin respectively. The linearity observed for Remogliflozin is in the range of 2-10µg/ml and for metformin is in the range of 10-50µg/ml. The accuracy of methods was assessed by recovery studies and was found to be within the range of 99.80% for 99.98% for both Remogliflozin and metformin. The developed methods were validated with respect to linearity, accuracy (recovery), and precision. The method can be employed for estimation of pharmaceutical formulations with no interference from any other excipients and diluents. The results were validated as per ICH guidelines.

KEYWORDS: Remogliflozin, Metformin, ICH, Validation etc.

1.0 Introduction

Pharmaceutical product quality is of vital importance for patient safety. Pharmaceutical analysis is the branch of pharmacy that is responsible for developing sensitive, reliable and accurate methods for the estimation of drugs in pharmaceutical dosage forms.Remogliflozin that is 5-Methyl-4-[4-(1-methylethoxy)benzyl]-1-(1-methylethyl)-1H-pyrazol-3-yl 6-O-(ethoxycarbonyl)-β-D-glucopyranosideRemogliflozin etabonate is a drug of the gliflozin class for the treatment of non-alcoholic steatohepatitis and type 2 diabetes.Remogliflozin etabonate helps in removing excess sugar from the body via urine. Metformin works by reducing the sugar production by cells in the liver and delays sugar absorption from the intestines[3,4].

Metformin chemically denoted as 1-carbamimidamido-N,N-dimethylmethanimidamide is a biguanide ant hyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM)[4] It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin may induce weight loss and is the drug of choice for obese NIDDM patients. Use of metformin is associated with modest weight loss. When used alone, metformin does not cause hypoglycemia; however, it may potentiate the hypoglycemic effects of sulfonylureas and insulin. Its main side effects are dyspepsia, nausea and diarrhea. Dose titration and/or use of smaller divided doses may decrease side effects. Metformin should be avoided in those with severely compromised renal function (creatinine clearance < 30 ml/min), acute/decompensate heart failure, severe liver disease and for 48 hours after the use of iodinated contrast dyes due to the risk of lactic acidosis. Lower doses should be used in the elderly and those with decreased renal function. Metformin decreases fasting plasma glucose, postprandial blood glucose and glycosolated hemoglobin (HbA1c) levels, which are reflective of the last 8-10 weeks of glucose control. Metformin may also have a positive effect on lipid levels.[5,6]

In Combination therapy should be used as initial treatment for patients in whom the probability of achieving Diabetic control with monotherapy is low. Given the number of antidiabetic agents available, the number of potential combinations is large. However, rational choices should be based on some requirements. This research focus on these requirements with specific interest in combination including insulin-diabetic system blockerstablet of Remogliflozin plus metformin hydrochloride was marketed under the name REMO M for use in patients when treatment with both Remogliflozin and metformin is appropriate.[7,8]

Analytical methods reported for quantitative determination of REMO individually in pharmaceutical formulations or biological fluids are high-performance liquid chromatographic HPLC,[9-15] UV,[16-19] and methods reported for quantitative determination of MET individually in pharmaceutical formulations or biological fluids are HPLC,[9-15] UV,[16-19] and some of the reported for quantitative determination method in combination of REMO and MET

Fig.No. 1. Structure of Remogliflozin

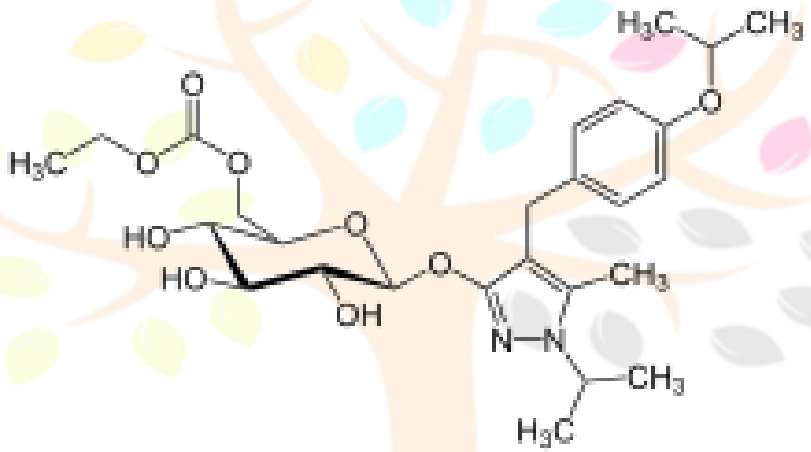
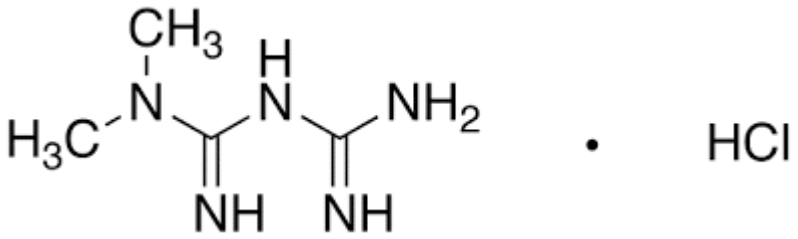


Fig.No. 2. Structure of Metformin



2.0 Materials and Methods

2.1 Instruments

Shimadzu UV-1800 double beam spectrophotometer was used to record the spectra of sample and reference solutions using pair of quartz cells of 10mm path length. All weighing was carried out on Sansui Vibra DJ-150S-Sweighing balance.Sonicator of Fast Clean is used for the purpose of sonication, Filter papers of Sartorius Stedim Biotech of grade 292 are used for the filtration purpose.

2.2 Chemicals

Remogliflozin(10mg) and metformin (50mg) pure drugs were obtained as a gift sample from Glenmark Pharmaceuticals Pvt Ltd. The combined formulation tablet Remo M (Glenmark Pharmaceutical Ltd., India.) (100 mg/500 mg) the two drugs purchased from local pharmacy. Analytical grade methanol and other chemicals were purchased from Merck Chemicals Pvt. Ltd. Mumbai.

2.3 Preparation of stock solution and selection of wavelength

2.3.1Remogliflozin Standard Stock Solution [R]:

An accurately weighed quantity of REMO (10 mg) was taken in 10mL volumetric flask and dissolved in methanol (10 mL) with the help of ultra-sonication for about 10 min. Then the volume was made up to the mark using methanol to get Remogliflozin standard stock solution (1 mg / mL).

2.3.2Remogliflozin Working Standard Solution [R_1]:

Remogliflozin standard stock solution [R_1] (1 mL) was diluted to 10 mL using 60% v/v methanol to get working standard solution (100 µg / mL)

2.3.3 Metformin Standard Stock Solution [M]:

An accurately weighed quantity of met (10 mg) was taken in 10mL volumetric flask and dissolved in methanol (10 mL) with the help of ultrasonication for about 10 min. Then the volumewas made up to the mark using methanol to get metformin standard stock solution(1 mg / mL).

2.3.4 MetforminWorking Standard Solution [M_1]:

Metformin standard stock solution [M_1] (1 mL) was diluted to 10 mL using 60% v/v methanol to get working standard solution (100 µg / mL)

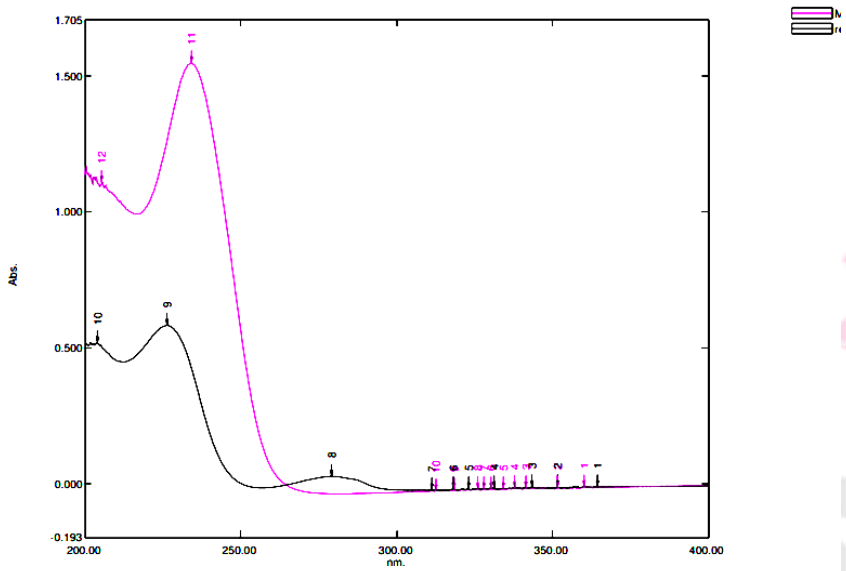
2.4 Determination of λ Max of Individual Component

An appropriate aliquot portion of REMO aliquots were transferred to two separate 10 mL volumetric flasks, the volume was made up to the mark using 60 %v/v methanol to obtain REMO (10 µg/mL) and MET(50 µg/mL).

Drug solutions were scanned separately between 200 nm to 400 nm.The overlay spectra of both drugs were recorded and two wavelengths 234nm ($\lambda_{max}$of metformin) and 258 nm(λ_{max} of Remogliflozin) were selected for further study.

2.4.1 Overlay spectra of Remogliflozin and Metformin

The overlay spectrum of both drugs was recorded (Fig.4) and two wavelengths 234nm (λ max of MET) and 258 nm (λ max of REMO) were selected for further study.



Graph No. 1 Overlay spectra of Remogliflozin and Metformin

2.5.1 Linearity study for Remogliflozin

An accurately measured aliquot portion of working standard solution of REMO [R1] was transferred to seven separate 10 mL volumetric flasks. The volume was made up to the mark using 60% v/v methanol to obtain concentrations (2-10 µg/mL). Absorbance of these solutions was measured at 234 nm.

2.5.2 Linearity study for Metformin

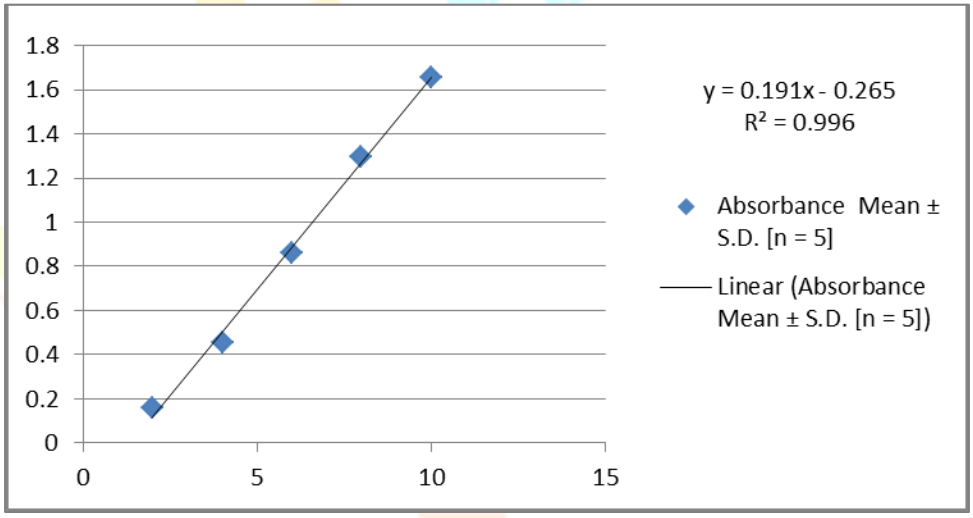
Accurately measured aliquot portions of working standard solution of MET [M_1] were transferred to seven separate 10 mL volumetric flasks. The volume was made up to the mark using60% v/v methanol to obtain concentrations (10-50µg/mL).Absorbance of these solutions was measured at 258 nm, calibration curve was plotted, absorbance vs concentration. The results are shown in the

Table No. 1 Regression and Optical characteristics of REMO and MET

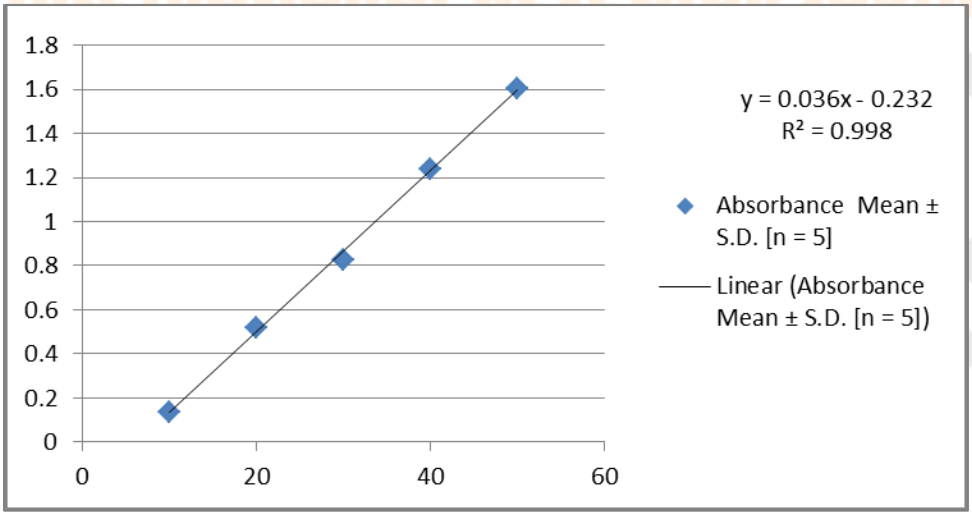
Parameters	Remogliflozin	Metformin
Beer's law limit (µg/ml)	2-10µg/ml	10-50 µg/ml
Regression Coefficient(R^2)	0.996	0.998

Regression equation	y= 0.191x -0.265	y= 0.036x- 0.232
Slope	0.191	0.036
Intercept	0.265	0.232

The study of regression and optical characteristics of REMO and MET are carried out in which Regression Coefficient (R^2) of REMO is 0.996 and of MET is 0.998. The slope of REMO 0.191 and slope of MET is 0.036 with Intercept of REMO 0.265 and for MET 0.232, therefore, concentration vs absorbance are fairly linear between both co-ordinates by statistical manner and obey ICH guidelines.



Graph No.2 Calibration curve of Remogliflozin at234 nm



Graph No.3 Calibration curve of metformin at258 nm

Estimation of Laboratory mixture by proposed method

2.6 Method: Simultaneous Estimation Method

If a drug sample contains two absorbing drugs (X and Y) each of which absorbs at the $\lambda_{max}$of the other,then it may possible to estimate both drugs by this method. The scanning spectra of 10µg/ml solution of Remogliflozin and metformin show clear peaks at234nmand258nmrespectively.

Amount of each drug was estimated using following equations,

$$C_x = \frac{A_2 \times a_{y_1} - A_1 \times a_{y_2}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}}$$

$$C_y = \frac{A_1 \times a_{x_2} - A2 \times a_{x_1}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}}$$

Where;

A1 and A2 are the absorbance of diluted mixture at λ_1 and λ_2

Cx and Cy are the concentration of X and Y respectively

ax1 and ax2 are absorptivities of X at λ_1 and λ_2 respectively

ay1 and ay2 are absorptivities of Y at λ_1 and λ_2 respectively

The results are determined in the Table No. 2

Table No. 2. Results of Estimation of REMO and MET in standard laboratory mixture

Analyte	**% Concentration estimated (Mean ± S.D)**	**% R.S.D**
Remogliflozin	99.98± 1.253	1.275
Metformin	99.93 ± 1.8900	1.8933

The estimation of REMO and METin standard laboratory mixture are carried out in which % concentration of REMO and MET were found to be 99.98 and 99.93 respectively. Those results are fairly accurate by statistical manner and are as per ICH guidelines.

2.7 Application of proposed method for Estimation of drugs in tablets

Twenty "Remo M" Tablets containing REMO (100 mg) and MET (500mg) were weighed and ground to fine powder. A quantity of sample equivalent to REMO (10 mg) and MET (50 mg) was transferred into 100 mL volumetric flask containing methanol (60 mL), sonicated for 15 min and the volume was made up to the mark and filtered through whatman filter paper (No. 45). This solution was (1 mL) transferred to 10 mL volumetric flaks, dissolved and volume was adjusted to the mark. The absorbances of the solutions were measured at 234 nm and

258 nm against blank. The concentrations of two drugs in sample were determined by using simultaneous equations**.** The results are reported in the Table No.3.

(Table No. 3) Results of Estimation of REMO and MET in tablets dosage form.

Analyte	**Label claim(mg/tab)**	**% Label claim estimated(Mean±S.D)**	**% R.S.D**
Remogliflozin	10	99.76 ± 1.1356	1.1359
Metformin	50	99.73 ± 1.2105	1.2110

The results of Estimation of REMO and MET in tablets dosage shows the % purity 99.76 to 99.73 with SD and RSD bellow 2 which is fairly accurate by statistical manner and are as per ICH guidelines.

3.0 Validation of proposed method

The proposed method was validated as per ICH guidelines[17,18]

3.1 Accuracy (Recovery study)

Accuracy of proposed method was ascertained on the basis of recovery study performed by standard addition method. A known amount of standard drug solutions were added to the tablet powder to make final concentrations in the range of 80%, 100% and 120% and re-analyzed it by the proposed method. The absorbance recorded and the % recoveries were calculated using formula.

% Recovery = [A - B/ C] X 100

Where,

A = Total amount of drug estimated

B = Amount of drug found on preanalysed basis

C = Amount of Pure drug added

The results are shown in the Table No.4

(Table No. 4) Recovery study

Sr. No.	**QuantityTablet Powder Taken(mg)**	**Percentage%**	**% of Drug Recovered (n = 3)**	
			REMO	**MET**
1.	85	80	99.80	99.98
2.	85	100	99.82	99.84
3.	85	120	99.97	99.81

The results of Recovery study of REMO and MET are found to be fairly accurate between 99.80% for 99.98% various concentrations under observation by statistical way and are obey ICH guidelines.

3.2 Precision

Precision was determined as intra-day and inter-day variations. Intra-day precision was determined by analyzing Remogliflozin (2, 4, and 6 µg/mL) and metformin (10, 20, and 30 µg/mL) for three times on the same day. Inter-

day precision was determined by analyzing the same concentration of solutions for three different days over a period of week. The results are shown in the Table No. 5.

(Table No.5)Precision Study

Precision	REMO	% RSD	MET	% RSD
Inter-day, *n*=3	5.68±0.7535	±0.7298	29.94±0.4752	±0.4356
Intra-day, *n*=3	5.98±0.4763	±0.5834	19.96±0.4536	±0.0791

The Precision Study of REMO and MET were carried out and Results are found to be fairly accurate by statistical manner and obeys ICH guidelines.

3.3 Ruggedness

Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by two different analyst using same operational and environmental conditions. The results are shown in Table No. 6.

(Table No. 6) Ruggedness study

	REMO10 µg/mL		MET 50 µg/mL	
	Amount Found in µg/mL Mean ± S.D. (n = 3)	% RSD	Amount Found in µg/mL Mean ± S.D.(n = 3)	% RSD
Analyst-I	9.96± 0.043	0.0208	49.98± 0.0321	0.0456
Analyst-II	9.98± 0.052	0.0592	49.79± 0.060	0.2015
Day-I	9.90±0.075	0.0856	49.99± 0.041	0.0318
Day-II	9.92±0.100	0.1252	49.89± 0.049	0.0623

The Ruggedness study of REMO and MET are carried out and results are found to be fairly accurate by statistical manner and obeys ICH guidelines.

3.4 LOD: Limit of detection of REMO and MET were found to be 0.7076 µg/mLand2.4932 µg/mL respectively.

3.5 LOQ: Limit of quantitation of REMO and MET were found to be 2.1443 µg/mL and 7.555 µg/mL respectively.

4.0 Results and Discussion

A simultaneous UV Spectrophotometric Estimation method was developed for Remogliflozin and metformin. The method employs 234 nm as λ1 and 258 nm as λ2 for formation of equations. Remogliflozin and metformin obeys Beer's law in the concentration range 2-10µg/ml (R^2=0.996) and 10-50 µg/ml (R^2=0.998) respectively. The mean

recovery for Remogliflozin and metformin was found to be 99.80% for 99.98% respectively. The developed method were validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values.

5.0 Conclusion

The proposed simultaneous UV Spectrophotometric Estimation method presented in this paper has advantages of simplicity, accuracy, precision and convenience for quantitation of Remogliflozin andmetformin. The proposed method can be used for the quality control of Remogliflozin and metformin in typical laboratories.

6.0 Acknowledgement

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