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Development and Validation of Uv Spectroscopic Method for Determination of Remogliflozin Etabonate in Bulk and Tablet Dosage Form

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ABSTRACT

A Simple, Precise, Accurate and Economical UV spectrophotometric method was developed and validated for estimation of Remogliflozin Etabonate in bulk and tablet form. The drug was highly soluble in methanol, so it was selected as the solvent system. The detection wavelength of Remogliflozin Etabonate was found to be 229 nm. The linearity of Remogliflozin Etabonate was found to be in range of 5 -25 µg/ml with correlation coefficient value 0.999.The linear regression equation obtained by least square regression method, were y = 0.0537x-0.04443. The absorbance was found to increases linearly with increasing concentration of Remogliflozin Etabonate. The correlation coefficient of Remogliflozin Etabonate was found to be 0.999. The LOD and LOQ were found to be 1.961 µg/ml and 4.200 µg/ml respectively. Mean recovery of Remogliflozin etabonate was found to be in the range of 99.29% to 101.06% signifies the accuracy of method. The methods was also found to be precise as % RSD was less than 2. This method was validated as per ICH Q2 (R1) guidelines. The proposed methods were found to be accurate, specific and reproducible which can be effectively applied to pharmaceutical dosage form.

KEYWORDS: Remogliflozin Etabonate, , Method Development, Validation.

I. INTRODUCTION:

Rmogliflozin Etabonate is Chemically (2R,3S,4S,5R,6S)-2-(Hydroxymethyl)-6-[5-methyl-1-propan-2- yl-4-[(4-propan-2-yloxyphenyl)methyl]pyrazol-3-yl]oxyoxane- 3,4,5-triol. It is an orally available prodrug of remogliflozin , a benzylpyrazole glucoside-based inhibitor of renal sodium-glucose co-transporter subtype 2 (SGLT2) with antihyperglycemic activity. upon administration and absorption , the inactive prodrug is converted toits active form Remogliflozin and acts selectively on the sodium-glucose co-transporter subtype 2 (SGLT2). Remogliflozin inhibits the sodium-glucose transport proteins (SGLT), which are responsible for glucose reabsorption in the kidney.[1-2] Some methods are chromarographic method is combination with other drugs.[3-5] So aim of the work is To develop simple ,rapid economics UV spectroscopic method for estimation of Remogliflozin Etabonate. To validate developed UV spectroscopy method as per ICI guideline Q2 R1 Apply a new simple UV spectroscopy method to marketed formulation of Remogliflozin Etabonate. Remo-Zen is marketed formulation of Remogliflozin Etabonate and used in this research work.

II. MATERIAL AND METHODS

Chemical and Reagents

Remogliflozin Etabonate was Purchased from Benzchem Enterprise, Vadodara. Methanol was purchased from Finar limited.

Instrumentation

Double beam UV-visible spectrophotometry (model-1800) having two matched quartz cells with 1 cm light path.

Preparation of Solution

Preparation of Stock solution:

10 mg of Remogliflozin Etabonate was weight & dissolved in sufficient volume of Methanol and sonicated for 10 mins. Then volume was made up to the mark in 100 ml volumetric flask with methanol to obtain final concentration (100 µg/ml) of Remogliflozin Etabonate. The solution is used to development and validation of method by making further dilution

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Preparation of Standard solutions for Calibration Curve :

From this stock solution, 0.5 ml, 1ml, 1.5ml, 2ml, 2.5ml were withdrawn out and makeup was done with methanol up to mark of 10 ml of measuring cylinder in order to get appropriate final concentration of 5 µg/ml, 10µg/ml,15µg/ml , 20 µg/ml and 25µg/ml. absorbance was taken at λ max 229 nm. Averages of such 5 sets of Values were taken for standard calibration curve, and the calibration curve was plotted.

Method Validation

Linearity and range:

Linearity of developed UV spectrophotometer was studied by obtaining calibration curve of Remogliflozin Etabonate at five different concentration levels ranging from 5-25 µg/ml. the linearity response was determined by analysing 5 independent level of calibration curve.Table 5.5 show the Linearity data of Remogliflozin Etabonate. The linearity curve of Remogliflozin Etabonate is shown in figure 5.2. The Equation of regression line was **y =** 0.0537x-0.04443. The correlation coefficient value was found to be 0.999.

Precision:

Precision of the method was determined by evaluating repeatability, intraday and Inter day precision. Repeatability was analysed by selecting middle concentration 15 µg/ml and performed3 independent time. Intraday and Inter-day variation was analysed by selecting three concentrations which were 10, 15 and 20 µg/ml from linearity range. Intraday analysis was carried on same day whereas inter day analysis was carried on three different days in replicates of three. The respective peak areas for different concentrations were reported in Table 5.6, 5.7 & 5.8 express precision data for the method in terms of % RSD.

Accuracy:

To study the reliability, suitability and accuracy of the method, recovery studies were carried Out. To the formulation equivalent to 15mg of Remogliflozin Etabonate the levels of 80%, 100% and120% were added. The concentration of drugs present in resulting solution was determined Using assay method; percentage recovery and percentage RSD were calculated. The results For the recovery study are given in Table 5.9

Ruggedness:

Ruggedness is reproducibility under normal but variable laboratory conditions. It is done by 2 Methods. In one method, three working standard dilutions by 2 different analysts Were prepared and tested their absorbance at fixed wavelength in the same equipment In another method, three working standard dilutions were prepared by the same Analyst and the measurement of absorbance was done at 2 different systems. The results for The ruggedness are given in Table 5.10

Robustness:

Robustness was determined by performing the same concentration of solution at Different wavelengths 229nm. The analysis showed %RSD less than 2 and indicates That the method developed is robust Table 5.11

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

The LOD and LOQ were calculated from the standard deviation of Y- intercept and mean slop of the calibration curve of Remogliflozin Etabonate using the formula as given below: The result if LOD and LOQ shown in table 5.12

LOD = 3.3 × $SD/Slope$

LOQ = 10 × $SD/Slope$

Where, SD = the standard deviation of Y-intercept of 5 calibration curves. Slope = the mean slop of the 5 calibration curves.

Assay

Application of the proposed method for analysis of Remogliflozin Etabonate in marketed formulation.Assay data are shown in 5.13.20 tablets were weighed and flattened into powder.powder weight equivalent to 10 mg of Remogliflozin Etabonate were transferred into 100 ml volumetric flask.50ml of solvent was added and sonicated for 20 minutes.Then the final volume was diluted up to mark with the solvent methanol and filtered.2ml of the above filtrate was tranferred into 25ml volumetric flask,and the final volume was adjusted up to mark with the same solvent to get sample solution with the concentration of 100µg/ml of drug.The absorbance of resultiong solution was measured at 229nm.The amount of drug was found to be of label claim respectively.Finally the developed new simple equation method was applied successfully to the marketed tablet dosage form and assay results were indicating that this method is applied to estimation of dosage form.

III. RESULT AND DISCUSSION:

Selection of Detection wavelength Λmax by UV Spectroscopy:

Remogliflozin Etabonate is soluble in organic solvents like Methanol, Ethanol and DMSO

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but economically Methanol is more preferable So, methanol was selected throughout the study. Remogliflozin Etabonate 10 µg/ml of standard solution was scanned in between 200 nm to 400 nm and showed Maximum absorption at 229 nm by UV spectrophotometer.

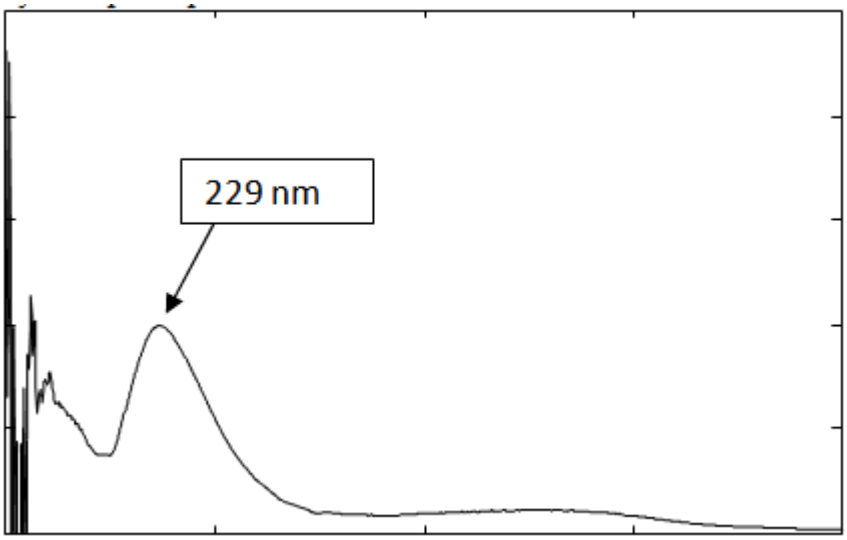

Fig.1 UV Spectra of Remogliflozin Etabonate

Linearity:
Linearity of developed UV spectrophotometer was studied by obtaining calibration curve of Remogliflozin Etabonate at five different concentration levels ranging from 5-25 µg/ml. the linearity response was determined by analysing 5 independent level of calibration curve. Table 1 show the Linearity data of Remogliflozin Etabonate. The linearity curve of Remogliflozin Etabonate is shown in figure 3. The Equation of regression line was y = 0.0537x-0.04443. The correlation coefficient value was found to be 0.999.

Table 1 Linearity data

Sr. No.	Concentration(µg/ml)	Absorbance (Mean ± SD)(n=5)	% RSD
1	5	0.2122±0.0019	0.906
2	10	0.4964±0.0020	0.417
3	15	0.7778±0.0023	0.306
4	20	1.0262±0.0019	0.187
5	25	1.2880±0.0029	0.226

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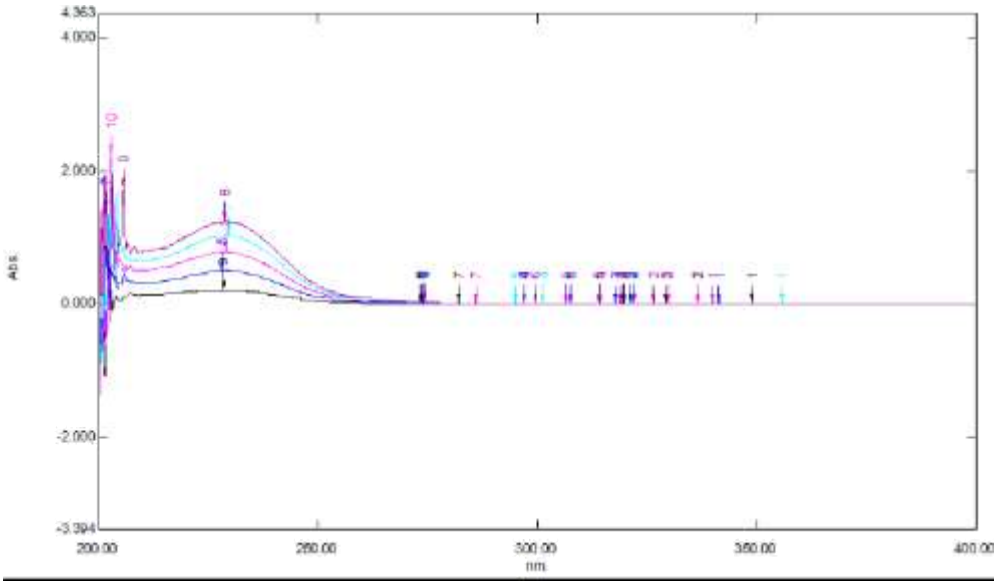

Fig 2. Linearity Graph

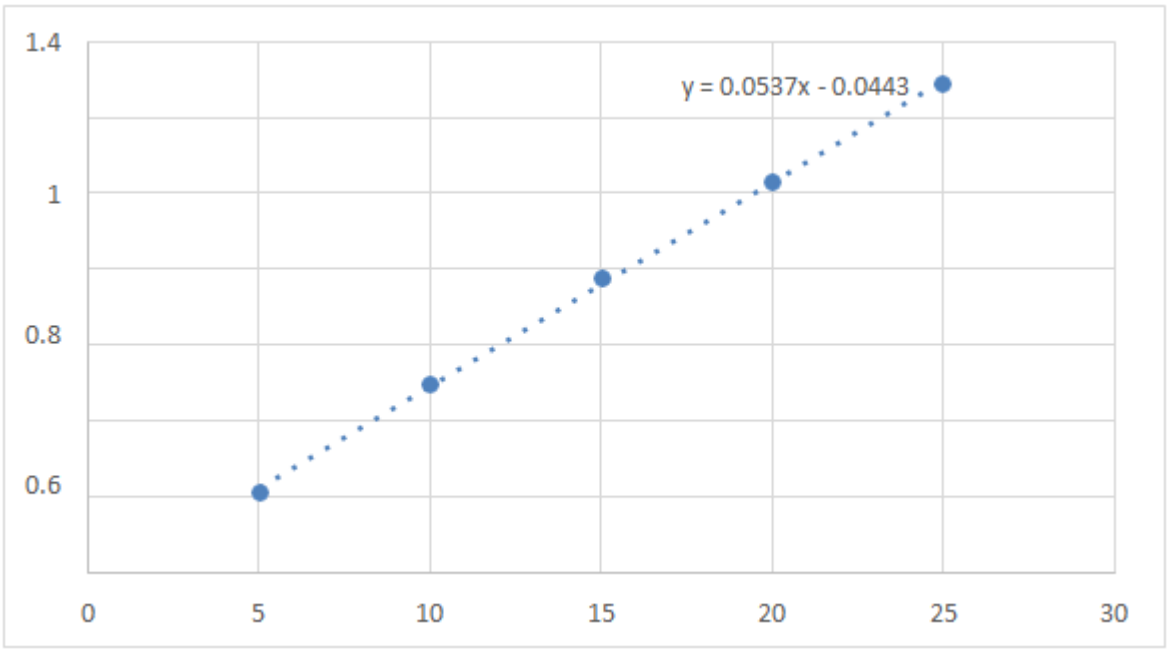

Fig 3 Linearity curve of Remogliflozin Etabonate (5 -25 µg/ml)

Precision:

Repeatability: The data for repeatability for Remogliflozin Etaboanate solution ispresented in table 2.

Table 2 Repeatability data

Concentration (µg/ml)	Absorbance
	0.772

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15	0.775
	0.774
	0.773
	0.774
	0.774
Average	0.773
SD	0.001
% RSD	0.133

Intraday: The data for intraday precision for Remogliflozin Etabonate solution ispresented in table 3.

Table 3 Intraday Precision

Sr. No.	Concentration(µg/ml)	Absorbance (Mean ± SD)(n=3)	% RSD
1	10	0.4853±0.0015	0.314
2	15	0.7753±0.0015	0.197
3	20	0.9963±0.0015	0.153

Interday: The data for intraday precision for Remogliflozin Etabonate solution is presented in table 4

Table 4 Interday data

Sr. No.	Concentration (µg/ml)	Absorbance (Mean ± SD) (n=3)	% RSD
1	10	0.4903±0.0045	0.919
2	15	0.7813±0.0049	0.631
3	20	0.9853±0.0136	1.381

Accuracy: Accuracy of the method was determined by recovery study from formulation at three level of standard addition. The result are shown in table 5.

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Table 5 Accuracy data

Sr. No.	Spiking Level (%)	Concentration of formulation (µg/ml)	Amount spiked (µg/ml)	Total Concentration (µg/ml)	Amount recovered (µg/ml) (Mean ± SD) (n=3)	% Recovered drug	% RSD
1	80	7.5	4.5	12	11.9 ± 0.002	99.25	0.249
2	100	7.5	7.5	15	15.2 ± 0.001	100.01	0.197
3	120	7.5	10.5	18	17.4 ± 0.001	101.06	0.163

Ruggedness: : Ruggedness was checked by different two analyst. The result are shown in table 6.

Table 6 Results of Ruggedness

Concentration (µg/ml)	Absorbance (n=6) by Analyst 1	Absorbance (n=6) by Analyst 2
15	0.844	0.840
	0.849	0.837
	0.85	0.845
	0.852	0.847
	0.856	0.850
	0.855	0.851
Average	0.851	0.845
SD	0.004	0.005
% RSD	0.514	0.656

Robustness: Robustness was determined on different wavelength 227 and 229nm and the result are shown in

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table 7.

Table 7 Results of Robustness

Concentration (µg/ml)	Wavelength(nm)	Sample Absorbance (n=5)	Absorbance (Mean ± S.D)	% RSD
15	227	0.772	0.780±0.006	0.7803
		0.776		
		0.783		
		0.779		
	231			
		0.852	0.845±0.008	0.947
		0.839		
		0.855		
		0.859		
		0.806		

LOD and LOQ : The LOD and LOQ for Remogliflozin Etabonate were found to be 1.961 µg/ml and 4.265 µg/ml resepectively.The results are shown in table 8.

Table 8 Results of LOD and LOQ

Parameter	Value
Mean slope	0.053
SD of intercept	0.031
LOD (µg/ml)	1.961
LOQ (µg/ml)	4.265

Assay: Application of the proposed method for analysis of remogliflozin etabonate in marketed formulation. Assay data of Marketed formulation is shown in table 9.

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Table 9 Assay data of Marketed formulation

Sr.No	Label Claim (µg/ml)	Amount of Drug Found in µg	%Assay
1	100	99.75	99.75
2	100	100.20	100.20
3	100	99.82	99.82
Mean SD(n=3)		99.92±0.02	99.92±0.02
%RSD			0.242

IV. CONCLUSION:

Remogliflozin Etabonate is an Antidiabetic drug used to improve glycemic control in patients with type 2 Diabetes. A sensitive UV spectrophotometric method was developed for the estimation of Remogliflozin Etabonate in bulk and pharmaceutical dosage form. Validation of the developed method was done as per the ICH guidelines Q2 (R1).

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