Original Research Article

Selective SGLT2 Inhibitor's Estimation and Validation from Galenical Form by RP-HPLC Method

ABSTRACT:

Aim: The recent analysis is required to do novel and simple, sensitive, precise, efficient, instant and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method for estimation of antidiabetic drug in the unit dosage form. Validation of this method is also planned to make it suitable for the actual use.

Study Design: Department of Pharmaceutical Chemistry, Datta Meghe Institute of Medical Sciences deemed to be university, Wardha in collaboration with Balkh university, Mazar-e-sharif, Afghanistan between August 2021 and December 2021.

Methodology: In that article develop the method and validate it by estimation of antidiabetic drugs in solid dosage form by RP-HPLC, by using System suitability test, Repeatability, Precision studies (Intra-day and Interday/Intermediate), Linearity/Calibration studies, Robustness, Force degradation, Specificity, Drug recovery/accuracy studies.

Results: as per ICH guidelines, the performance if system suitability in remogliflozin were achieved all guidelines; in that ,tailing factor(*T*),separation factors(α),theoretical plates(*N*),capacity factor (*k'*), resolution (*R*) and RSD (%). The validated stress degradation studies under thermal, oxidative, alkali and acid , in few degradation products for remogliflozin (REM).

Conclusion: From the results we conclude that, this novel technique which validated for exploration by reverse phase high performance liquid chromatography (RP-HPLC) should be used for routine quality control of remogliflozin (REM) prediction from developed formulation.

Key Words:

I.INTRODUCTION

According to previous research work, management of glycaemia control and reducing postprandial glucose excursions means change in concentration from before to after a meal can lower the risk of diabetic complications, e.g. Decrease probability of myocardial infarction means decreases or stops to the coronary artery of the heart, renal disease and retinopathy(1,2).the clinical management of T2DM is still difficult to manage, also with most of patients failing to reach and maintain their target of glycemic levels in practise. (3).Under normal physiological conditions when the glomerular filtrate reaches the proximal tubule, glucose is primarily reabsorbed through the active sodium dependent glucose transporter2 (SGLT2) located on the apical or luminal membrane of the epithelial cell in the S1 segment (4-6). SGLT1 is a high-affinity,low-capacity glucose/galactose co-transporter primarily expressed in the intestine and in the kidney (7,8). Together, SGLT1 and SGLT2 are responsible for the active reabsorption of glucose

across the renal luminal membrane (9,10). In humans, genetic alterations in SGLT2 increase renal glucose excretion (upto200g/day) with no apparent adverse effects on renal function or carbohydrate metabolism (11).

Remogliflozin etabonate is the ester prodrug of remogliflozin(12), whichistheactiveentity that selectively inhibits SGLT2. Remogliflozin etabonate causes a concentration dependent increase in urinary glucose excretion in mice and rats (12, 13). Unlike earlier SGLT inhibitors, such as phlorizin and T-1095, remogliflozin displays a high level of selectivity for SGLT2 over SGLT1 (14, 15).

H3PO4 in fluid, also known as extraction buffer, has been the most frequently used solvent phase. A phosphate buffer's pH can be easily adapted by using mono-, di-, or tribasic phosphate salts. When phosphate salts are used, however, To eliminate non - soluble molecules, solution was stirred through 0.22m filter paper. Other non-UV active acids or bases could be used to alter peak shape and accumulation. (16).

High Performance Liquid Chromatography (HPLC) is more efficient as compaired to gas chromatography (GC) since, it is not limited to volatile and thermally stable samples, and the choice of mobile and stationary phases is wider(17,18).

The pH of the solution is usually the deciding factor when selecting a buffer. The pH range for reversed phase on silica-based filling is typically 2 to 8. It is critical that the buffer has a pH that is close to the requested pH because buffers control pH best at about there pKa. A rule must select a buffer with a pKa value. (19 -23).

Resolution, selectivity, and efficiency are all affected by the mobile phase. An aquatic buffer and a non-UV active water miscible organic solvent make up the mobile phases in reverse phase chromatography. The effects of the lipophilic and hydrophilic phases, as well as the amounts in which they are mixed, will influence the drug molecule's analysis. The synthetic nature of the analyte and the hydrophobicity of the analytes in the combination determine the mobile-phase and gradient parameters, respectively. The aqueous buffers are in a state of flux. The mobile phase protonates free silanols on the column at low pH, which lowers peak tailing and decreases peak tailing. Basic analytes are prepared by the reaction at low pH; when ionised, the particles become ionised. (24).

II.MATERIAL AND METHODS

Thehigh-performance liquid chromatography (HPLC) of Shimadzu SCL-10AVP in built with binary pump(LC-10ATVP),UV detector(SPD-10AVP),Rheodyne20µl loop capacity manual injector(P/N77251) was used throughout the analysis. The LC-Solutions software was used to interpret the HPLC reports. Water-Symmetry® C18, 5µm; 150 x 4.6 mm ID., column purchased from(Newcastle-UK)was used throughout the analysis. Digital weighing balance (ME-204)purchased from Mettler-Toledo(USA), ultra-sonicator Labman®purchased from Ultra Chrom Ltd, India. Digital pH meter from Mettler-Toledo was purchased from(Mumbai- India). 50 µ micro-syringe was purchased from Hamilton USA. 0.20µ and 0.45µ nylon membrane filters were purchased from Phenomenex®Mumbai, India.

2.1Standard stock solutions of REM

Standard stock solutions of REM (1 mg mL-1) were prepared differently by dissolving 10 mg of the drug in acetonitrile-water (2:1 v/v) using a 20 mL volumetric flask and completing the final volume by adjusting with either acetonitrile and water, based on their solubility in particular solvents. Furthermore, freshly A sonicator was used to sonicate the stock solution that had been obtained for 10-20 minutes and filtered through 0.20µ nylon filters. Required serial dilution was made for evaluating the validation studies.

2.2Working Stock Solution

Working stock solution of REM (40 µg mL-1) was made by finishing to volume with the organic phase after samples were diluted of 4 mL of its sample solution in a volumetric flask and diluted.

2.3Chromatography Condition

Separation was carried using an isocratic elution based on water-acetonitrile (40:60, v/v) as a mobile phase on a Water-Symmetry C18 column (150 mm 4.6mm, 5m). At 230 nm, the ultraviolet detector was turned on. Leading up to use, the buffer mixture was filtered through such a 0.2 m nylon membrane filter and sonicated in an ultrasonic bath for 10-20 minutes. The aqueous layer was pumped through the column at a rate of 1.1 mLmin-1. The column temperature was determined to 28 degrees Celsius, and the added dropwise was 20 litres.

2.4Sample preparation for accuracy/drug recovery studies

10-20 Remogliflozin100mg tablets were independently weighed, grinded, and mixed in a mortar. An weighed accurately amount of finely powdered Remo Tablet® 100mg: sonicated until dissolved in 100mL of acetonitrile and water. For each laboratory activity, the solutions were filtered, accompanied by serial dilutions to the mandatory concentration levels using the solvent system and the calibration curve method.

Sample Preparation for Linearity / Calibration studies

Accurately measured aliquots of stock solutions remogliflozin100mg, respectively were transferred separately into a series of 10mL volumetric flasks. The final volume was adjusted with same mobile phase, and then 20µL were injected into HPLC. A calibration curve (linearity graph) was plotted by calculating peak are against concentration.

Precision of the three methods

Three similar concentrations of the remogliflozin 100mg (250ppm) solution were analyzed three times, within the same day (intraday precision). (Table 5,6) Also the mentioned concentrations were analyzed on three successive days using the same procedure to determine the intermediate precision. (Figure 2)

Robustness for the chromatographic method

To analyze the influence of the flow velocity, the solvent system flow rate was changed by 2 decimal points from 1.1 mL.min-1 to 1.3 mL.min-1 and to 0.9 mL.min-1; similarly, the variation of organic modifier used as acetonitrile was changed by 2% from 60% to 62 percent and 58 percent to monitor the peak area as well as retention time. Finally, the effect of wavelength was studied by varying the wavelength from 230 to 223nm and testing and evaluating distinctions in standard calibration characteristics such as retention time, peak tailing, capacity factor, resolution, and theoretical plates.

III.RESULTS

It is the first-time estimation of remogliflozin (REM) was attempted on Water-Symmetry® C18, 5µ column; 150 x 4.6 mm exhibited the good peak symmetry and height. Moreover, it improved the capacity factor (k') and theoretical plates (*T*) at 230 nm UV detection. Importantly, no any article has been published yet to report the analysis of remogliflozin on C18 column. Therefore, this novel analytical method, proved effectively the estimation of remogliflozin with acceptable all criteria given by ICH guidelines and US-FDA. Chromatography.

Parameter-Analytes: Remogliflozin (250ppm), Column: Water-Symmetry C18;5µ,150X4.6mm.ID.Mobile Phase: Water-Acetonitrile; 40:60v/v ,Flow rate:1.1mL.min^{-1} ,Elution mode: Isocratic elution mode, Wavelength selected: 230nm, Temperature: Room temperature, Run time: 10minutesRetention time: remogliflozin (4.90min).(Figure .1.)

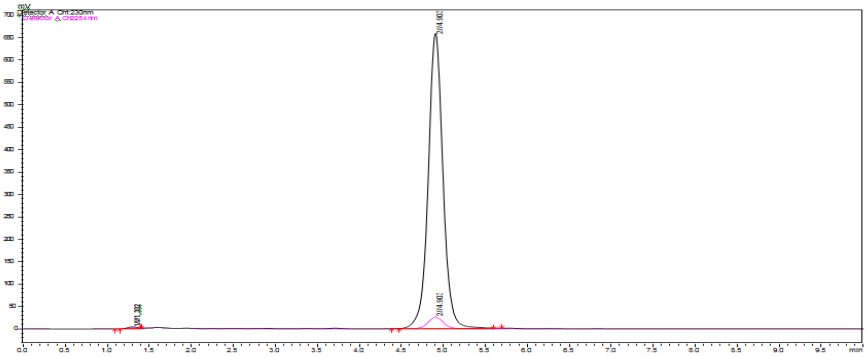


Figure 1: Chromatograph of remoglifliozin (4,90min) at flow rate 1.1 ml.min^{-1}

Peak	Ret. Time	Area	Height	Area%	T Plate	Resolution	k'	Tailing F.	Separation
1	1.336	48103	3643	0.5599	256.416	--	0	1.507	0
2	4.449	8542803	770826	99.4401	3901.801	10.066	2.331	1.054	0

Table 1 Method development parameters of remogliflozin

3.1 SystemsuitabilitytestsforRemogliflozin

System suitability test reveals the factors such as, the oretical plate(N),capacity factor (k'), resolution(R),separation factor (α), tailing factor(*T*),Mean ±SD and RSD % which should in acceptable range for at least 6 successive injections of same analytic. Table No.2 represents the systems suitability studies for remogliflozin.

Table2: System suitability data of remogliflozin

System suitability	Remogliflozin	Acceptable

parameters	(REM)	Values
Theoretical plates (*N*)	3874	> 2000
Capacity Factor (*K'*)	2.766	> 1.5 - <10
Resolution (*R*)	---	≥ *2*
Selectivity/Separation factor (α)	0.00	>*k'*
Asymmetry/Tailing factor (*T*)	1.03	> 2
Retention time (*tR*)	4.903 min.	>*k'*
Wavelength of Detection (nm)	230 nm	> 200 nm
Repeatability (%RSD)	0.20	< 2
Intra-Day Precision (%RSD)	0.20 – 0.84	< 2
Inter-Day Precision (%RSD)	0.07 – 0.88	< 2
Linearity range	15.65 – 250 µg.ml^{-1}	NA
Regression equation	Y= 35958x - 43049	NA
SE of intercept (S_e)	92429.58	NA
SD of intercept (S_a)	41335.76	NA
Correlation Coefficient (r^2)	0.998	NA
LOQ[a] (µg.mL^{-1})	3.79 µg.ml^{-1}	NA
LOD[a] (µg.mL^{-1})	11.49 µg.ml^{-1}	NA

3.2 Method Validation

3.2.1 Repeatability

The freshly prepared stock solution of remogliflozin of same concentrations (250 µg.mL^{-1}), were evaluated for six injections within the same day. The % RSD was calculated and found it is less than 2%; shown in (Table:3).

Table 3: Repeatability data of REM:-

S. No.	Remogliflozin
	PeakArea;Conc. 250ppm
1	8054477
2	8022523
3	8056201
4	8066521
5	8052996
Mean	8050543
STD. DEV.	16537.25
RSD (%)	0.20

3.2.2Intradayprecision

The freshly prepared stock solutionofREMofthreereplicate soother same concentrations;250ppmweretestedand evaluated within the same day (intra-day precision). The %RSD was calculated and found lessthan2%;shownin(Table4).

Table 4: Intraday Precision data of remogliflozin

Drug Name: Remogliflozin (REM)				
S. No.	Concentration (ppm)	Area	STDEV	%RSD
1	250 ppm	8071715	21528.49	0.26
	250 ppm	8029231		
	250 ppm	8044410		
	250 ppm	8151020		
	250 ppm	8261006		

2	250 ppm	8278611	69145.16	0.84
3	250 ppm	8164001	16684.59	0.20
	250 ppm	8135719		
	250 ppm	8134523		
Range of %RSD				0.20 – 0.84

3.2.3 Interday (intermediate)precision

The stock solution of REM of three replicates of three different concentrations; 250 ppm, were tested and evaluated in three successive days (interday/intermediate precision). The %RSD was calculated and found less than 2%; shown in (Table 5).

Table 5: Interday (intermediate) Precision data of remogliflozin

Drug Name: Remogliflozin (REM)				
S. No.	**Concentration (ppm)**	**Area**	**Mean ± SD**	**%RSD**
DAY 1	250 ppm	8194200	15201.58	0.18
	250 ppm	8185512		
	250 ppm	8164624		
DAY 2	250 ppm	8051316	6104.97	0.07
	250 ppm	8039132		
	250 ppm	8045913		
DAY 3	250 ppm	8131021	72354	0.88
	250 ppm	8231003		
	250 ppm	8271612		
Range of %RSD				0.07 – 0.88

The above-mentioned concentrations were analyzed on three successive days using, the procedure mentioned under section. The % RSD was calculated and the results are shown in (Table 2).

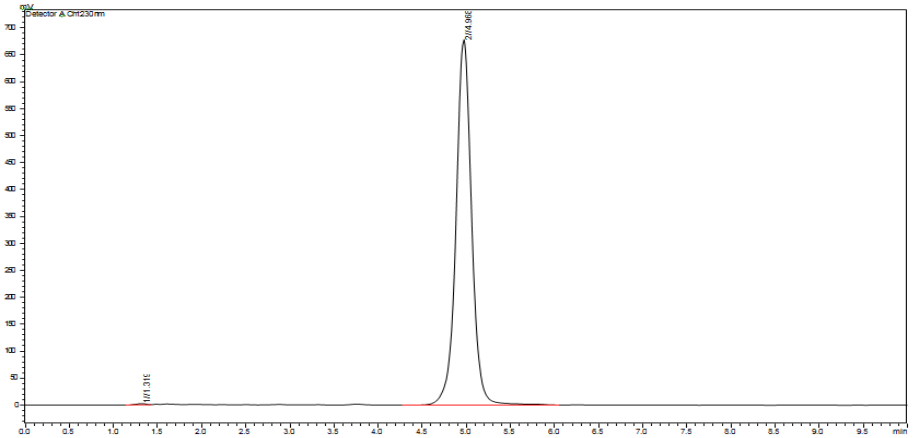


Figure 2: Remoglflozin 250 PPRECD 2nd

3.2.4. Linearity

Under linearity or calibration studies, a line relationship between are under peak values and selected drug concentration (µg.mL.min^{-1})was plotted for five chosen concentrations of each drug. The linearity of the calibration curves was validated by the high value of correlation coefficient, acceptable values of regression coefficient, standard deviation of the slope and standard deviation of the intercept; shown in (Table:6).

Limit of detection (LOD) which represents the concentration of analyte at S/N ratio of 3.3 and limit of quantification (LOQ) at which S/N is10 were determined and results are given in (Table :6). Low values of LOD and LOQ indicate sensitivity of the applied method for determination of the mentioned drugs in tablets.

Table .6; Linearity data of remogliflozin

Name of Drug**; Remogliflozin (REM)**			
S. No.	Concentration (µg.mL-1)	Area	Average (Mean)
	250 PPM	8962176	

1	250 PPM	8949721	8955948
2	125 PPM	4411430	4494852
	125 PPM	4578275	
3	62.5 PPM	2124505	2092642
	62.5 PPM	2060779	
4	31.25 PPM	931288	969040
	31.25 PPM	1006792	
5	15.62 PPM	682023	689029
	15.62 PPM	696035	
6	Regression Equation		Y= 35958x - 43049
7	Correlation coefficient (R^2)		0.998
8	Std. Error of intercept		92429.58
9	Std. Dev. of intercept		41335.76
10	LOQ		3.79 µg.ml^{-1}
11	LOD		11.49 µg.ml^{-1}

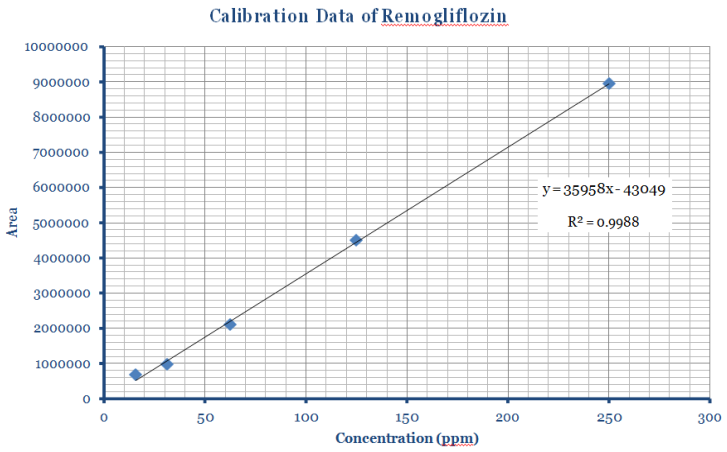


Figure 3: Calibration curve of remogliflozin

3.2.5. Robustness for the chromatographicmethod

From all above studies, after making deliberated changes in flow rate (± 0.2mL.min^{-1}), organic modifier concentration; acetonitrile (±2%) and wavelength(±2nm)have not made any significant changes in resolution, capacity factor and tailing factor. None the less, it seems minute changes in robustness studies makes significant changes in the cortical plate counts. Robustness studies for REM displayed in tableNo.8. Finally, the wavelength was changed by±2nm wavelength and results were reported in Table7.

Table 7: Robustness data of Remogliflozin

Content	F. (-0.2 ml.mL^{-1})	F (+0.2 ml.mL^{-1})	A (-2 ml)	A (+2 ml)	WL (-2 nm)	WL (+2 nm)
Resolution	----	-----	----	----	----	----
Tailing factor	1.05	0.97	1.01	0.99	1.01	1.01
Capacity factor	2.32	3.31	2.77	2.75	2.76	2.75
Theoretical plates	3834	4259	3835	4174	3999	4027

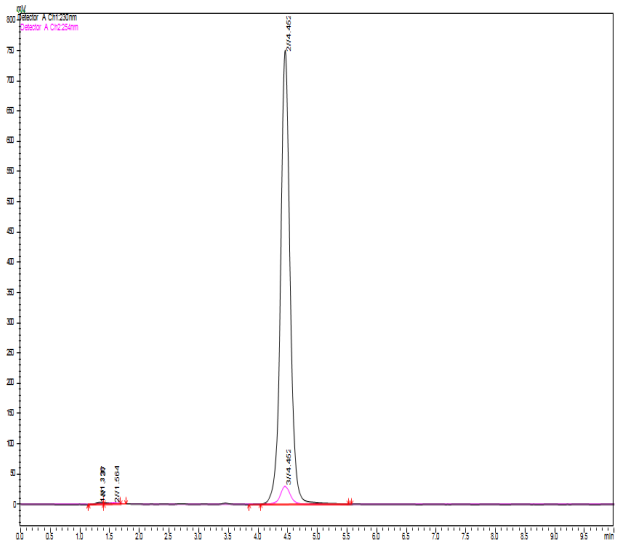
Table No. 8: Effect of flow rate on remogliflozin

Peak #	Ret. Time	Area	Height	Area %	T.Plate#	Resolution	k'	Tailing F.	Separation
1	1.337	43155	3225	0.5115	208.989	--	0	1.487	0
2	4.452	8393031	751322	99.4885	3834.979	9.473	2.329	1.051	0

Table.9: effect of organic modifier (-2%) on Remogliflozin

Peak #	Ret. Time	Area	Height	Area%	T.Plate #	Resolution	k'	Tailing F.	Separation

1	1.596	24385	2410	0.2314	479.882	--	0	--	0
2	1.783	4543	928	0.0431	582.844	0.638	0.117	--	0
3	6.02	10507628	706789	99.7255	3835.829	12.384	2.772	0.998	23.618



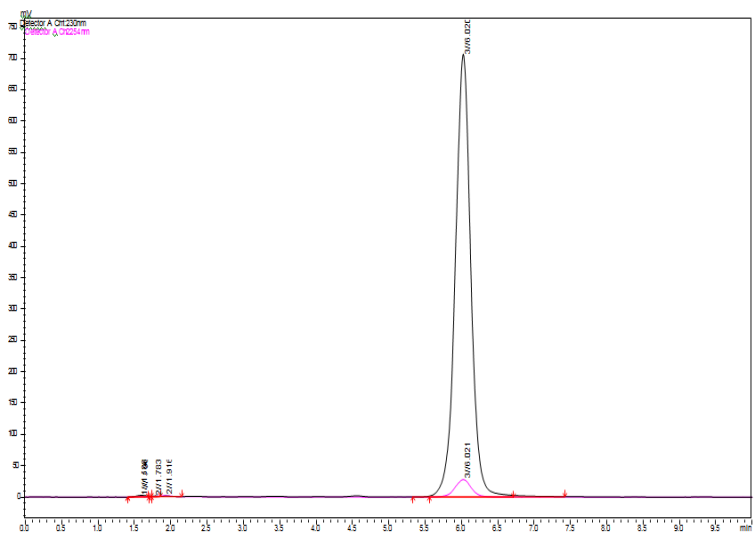


Figure 4: Chromatograph of REM (4.45 min) at flow rate 0.9 mL. min^{-1}Figure 5: robustness studies for REM

3.2.6 Accuracy

Accuracy of the results was calculated by % recovery of 5 different concentrations of each drug. The results including the mean of the recovery and standard deviation are shown in (Table 2).

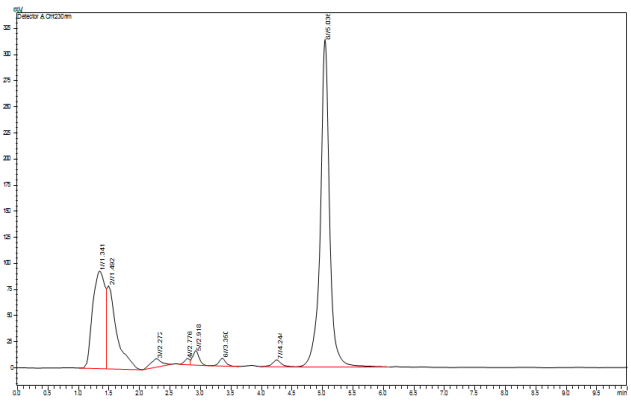
Table 10: Accuracy data of REM

Conc. (%)	S. No.	S. amt. (µg.mL^{-1})	D. added (µg.mL^{-1})	Amt. rec. (µg.mL^{-1})	% recovery	Mean±SD	% RSD
80%	1	100	80	169.45	94.14	97.11±2.47	2.57
	2	100	80	177.99	98.89		
	3	100	80	171.14	95.32		

100%	1	100	100	187.33	93.67	94.60±1.38	1.46
	2	100	100	187.89	93.94		
	3	100	100	192.41	96.20		
120%	1	100	120	214.63	97.56	97.14±0.85	0.88
	2	100	120	211.56	96.16		
	3	100	120	214.99	97.72		

Table11: Force degradation studies

	Remogliflozin (REM)		Degradants of REM
Conditions	% Area Std.	% degradation	No. of degradants
Acid (0.1N/M HCl) + 60˚C + 12 Hrs.	56%	44%	7
Base (0.1N/M NaOH) + 60˚C + 12 Hrs.	69%	30%	1
Thermal (60˚C) + 12 Hrs.	87%	13%	7
Oxidation (3-6% H_2O_2) + Room Temp.	10%	90%	1 at t0



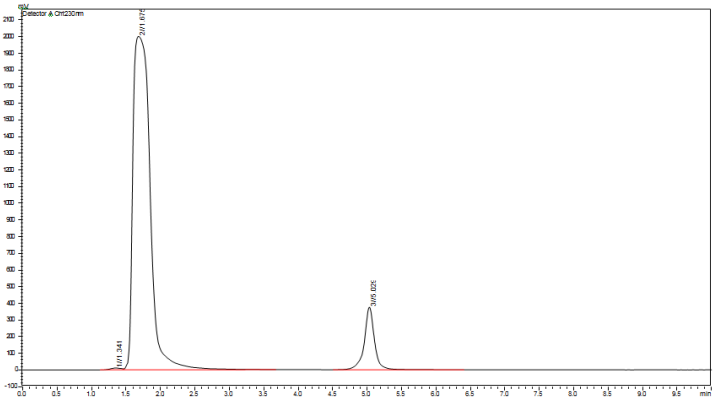


Figure No.6: REM force degradation studies at 0.1N HCl **Figure No. 7: REM force degradation studies with 3% H_2O_2**

IV.DISCUSSION

From all above results and discussion, it has be enconcluded that the developed analytical method for the estimation of remogliflozin (REM) in both bulk and tablet formulation has obliged the ICH guidelines. As per the ICH guidelines,the developed method has complied the linearity range (calibration data), accuracy/drug recovery studies (%), repeatability, precision studies (intraday and interday/intermediate), and robustness. Moreover, as per the ICH guidelines, the system suitability test performed for remogliflozin has achieved all guidelines; including, tailing factor(*T*),separation factors(α),the cortical plates(*N*),capacity factor (*k'*), resolution (*R*) and RSD (%). The validated stress degradation studies under thermal, oxidative, alkali and acid as certained few degradation products for remogliflozin (REM).

V.CONCLUSION

Above all results we, may conclude that, this developed and validated method for investigation by reverse phase high performance liquid chromatography (RP-HPLC) can Above all results we, may conclude that be used for routine analysis of estimation of remogliflozin (REM) from marketed formulation.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Holman, R. et al. 2008. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J

Med,359:1577–1589.

2. Lancet. 1998. Effect of intensive blood glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group, 352:854–865.
3. Giugliano, D. et al. 2009. Is the current therapeutic armamentarium in diabetes enough to control the epidemic and its consequences? What are the current shortcomings? Acta Diabetology, 46:173–181.
4. Kanai, Y. et al. 1994. The human kidney low affinity Na+/glucose co-transporter SGLT2. Delineation of the major renal resorptive mechanism for D-glucose. Journal of Clinical Investigation, 93:397–404.
5. Wells, RG. et al. 1992. Cloning of a human kidney cDNA with similarity to the sodium-glucose-transporter. American Journal of Physiology,263:459–F465.
6. You, G. et al., 1995. Molecular characteristics of Na (+)-coupled glucose transporteosinadultandembryonicratkidney.JournalofBiologicalChemistry,70: 29365–29371.
7. Bakris, GL.etal.2009. Renal sodium-glucose transport: role in diabetes mellitus and potential clinical implications.KidneyInt,75:1272–1277.
8. Nishimura,M.&Naito,S.2005.Tissue-specific mRNA expression profiles of human ATP-binding cassette and solute carrier transporter super families. Drug Metabolism and Pharmacokinetics, 20:452–477.
9. Idris, I. & Donnelly, R. 2009. Sodium-glucose co-transporter-2 inhibitors: an emerging new class of oral antidiabetic drug. DiabetesObesity&Metabolism,11:79– 88.

   Wright, EM. & Turk E.2004. The sodium/glucose co-transport family SLC5. Pflueger'sArchives, 447:510–518.
10. Santer,R.etal. 2003. Molecular analysis of the SGLT2 gene in patients with renal glucosuria. Journal of American Society of Nephrology,14:2873–2882.
11. Fujimori, Y.2008. Remogliflozin etabonate, in a novel category of selective low- affinity sodium glucose co-transporter (SGLT2) inhibitors, exhibits antidiabetic efficacy in rodent models.Journal of Pharmacological Expert Therapy,327:268–276.
12. Harrington, WW. et al. 2008. Remogliflozin etabonate, potent and selective sodium-dependent glucose transporter 2 antagonists, produced sustained metabolic effects in zucker diabetic fatty rats. Diabetes, 58:529-535.

13. Wright, EM. 2002. Molecular basis for glucose-galactose malabsorption. Cellular Biochemistry and Biophysics, 36:115–121.
14. Sigafoos, et al. 2012. Assessment of the Drug Interaction Risk for Remogliflozin Etabonate, a Sodium-Dependent Glucose Cotransporter-2 Inhibitor: Evidence from In Vitro, Human Mass Balance, and Ketoconazole Interaction Studies. Drug Metabolism and Disposition, 40: 2090–2101.
15. Kaushal, C. and Srivastava, B.2010. A Process of Method Development: A Chromatographic Approach.J Chem Pharm Res,2(2):519-545.
16. Lindholm,J.2004. Development and Validation of HPLC Method for Analytical and Preparative Purpose. Acta Universities Upsaliensis Uppsala: 13-14.
17. Jeffery, GH., Bassett, J., Mendham, J., Denny, RC., Vogel's Textbook of Quantitative Chemical Analysis, fifth edition, Long man scientific & technical:213-220.
18. Understanding pH Buffers: which one to use, and at what concentration: available from:www.laserchrom.co.uk.
19. Technical Tips: Selecting Buffers pH in Reversed-phase HPLC: available from: download.5117.com/data/file/30.pdf
20. Reversed-phase HPLC Buffers: High Quality Buffers (solutions, solids or concentrates): available from:ccc.chem.pitt.edu/wipf/web/HPLC_RP_buffers.pdf.
21. Buffers and Buffering Capacity: available from:www.bartek.ca.
22. Chandra, M. Buffers: A guide for the preparation and use of buffers in biological system: available from:www.calbiochem.com.
23. Ngwa, G.2010. Forced Degradation Studies. Forced Degradation as an Integral part of HPLC Stability Indicating.