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SIMULTANEOUS QUANTIFICATION AND FORCE DEGRADATION STUDIES OF REMOGLIFLOZIN, EVOGLIPTIN, AND METFORMIN BY HILIC TECHNIQUE

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Abstract: This research paper presents a study on the simultaneous quantification and force degradation analysis of Remogliflozin, Evogliptin, and Metformin using the hydrophilic interaction liquid chromatography (HILIC) technique. The solubility of the three drugs was determined, and acetonitrile was selected as the solvent for further analysis. The wavelength of 210 nm was chosen for analysis due to the highest absorption peak intensities. Stock standard solutions were prepared for each drug, and working standard solutions were then prepared for the quantification studies. The HILIC technique, specifically using the Acclaimed Mix-Mode HILIC-1 column, was employed for the separation of the analytes. Various mobile phases were tested, and the optimized chromatographic conditions were determined. The linearity of the method was evaluated, and calibration curves were constructed for each drug. The accuracy of the method was assessed by analyzing samples from marketed formulations, and the results showed high drug recovery within the acceptable range. The validation parameters, including accuracy, precision, sensitivity, robustness, specificity, and selectivity, were evaluated according to ICH guidelines. The developed HILIC method demonstrated reliable quantification of Remogliflozin, Evogliptin, and Metformin in bulk samples and exhibited suitable analytical performance for future applications.

Index Terms **- Remogliflozin, Evogliptin, Metformin, HILIC, simultaneous quantification, force degradation, validation.**

1. INTRODUCTION

In recent years, there has been an increasing prevalence of type 2 diabetes mellitus (T2DM), a chronic metabolic disorder characterized by hyperglycemia and insulin resistance. The management of T2DM involves the use of multiple drugs targeting different aspects of glucose metabolism to achieve optimal glycemic control. Remogliflozin, evogliptin, and metformin are three commonly prescribed antidiabetic drugs that have demonstrated efficacy in lowering blood glucose levels through different mechanisms of action.

Remogliflozin belongs to the sodium-glucose co-transporter 2 (SGLT2) inhibitor class, which inhibits glucose reabsorption in the kidneys, leading to increased urinary glucose excretion. Evogliptin, on the other hand, is a dipeptidyl peptidase-4 (DPP-4)

inhibitor that enhances the action of incretin hormones, stimulating insulin secretion and reducing glucagon levels. Metformin, a biguanide, works by suppressing hepatic glucose production and improving insulin sensitivity in peripheral tissues.

Simultaneous quantification of multiple antidiabetic drugs in pharmaceutical formulations and biological samples is essential for assessing their individual and combined effects in the treatment of T2DM. Additionally, force degradation studies play a crucial role in evaluating the stability and potential degradation pathways of these drugs under various stress conditions, providing important information for their formulation and storage.

Analytical techniques play a pivotal role in the quantification and degradation studies of pharmaceutical compounds. In this study, the hydrophilic interaction liquid chromatography (HILIC) technique is employed for the simultaneous quantification and force degradation studies of remogliflozin, evogliptin, and metformin. HILIC offers distinct advantages over other chromatographic techniques, particularly for polar and hydrophilic compounds. By utilizing a polar stationary phase and a mobile phase with a high organic content, HILIC allows for efficient separation and retention of highly polar and ionic analytes.

The HILIC technique offers improved selectivity, sensitivity, and resolution for the simultaneous determination of these antidiabetic drugs. This method is particularly suitable for analyzing the highly polar metformin, ionic polar evogliptin, and neutral polar remogliflozin in a single analysis. Moreover, the HILIC technique enables the use of organic modifiers such as methanol or acetonitrile, which further enhances the separation efficiency of the target compounds.

The objectives of this study are to develop a robust and reliable HILIC method for simultaneous quantification of remogliflozin, evogliptin, and metformin, and to investigate their force degradation behavior under various stress conditions. The simultaneous determination of these drugs will provide valuable insights into their co-administration, potential drug-drug interactions, and stability profiles. Ultimately, this research aims to contribute to the advancement of pharmaceutical analysis in the field of diabetes management, supporting the development of safe and effective antidiabetic therapies.

2. MATERIALS AND METHODS

Solubility Study: The solubility of metformin, remogliflozin, and evogliptin was investigated in various solvents. Acetonitrile and methanol were found to be suitable solvents as they exhibited good solubility for all three compounds. Therefore, acetonitrile was selected for further analysis.

Selection of Wavelength for Analysis: The wavelength of 210nm, which corresponded to the highest absorption peak intensities of metformin, remogliflozin, and evogliptin, was chosen for their estimation. Subsequent simultaneous determinations were performed at this wavelength.

Preparation of Stock Standard Solution: Separate stock standard solutions of metformin, remogliflozin, and evogliptin were prepared. Approximately 100mg of each compound was accurately weighed and dissolved in two different 100ml calibrated volumetric flasks containing 25ml of acetonitrile. The solutions were sonicated for 10 minutes and then made up to the calibrated mark with acetonitrile to obtain a concentration of 1000µg/ml for each compound.

Preparation of Working Standard Solution: Working standard solutions of metformin, remogliflozin, and evogliptin were prepared by diluting an accurate volume of the stock standard solutions. Specifically, 4.0 mL of remogliflozin was diluted with 10 mL of solvent to obtain a concentration of 40µg/mL, 8.0 mL of evogliptin was diluted with 10 mL of solvent to obtain a concentration of 80µg/mL, and 4.0 mL of metformin was diluted with 10 mL of solvent to obtain a concentration of 40µg/mL for remogliflozin, evogliptin, and metformin, respectively.

Selection of Chromatographic Layer: The hydrophilic interaction liquid chromatography (HILIC) technique was chosen for the quantification and force degradation studies. Previous attempts using a reverse phase technique with C18 columns did not achieve the desired separation. Considering the polar nature of metformin, along with the ionic polar evogliptin and neutral polar remogliflozin, a moderately higher composition of organic modifiers such as methanol or acetonitrile was deemed necessary.

Selection of Analytical Column: The acclaimed Mix-Mode HILIC-1 column (5µ, 150 x 4.6 mm id) was utilized throughout the analysis.

Selection of Solvent System: Homologous mixtures of metformin, remogliflozin, and evogliptin were eluted using various mobile phases. The following solvent systems were evaluated: i) Solvent A: 15mM ammonium acetate, Solvent B: MeOH-ACN (20:80 v/v), **Column:** Zodiac C18 (5µ, 150 x 4.6 mm id) ii) Solvent A: 10mM ammonium formate, Solvent B: MeOH-ACN

(20:80 v/v), Column: Zodiac C18 (3µ, 150 x 4.6 mm id) iii) Solvent A: 15mM ammonium acetate, Solvent B: Acetonitrile (100%), Column: Acclaimed Mix-Mode HILIC-1 (5µ, 150 x 4.6 mm id) iv) Solvent A: 15mM ammonium acetate, Solvent B: AA-ACN (25:75% v/v), pH 5.5, Column: Acclaimed Mix-Mode HILIC-1 (5µ, 150 x 4.6 mm id)

Prior to application, the solvents were sonicated for 30 minutes and filtered through 0.45µ nylon membrane filters purchased from Phenomenex® Mumbai, India.

Optimization of Solvent System: The solvent system consisting of 15mM ammonium acetate (Solvent A) and AA-ACN (25:75% v/v) (Solvent B) provided a well-optimized chromatogram. The column used was Acclaimed Mix-Mode HILIC-1 (5µ, 150 x 4.6 mm id) with a pH of 5.5. Gradient elution was performed using an isocratic elution mode with 20mM AA-ACN (25:75%) as the mobile phase at a flow rate of 1 mL/min.

Optimization of a solvent system

Finally get the good optimized chromatogram with 15 Mm ammonium acetate B; AA-ACN (25:75% v/v) as a mobile phase, Column: Acclaimed Mix-Mode HILIC-1(5µ, 150 x 4.6 mm id); pH 5.5. Gradient elution: isocratic elution mode with 20mM AA-ACN(25:75). Flow rate 1 mL/min.

3. RESULTS AND DISCUSSION

The chromatographic separation was performed on Acclaimed Mix-Mode HILIC-1(5µ, 150 x 4.6 mm id) at an ambient temperature. The samples were eluted using 15 Mm ammonium acetate B; AA-ACN (25:75% v/v) as a mobile phase at a flow rate 1.0mL/minute. The common wavelength of absorption of metformin, remogliflozin and evogliptin was found to be 210nm. The chromatograms of the prepared standard stock solutions of remogliflozin, evogliptin and metformin were recorded under optimized chromatographic conditions.

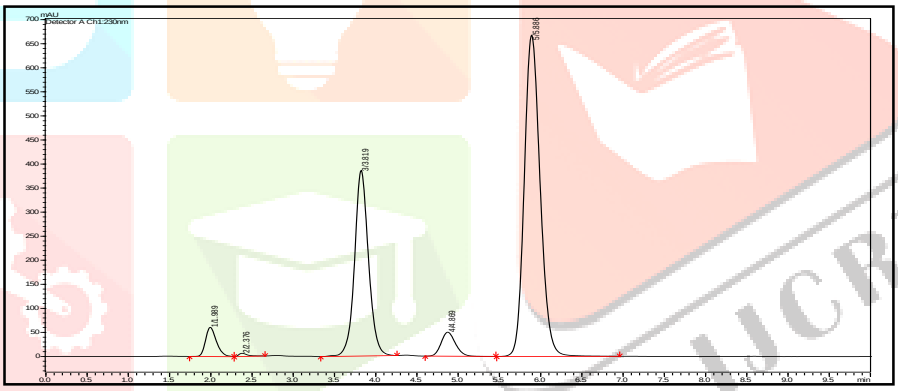

Figure No. 3.4a.i: Optimized Spectra of remogliflozin, evogliptin and Metformin

Table No. 3.4.1: Optimized chromatographic criterion

Chromatographic Mode	Chromatographic Conditions
HPLC System	Shimadzu SCL-10A$_{VP}$ inbuilt with binary pump (LC-10AT$_{VP}$), UV detector (SPD-10A$_{VP}$), Rheodyne 20µl/10µl loop capacity manual injector (P/N 77251)
Detector	UV (DAD)
Column	Acclaimed Mix-Mode HILIC-1(5µ, 150 x 4.6 mm id)
Mobile phase	15 Mm ammonium acetate B; AA-ACN (25:75% v/v)
Detection wavelength	210 nm
Flow rate	1.0 ml/min.
Temperature	Ambient
Injection Volume	10 µL
Data analysis	Empower 3 software

3.4.10 System suitability parameters

The homogenous mixture of freshly prepared stock solution of equal concentration of remogliflozin, evogliptin and metformin were injected 6 times to determine the closeness of results achieved for relative standard deviation (RSD) in percentage; The calculated values should always less than 2%. Moreover, other system suitability parameters including, retention

or capacity factor (k'), resolution (Rs) and theoretical plates (N), tailing factor/peak asymmetry (As) and separation factor were tested and evaluated.

Table No. 3.4.2: System suitability parameters

Parameters	Remogliflozin (REM)	Evogliptin (EVO)	Metformin (MET)
Retention time(Rt) min	3.81 min.	4.86 min.	5.88 min.
Theoretical Plates	2452	3601	4381
Tailing factor	1.08	1.18	1.17
Resolution (*R*)	---	3.31	2.99

3.4.11 Linearity or Range

The linearity/calibration studies of HPLC-DAD method represents its ability to explicit the results that should proportional to the known concentration of studied analytes within the selected range of 20, 10, 5, 2.5 and 1.25 µg/ml, 5, 2.5, 1.25, 0.75 and 0.37 µg/ml and 100, 50, 25, 12.5 and 6.25 µg/mL against the peak area (mAu) for REM, EVO and MET. Therefore, over the known concentrations of REM, EVO and MET their corresponding area were found highly proportional since as noted their regression coefficients (R^2) were exactly 1 for remogliflozin and metformin and 0.999 for evogliptin **(Figure No. 3.4b.i- 3.4b.iii and Table No. 3.4.3- 3.4.5).**

Table No. 3.4.3: Linearity data of remogliflozin

Name of Drug remogliflozin		
S. No.	**Concentration (µg.mL^{-1})**	**Peak Area**
1	20	2776398
2	10	1403351
3	5	713758
4	2.5	379636
5	1.25	190592
Regression Equation		y = 137521x + 26959
Correlation coefficient (R^2)		1
Std. error of intercept		4789.20779
Std. Dev. Of intercept		10708.99418
LOQ		0.78µg/ml
LOD		0.23µg/ml

n= number of determinations

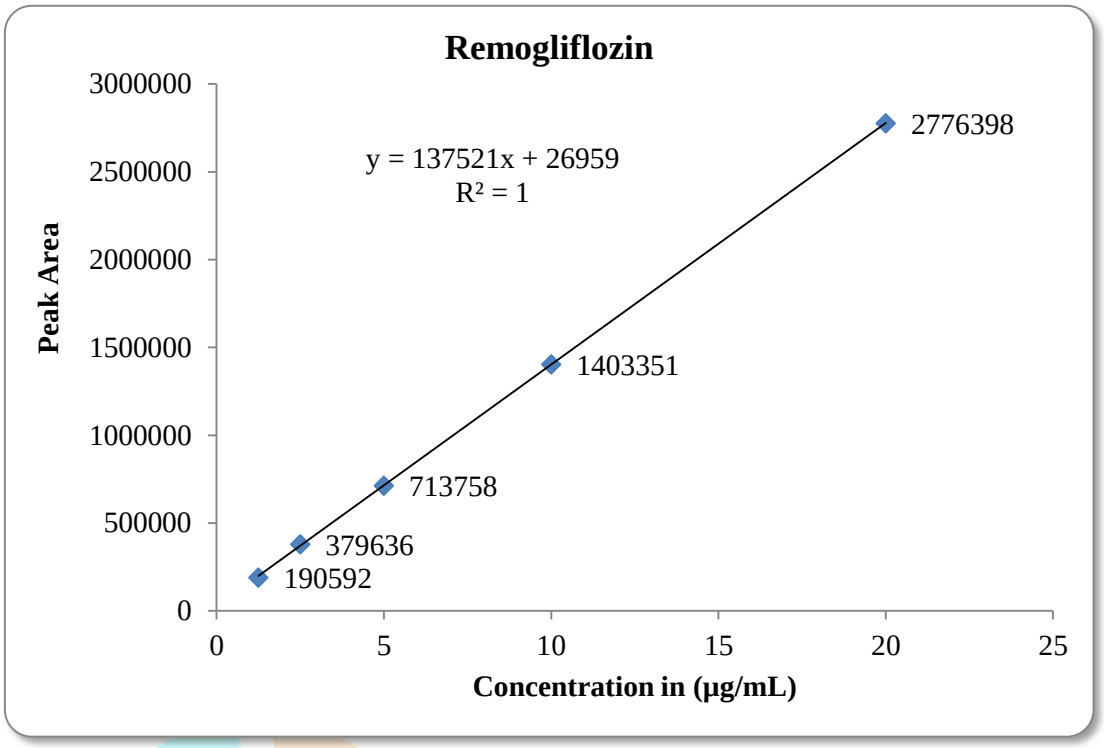

Figure No. 3.4b.i; Linearity spectra of Remogliflozi

Table No. 3.4.4: Linearity data of evogliptin

Name of Drug evogliptin		
S. No.	**Concentration (µg.mL^{-1})**	**Peak Area**
1	5	370734
2	2.5	183959
3	1.25	102954
4	0.75	48822
5	0.37	26304
Regression Equation		y = 73032x + 5055.1
Correlation coefficient (R^2)		0.9992
Std. error of intercept		3113.822334
Std. Dev. Of intercept		7627.275868
LOQ		0.43µg/ml
LOD		0.13µg/ml

n= number of determinations

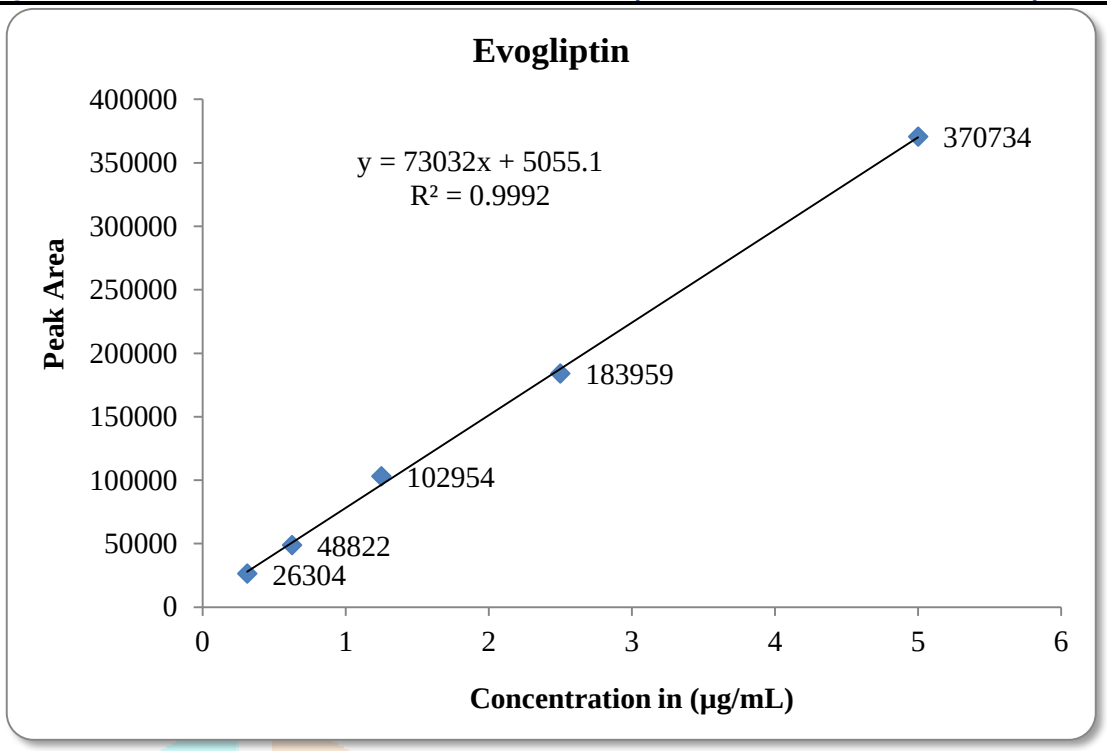

Figure No. 3.4b.ii: Linearity spectra of Evogliptin

Table No. 3.4.5: Linearity data of metformin

Name of Drug: metformin		
S. No.	**Concentration (µg.mL^{-1})**	**Area**
1	100	5579866
2	50	2804013
3	25	1438872
4	12.5	752037
5	6.25	379477
Regression Equation		y = 55304x + 47830
Correlation coefficient (R^2)		1
Std. error of intercept		8895.354744
Std. Dev. Of intercept		21789.0802
LOQ		1.61µg/ml
LOD		0.48µg/ml

n= number of determinations

3.4.12 Analysis of bulk samples

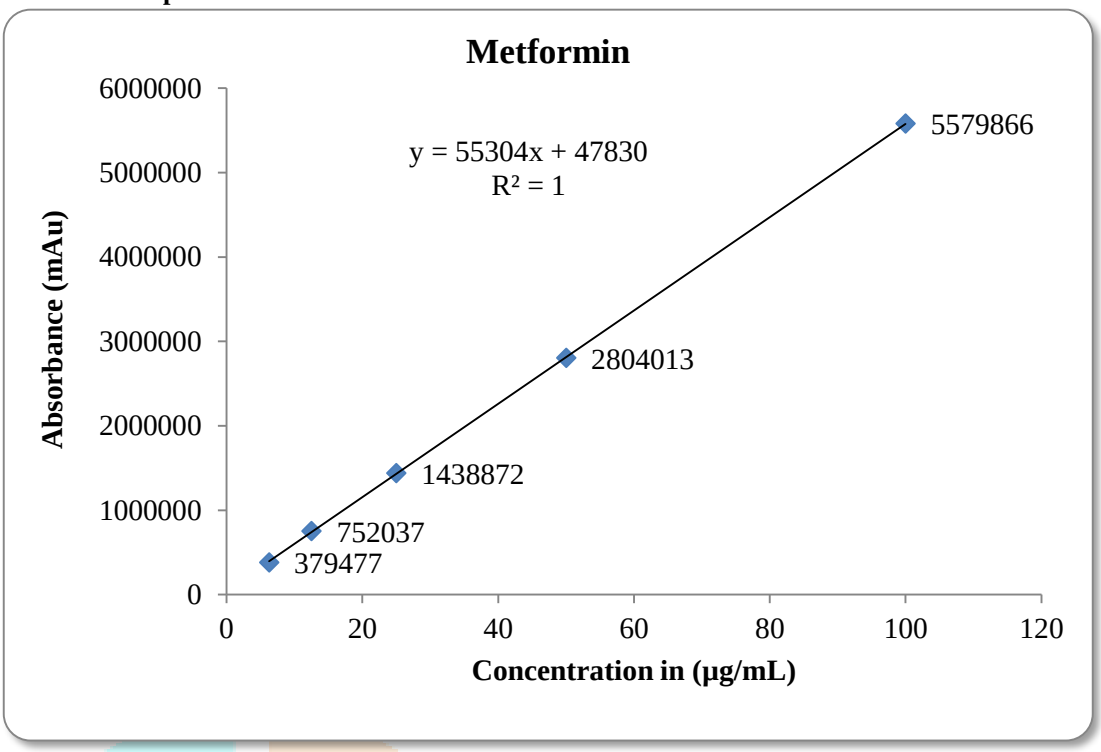

Figure No. 3.4b.iii: Linearity spectra of metformin

Remogliflozin, Evogliptin and Metformin (20mg 5mg and 100mg), were accurately weighed and transferred in 100 mL of the calibrated volumetric flask; solubilized in acetonitrile, and the volume was diluted to the mark of a calibrated volumetric flask with same to have 200 µg/ml, 50 µg/ml and 1000 µg/ml concentrations of Remogliflozin, Evogliptin and Metformin. The suitable volumes (1.0ml dilute to 10ml) of this were diluted with a solvent system to get the final concentrations of 20 µg/ml, 5 µg/ml and 100 µg/ml of Remogliflozin, Evogliptin and Metformin that was analysed according to the procedure of chromatographic conditions; the peak areas of both analytes were estimated, and the findings are presented in **Table No 3.4.6.**

Table No. 3.4.6: Analysis (Assay) of Remogliflozin, Evogliptin and Metformin in bulk sample

Drugs	Amount taken [µg/mL]	Amount found [µg/mL] ± SD	% Amount found	% RSD {n=6}
Remogliflozin	20	19.97 ± 0.03	99.97 ± 0.32	0.42
Evogliptin	5	5.02 ± 0.19	100.08 ± 0.08	0.35
Metformin	100	101.09± 0.11	101.09± 0.11	0.57

n= number of determinations

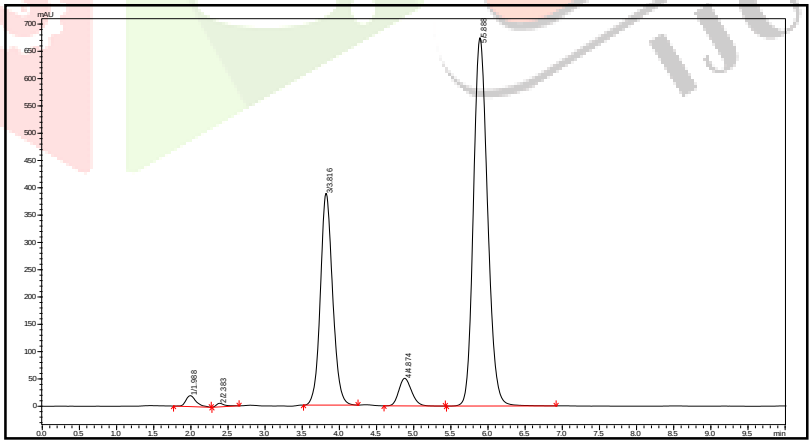

Figure No. 3.4c.i: Analysis (Assay) spectra of Remogliflozin, Evogliptin and Metformin in bulk sample

3.4.13 Validation:-

The design HILIC method for Remogliflozin, Evogliptin and Metformin was explored for accuracy, precision (intra- and inter-day, and repeatability), sensitivity (LOD and LOQ), robustness, specificity, and selectivity ICH reference.

A. Accuracy

Percentage drug accuracy of three different concentrations; 80%, 100% and 120% (injected thrice) to estimate the Remogliflozin, Evogliptin and Metformin from marketed formulation and results obtained have been reported in **Table No. 3.4.7- Table No. 3.4.9.** Accuracy can be studied by applying the calibration curve; the Y-intercept and the slope of the graph were used to determine the % drug recovery, attributed to the developed method for the simultaneous quantification of selected drugs or by comparing with similar concentration of reference standard.

As resulted, the achieved drug recovery of Remogliflozin, Metformin and Evogliptin, Metformin were in the range of 96.82-99.58, 100.16-102.18 and 96.38-101.09, respectively. As recommended by International conferences of Harmonization (ICH) guidelines the drug recovery should be within the range and the RSD in percentage should be less than 2%. Hence, the

calculated drug recoveries for simultaneous estimation of Remogliflozin, Evogliptin and Metformin represents the drug recovery were in the acceptance limit given by ICH guidelines.

able No. 3.4.7: Accuracy data of Remogliflozin

Drug Name: Remogliflozin etabonate			Drug content: 100 mg		Marketed formulation; Remo MV 500 Tablet		
Std. conc. (%)	**Std. (µg/ml)**	**Peak area**	**Drug (%)**	**Drug (µg/ml)**	**Peak area**	**Avg. peak area**	**Drug Rec. (%)**
100%	20 µg/ml	4537971	80	16	3560816	3514965	96.82
				16	3469114		
			100	20	4451020	4518725	99.58
				20	4586429		
			120	24	5341224	5362623	98.48
				24	5384021		
			Drug recovery Range (%) as per ICH = 100±10%				96.82–99.58%

Table No. 3.4.8: Accuracy data of Evogliptin

Drug Name: Evogliptin			Drug content: 5 mg		Marketed formulation: Valera M 500 Tablet		
Std. conc. (%)	**Std. (µg/ml)**	**Peak area**	**Drug (%)**	**Drug (µg/ml)**	**Peak area**	**Avg. peak area**	**Drug Rec. (%)**
100%	5	600834	80	4	488131	488131	101.55
				4	493441		
				4	482821		
			100	5	603526	613932	102.18
				5	624338		
				5	613932		
			120	6	724231	722127	100.16
				6	722127		
				6	720023		
			Drug recovery Range (%) as per ICH = 100±10%				100.16– 102.18%

Table No. 3.4.9: Accuracy data of Metformin

Drug Name: Metformin			Drug content: 500 mg		Marketed formulation; Remo MV 500 Tablet		
Std. conc. (%)	**Std. (µg/ml)**	**Peak area**	**Drug (%)**	**Drug (µg/ml)**	**Peak area**	**Avg. peak area**	**Drug Rec. (%)**
100%	100	8964174	80	80	7142691	7082173	98.76
				80	7021655		
				80	7082173		
			100	100	8928364	9061489.5	101.09
				100	9061489		
				100	9194615		
			120	120	10367834	10367834	96.38
				120	10714037		
				120	10021632		
			Drug recovery Range (%) as per ICH = 100±10%				96.38– 101.09 %

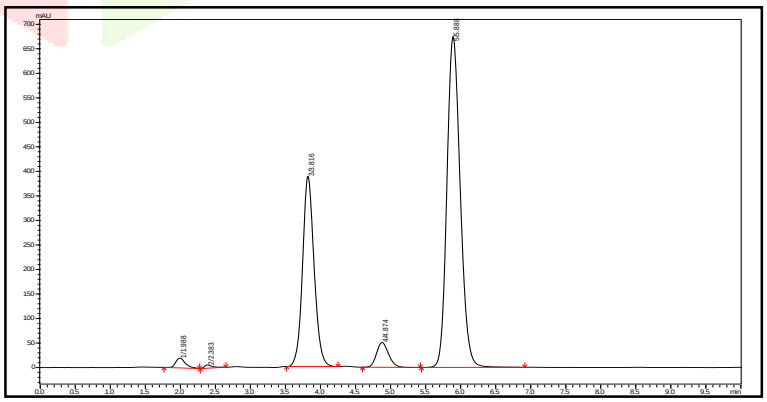

Figure No. 3.4d.i: Accuracy spectra by HILIC analysis of marketed formulation of Remo MV 500 (Concentration 80%)

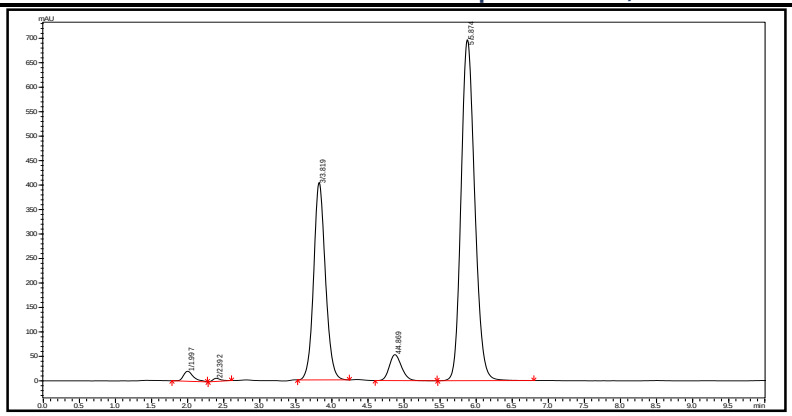

Figure No. 3.4d.ii: Accuracy spectra by HILIC analysis of marketed formulation of Valera M 500 (Concentration 100%)

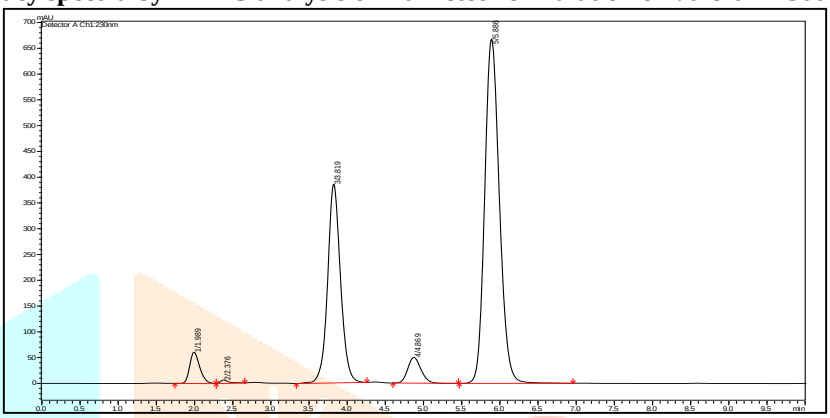

Figure No. 3.4d.iii: Accuracy spectra by HILIC analysis of marketed formulation of Remo MV 500 (Concentration 120%)

B. Repeatability studies

Implementing the procedure under chromatographic condition of experimental section, the homologous mixture of remogliflozin (40 selected analytes was injected six times with similar procedure within a same day. The% RSD was calculated and found it is less than 2% for remogliflozin (0.33%), evogliptin (1.30%) and metformin (0.41%).

Table No. 3.4.10: Repeatability data of REM, EVO and MET

Sr. No.	Peak Area; Conc. 20 µg/ml	Peak Area; Conc. 5 µg/ml	Peak Area; Conc. 100 µg/ml
1	4237971	560834	8964174
2	4230084	580207	8975041
3	4213869	561320	8901334
4	4200808	564947	8921736
5	4234048	564161	8907204
6	4221243	570623	8976044
Mean	**4223003**	**567015.3333**	**8940922.167**
STD. DEV.	**13966.5256**	**7349.915011**	**34671.40728**
RSD (%)	**0.33**	**1.30**	**0.41**

C. Precision (Intraday & Interday)

The precision of HILIC method represents its closeness to the agreement among the series of repetitive results, derived after multiple sampling of the same homogenous mixture of selected drugs under the given conditions. As displayed in **Table No. 3.4.11**; for intermediate variability for precision studies, this method is significantly precise over the testing range of remogliflozin, evogliptin and metformin. Moreover, the peak area of all studied samples was also correlated with selected concentration since as observed their percentage relative standard deviation (RSD) was less than 2%. Thus it reflects, the proposed method has acceptable precision with minimum variations and can be applicable for routine analysis.

C. i. Intraday (intermediate) precision studies of REM, EVO and MET:

Implementing the chromatographic procedure mentioned under experimental section, the homologous mixture of REM, EVO and MET of three replicates of similar concentrations; were tested within a same day. The percentage RSDs for all three drugs was calculated and they were found less than 2%. The results were shown in **Table No. 3.4.11- Table No. 3.4.13.**

Table No. 3.4.11: Intraday precision data of REM

Drug Name: Remogliflozin						
S. No.	**Concentration (µg/ml)**	**Area**	**Amount found(µg/ml)**	**% Amount found**	**Mean ± SD**	**% RSD**
1	20	4237971	19.96	99.81	12288.459	0.29
	20	4230084	19.97	99.98		
	20	4213869	20.07	100.38		
2	20	4200808	20.13	100.69	16765.315	0.40
	20	4234048	19.98	99.90		
	20	4221243	20.04	100.20		
3	20	4390298	19.84	97.20	37533.234	0.84
	20	4351020	19.00	95.03		
	20	4326059	19.69	99.46		
Range of %RSD						**0.29 - 0.84**

Table No. 3.4.12: Intraday precision data of EVO

Drug Name: Evogliptin						
S. No.	**Concentration (µg/ml)**	**Area**	**Amount found(µg/ml)**	**% Amount found**	**Mean±SD**	**%RSD**
1	5	560834	4.86	97.61	11047.383	1.95
	5	580207	4.94	98.88		
	5	561320	5.07	101.38		
2	5	564947	5.21	104.2	3525.9092	0.62
	5	564261	4.96	99.25		
	5	570623	5.03	100.6		
3	5	599450	4.94	98.88	7626.4402	1.26
	5	560526	4.85	97.53		
	5	564117	4.92	98.66		
Range of %RSD						**0.62 -1.95**

Table No. 3.4.13: Intraday precision data of MET

Drug Name: Metformin						
S. No.	**Concentration (µg/ml)**	**Area**	**Amount found (µg/ml)**	**% Amount found**	**Mean ± SD**	**%RSD**
1	100	8928364	99.60	99.60	39790.44931	0.47
	100	9061489	101.08	101.08		
	100	9194615	102.57	102.57		
2	100	8921736	99.52	99.52	36284.71553	0.43
	100	9007204	100.40	100.40		
	100	8976044	100.13	100.13		

3	100	8918273	99.48	99.48	70393.48489	0.79
	100	8928364	99.60	99.60		
	100	9044930	100.90	100.90		
Mean % RSD						**0.43 - 0.79**

C. ii. Interday (intermediate) Precision studies of REM, EVO and MET

Implementing the chromatographic procedure mentioned under experimental section (5.3), the homologous mixture of REM, EVO and MET of three replicates of similar concentrations (100 µg/ml) were tested and evaluated for three successive days (interday/intermediate precision). Furthermore, the percent RSD was calculated and found it is less than 2%; for all selected analytes in simultaneous HPLC-UV analysis **(Table No. 3.4.14- Table No 3.4.16)**

Table No. 3.4.14: Interday (intermediate) precision data of REM

Drug Name: Remogliflozin				
Sr. No.	**Concentration (µg/ml)**	**Area**	**Mean ± SD**	**%RSD**
DAY 1	20	4490298	37533.234	0.84
	20	4451020		
	20	4526059		
DAY 2	20	4585701	37429.70483	0.82
	20	4586439		
	20	4521243		
DAY 3	20	4544530	49524.65625	1.10
	20	4451020		
	20	4526059		
	Range of % RSD			**0.82-1.10**

Table No. 3.4.15: Interday (intermediate) Precision data of evogliptin

Drug Name: Evogliptin				
Sr. No.	**Concentration (µg/ml)**	**Area**	**Mean ± SD**	**%RSD**
DAY 1	5	599450	7626.440257	1.26
	5	603526		
	5	614217		
DAY 2	5	621967	1881.010455	0.30
	5	624338		
	5	620623		
DAY 3	5	617772	7414.895167	1.21
	5	603526		
	5	614217		
	Range of % RSD			**0.30 -1.26**

Table No. 3.4.16: Interday (intermediate) Precision data of metformin

Drug Name: metformin				
Sr. No.	**Concentration (µg/ml)**	**Area**	**Mean ± SD**	**%RSD**
DAY 1	100	8918273	70393.48489	0.79
	100	8928364		
	100	9044930		
DAY 2	100	8821736	36284.71553	0.43
	100	8907204		
	100	8876044		
DAY 3	100	9194615	133468.3649	1.47
	100	8928364		
	100	9044930		
	Range of % RSD			**0.43 -1.47**

D. Sensitivity

Furthermore, the limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the response and the slope of the regression equation; shown in **Table No. 3.4.3- Table No. 3.4.5.** As observed, the LOD 0.80 µg/ml, 1.60 µg/ml and 0.30 µg/ml and LOQ were 2.17 µg/ml, 5.34 µg/ml and 1.01 µg/ml for REM, EVO and MET respectively. These results signify that the selected wavelength 210 nm is more sensitive for remogliflozin and metformin and apparently less sensitive for evogliptin. However, this lower UV sensitivity was also observed for some other gliptin class of drugs including evogliptin, sitagliptin and saxagliptin.

Thus, the proposed method can be used for the routine HILIC analysis of either individual or simultaneous analysis of selected drugs from pharmaceutical drugs or biological fluids.

E. Robustness

Robustness of any HILIC method represents its ability to remain unaffected by small but deliberate changes in certain separation factors to ascertain its reliability during routine HILIC analysis. The variation in separation factors such as effect of temperature, flow rate, wavelength, column length, stationary phase particle size, pH, organic modifier composition in mobile

phase and injection volume have been considered. The effects of all these variables over changes in retention pattern including effects on capacity/retention factor (k'), resolution (Rs), tailing factor (*Tf*), separation factor, theoretical plates (N) and peak area can be monitored.

In this method, robustness studies was established by making deliberate changes in flow rate (1.0 ± 0.1 ml/minutes), organic modifier as acetonitrile (75±2% ml), and wavelength (210±2nm). As shown in results **Figures 3.4e.i -3.4e.vi**; variation in flow rate and organic modifier have made slight changes in retention pattern like increase in flow rate and organic modifier have reduce the retention time, retention factor and resolution whereas decreasing the same variables have marginally extended the retention time, capacity/retention factor (k'), resolution (Rs). As noted, these variations have not made any significant changes in theoretical plates and tailing factor of all selected drugs.

Therefore, as displayed in all **Figures (Fig 3.4e.i -3.4e.vi)** and **tables (Tables 3.4.17-3.4.25)** the robustness studies for simultaneous estimation of remogliflozin, evogliptin and metformin were almost unchanged which clearly depicts that the proposed HILIC method obliged all minimum requirements led by the ICH guidelines.

Table No. 3.4.17: Robustness data of remogliflozin

Variables	Remogliflozin			
	tR(min)	k'	Tf	N
Flowrate(+0.2mL.min-1)	3.16	0.92	1.14	2446
Flowrate(-0.2mL.min-1)	5.08	1.01	1	2755
Acetonitrile (+2%)	3.63	0.81	1.16	2491
Acetonitrile (-2%)	4.04	1.02	1.08	2541
Temperature(+2˚C)	4.04	1.04	0.98	2706
Temperature(-2˚C)	4.04	1.04	1.01	2730
Wavelength (-) 208nm	3.85	1.02	0.99	2706
Wavelength (+) 212nm	4.07	0.97	1.04	2541
Mean±S.D.	4.00 ± 0.63	0.97 ± 0.09	1.06 ± 0.08	

Table No. 3.4.18: Robustness data of evogliptin

Variables	Evogliptin				
	tR (min)	k'	Tf	Rs	N
Flowrate(+0.2mL.min-1)	4.04	1.46	1.2	3.36	3615
Flowrate(-0.2mL.min-1)	6.54	1.58	1.18	3.59	3755
Acetonitrile (+2%)	4.57	1.28	1.22	3.18	3670
Acetonitrile (-2%)	5.26	1.63	1.17	3.58	3815
Temperature(+2˚C)	5.22	1.63	1.18	3.57	3627
Temperature(-2˚C)	5.22	1.63	1.18	3.58	3635
Wavelength (-) 208nm	5.26	1.58	1.17	3.21	3755
Wavelength (+) 212nm	5.04	1.42	1.23	3.52	3670
Mean±S.D.	5.14 ± 0.84	1.54 ± 0.14	1.19 ± 0.02	3.48±0.17	

Table No. 3.4.19: Robustness data of metformin

Variables	Metformin				
	tR(min)	k'	Tf	Rs	N
Flowrate(+0.2mL.min-1)	4.89	1.97	1.18	2.98	4216
Flowrate(-0.2mL.min-1)	8.08	2.17	1.17	3.32	4814
Acetonitrile (+2%)	5.43	1.72	1.21	2.73	4300
Acetonitrile (-2%)	6.46	1.16	1.09	3.58	4744
Temperature(+2˚C)	6.42	2.24	1.18	3.32	4645
Temperature(-2˚C)	6.42	2.24	1.18	3.32	4677
Wavelength (-) 208nm	5.88	2.02	1.15	3.21	4774
Wavelength (+) 212nm	6.39	1.98	1.26	3.23	4808
Mean±S.D.	6.28 ± 1.09	1.92 ± 0.42	1.17 ± 0.04	3.21 ± 0.30	

Table No. 3.4.20: Robustness studies, effect of flow rate 1.2 ml/min

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.
1	1.644	151277	18092	1.2321	885.499	--	0	1.455
2	1.971	43244	6429	0.3522	1922.323	1.631	0.199	1.728
3	3.164	3848261	405655	31.3415	2446.837	5.477	0.925	1.144
4	4.049	526843	53101	4.2908	3615.224	3.367	1.462	1.202
5	4.896	7708842	692296	62.7834	4296.881	2.984	1.978	1.187

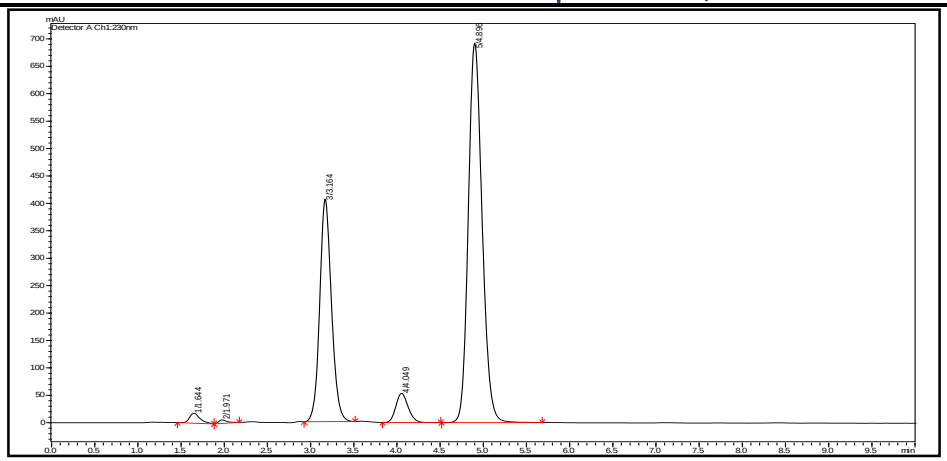

Figure No. 3.4e.i: Effect of flow rate 1.2 ml/min on REM, EVO and MET

Table No. 3.4.21: Robustness studies, effect of flow rat 0.8 ml/min

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.
1	2.53	284120	23870	1.4357	1143.558	--	0	1.579
2	3.055	68088	6208	0.3441	2124.637	1.862	0.208	1.647
3	5.086	6206012	411322	31.3605	2755.32	6.221	1.01	1.009
4	6.548	848840	52961	4.2894	3755.931	3.59	1.588	1.187
5	8.028	12382193	707638	62.5703	4814.898	3.325	2.173	1.179

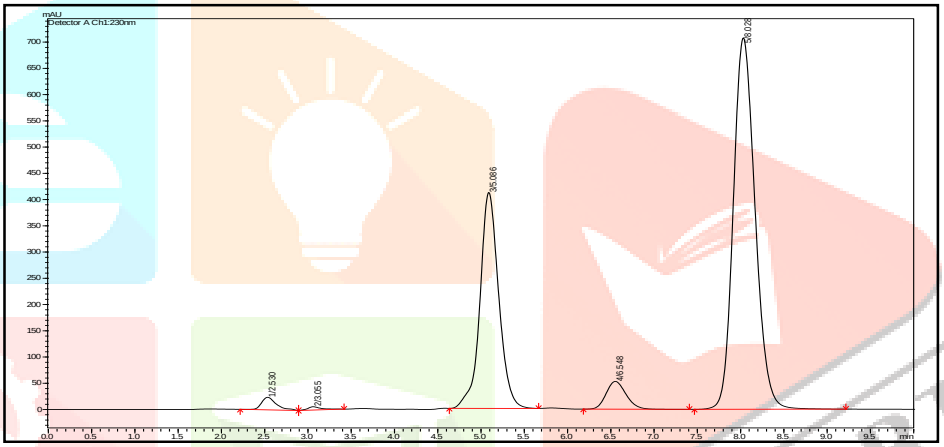

Figure No. 3.4e.ii: Effect of flow rate 0.8 ml/min on REM, EVO and MET

Table No. 3.4.22: Robustness studies, effect of solvent B composition

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.
1	1.998	175517	17893	1.1629	1084.075	--	0	1.44
2	2.381	55813	7071	0.3698	2205.04	1.721	0.192	1.867
3	3.63	4785571	437938	31.707	2491.996	5.059	0.817	1.164
4	4.573	639760	57470	4.2388	3670.855	3.181	1.289	1.221
5	5.439	9436430	758374	62.5215	4300.471	2.732	1.722	1.21

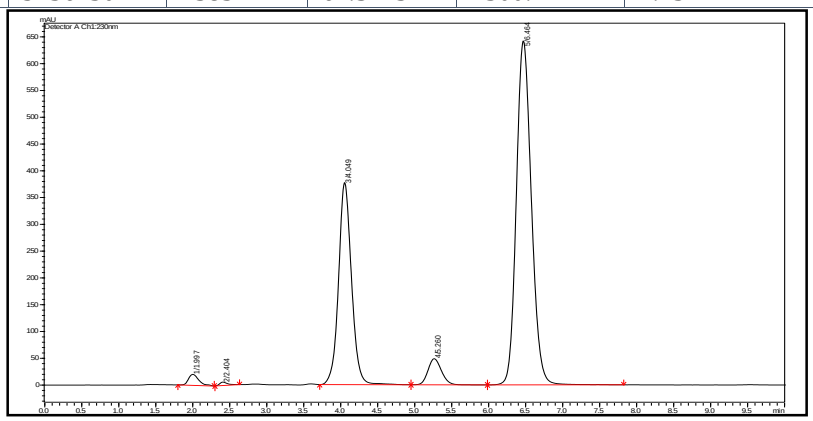

Figure No. 3.4e.iii: Effect of organic modifier, solvent B composition %on REM, EVO and MET

Table No. 3.4.23: Robustness studies, effect of solvent B composition

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.
1	1.997	198575	20444	1.3663	989.569	--	0	1.543
2	2.404	49094	6473	0.3378	2294.858	1.788	0.204	1.646

3	4.049	4596240	377042	31.6242	2541.499	6.303	1.027	1.089
4	5.26	616131	48690	4.2393	3815.825	3.66	1.634	1.177
5	6.464	9073903	642042	62.4325	4744.747	3.364	2.237	1.166

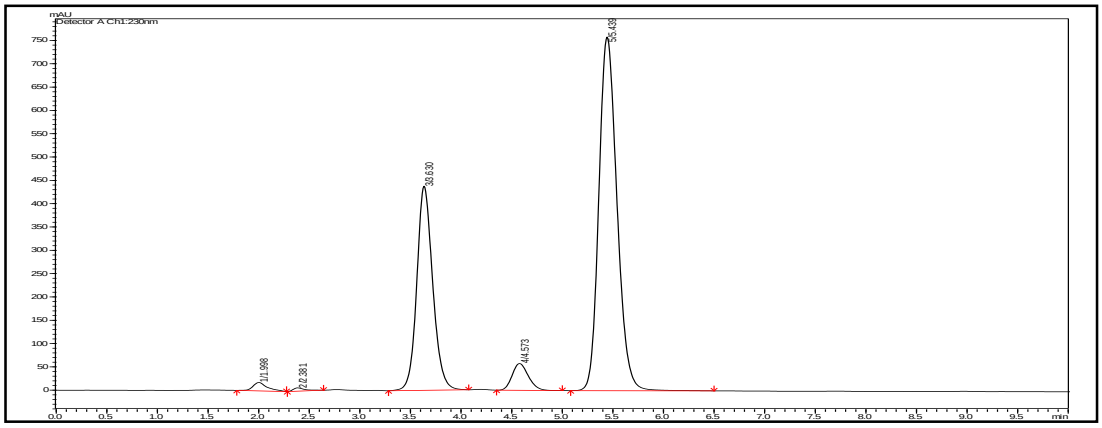

Figure No. 3.4e.iv: Effect of organic modifier, solvent B composition % on REM, EVO and MET

Table No. 3.4.24: Robustness studies, effect of wavelength 208 nm

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.
1	1.983	172980	17150	1.2035	976.995	--	0	1.566
2	2.394	60524	6952	0.4211	1995.019	1.759	0.208	2.053
3	4.049	4355617	359836	30.3043	2706.049	6.295	1.042	0.983
4	5.225	631573	48587	4.3942	3627.766	3.571	1.635	1.186
5	6.427	9152221	642366	63.6769	4645.491	3.32	2.242	1.182

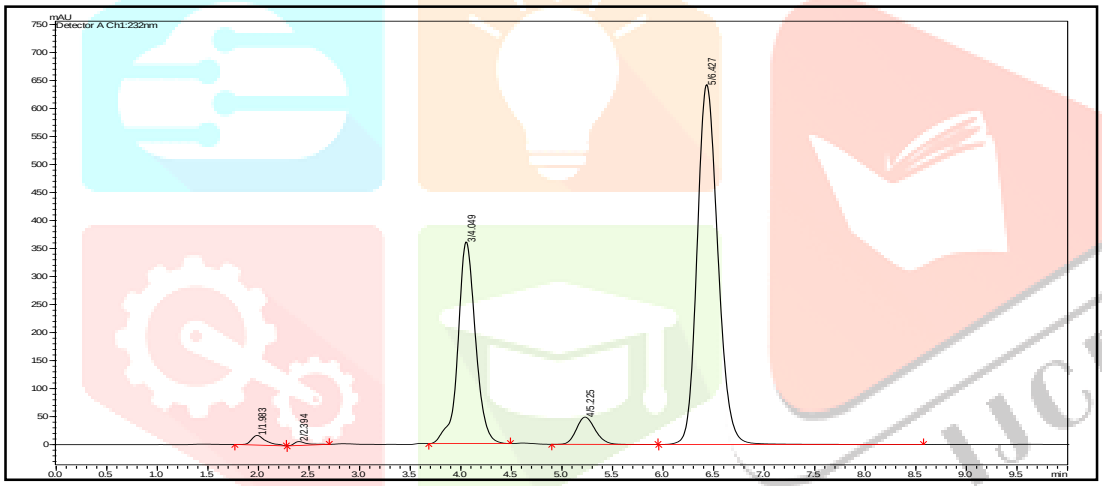

Figure No. 3.4e.v: Effect of wavelength at 208 nm on REM, EVO and MET

Table No. 3.4.25: Robustness studies, effect of wavelength 212 nm

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.
1	1.983	157713	15576	0.9564	969.424	--	0	1.62
2	2.395	62045	7094	0.3763	2001.375	1.756	0.208	2.05
3	4.049	5389574	449089	32.6851	2730.955	6.316	1.042	1
4	5.225	696304	53571	4.2227	3635.399	3.58	1.635	1.186
5	6.427	10183769	718273	61.7595	4677.243	3.327	2.241	1.184

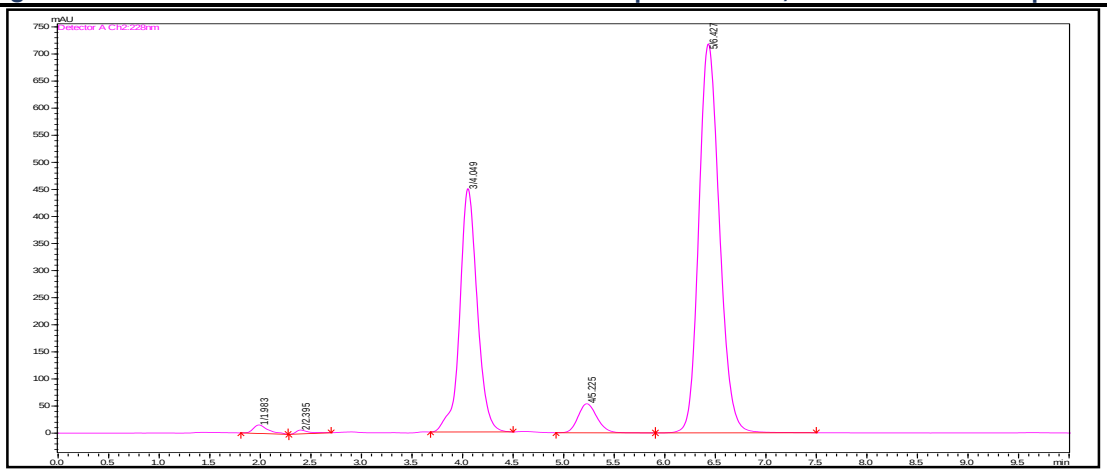

Figure No. 3.4e.vi: Effect of wavelength at 212 nm on REM, EVO and MET

F. Specificity and selectivity

Specificity is the ability to assess analytes of interest in the presence of component that may be expected to be present, such as impurities, degradants and matrix compound but there is no such compound was found. Selectivity of the analysis reduces interference from other compound in the sample matrix. The proposed method is quite good specific and selective. It was noticed that there was no specific intervention seen around Rt of both the drug and baseline exhibits a sustainable noise. Purity spectra are shown in **Figure No. 3.4f.i-3.4f.iii** for remogliflozin, evogliptin and metformin respectively.

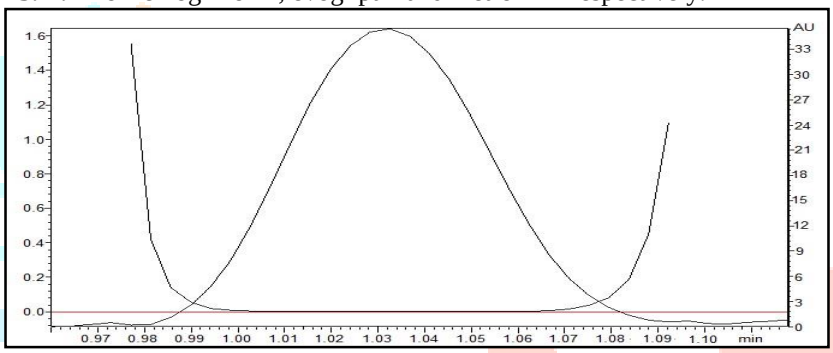

Figure No. 3.4f.i: Peak purity spectra of Remogliflozin

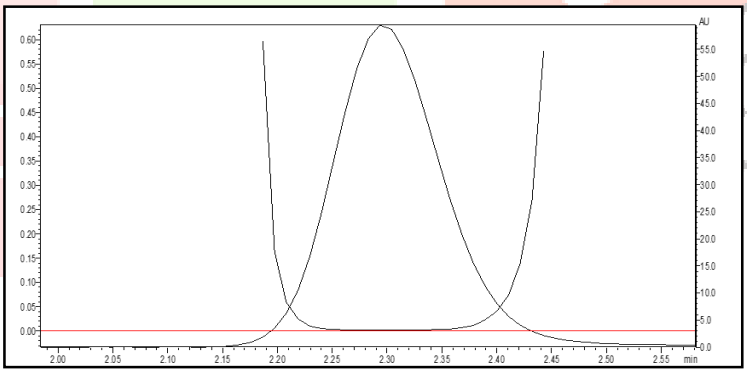

Figure No. 3.4f.ii: Peak purity spectra of Evogliptin

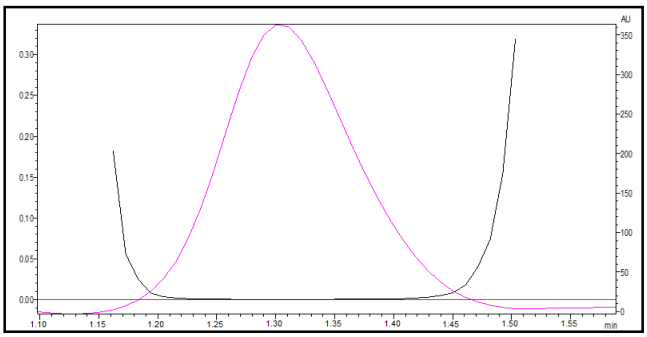

Figure No. 3.4f.iii: Peak purity spectra of Metformin

3.4.14 Force degradation studies

The present UHPLC method was used to address the intrinsic stability behaviour of the Sitagliptin and Metformin under distinct conditions of stressors. It was investigated according to the Q1A (R2) guideline of ICH references for hydrolysis, oxidation, thermal (dry heat and wet heat stress), and photolysis as per the references of Q1B. The stressors, the preference of their concentration, and samples' processing were predicated on a pre-developed research experiment. Subsequently, the Sitagliptin and Metformin were practically insoluble in water; thus, the stress studies were initiated by dissolving the stressor in methanol. The slight changes in mobile phase composition and flow rate were made to resolve all the potential degradants.

The forced degradation studies using HILIC technique revealed the possible degradation of selected drugs; remogliflozin, evogliptin and metformin under the given stress conditions including effects of acid (0.1N HCl) alkali (0.2 N NaOH), peroxide 3% H_2O_2), and thermal condition (45-60˚C).

A. Acidic hydrolysis

Acidic hydrolytic stress studies for Remogliflozin, Evogliptin and Metformin were carried out by precisely solubilizing 10 mg of Remogliflozin, Evogliptin and Metformin separately into calibrated flask consisting of 10 mL of 0.2 M methanolic HCl for remogliflozin and 0.1 M methanolic HCl for evogliptin and metformin. The resulting solutions were moved into a 50 mL RBF, attached with a reflux condenser, and refluxed at 45°C for three hour to 12 hours for remogliflozin, evogliptin and 60°C for 45 min to 12Hrs for metformin a thermostatic water bath. Adequate aliquots of stress-induced remogliflozin, evoglitpin and metformin samples (1.0 mL) were withdrawn and subjected to neutralization with equal concentrations of 0.2 M and 0.1 M methanolic NaOH solution. Afterward, 0.1 mL of resulting solutions were diluted with a solvent system to obtained the concentrations of 10 µg/mL Remogliflozin, Evogliptin and Metformin was addressed as per the design UHPLC method. The acid hydrolysis chromatograms are depicted in **Figure 3.4g.i.**

B. Alkaline hydrolysis

Alkaline hydrolytic stress studies for Remogliflozin, Evogliptin and Metformin were investigated using precisely solubilizing 10 mg of Remogliflozin, Evogliptin and Metformin separately into calibrated flask consisting of 10 mL of 0.2 M methanolic NaOH. Resulting solutions of Remogliflozin, Evogliptin and Metformin were preserved in the dark at room temperature for 3 days to avoid a certain level of substantial degradation due to light. Adequate aliquots of stress-induced Remogliflozin, Evogliptin and Metformin samples were withdrawn (1.0 mL) were withdrawn and subjected to neutralization with equal concentration 0.2 M methanolic HCl solution. Afterward, 0.1 mL of resulting solutions were diluted with a solvent system to obtain the concentration of 10 µg/mL Remogliflozin, Evogliptin, and Metformin was addressed. The chromatogram is depicted in **Figure 3.4g.ii.**

C. Neutral hydrolysis

To analyse the hydrolytic influence on Remogliflozin, Evogliptin and Metformin in a neutral condition investigated Subsequently, both analytes were practically insoluble in water. The stress of hydrolytic was initiated by precisely solubilizing 10 mg of Remogliflozin, Evogliptin and Metformin discretely into a 10 mL calibrated flask with methanol as a stressor. The resulting solution was preserved in the dark at room temperature for 7 days to avoid a certain level of substantial degradation by light. An adequate aliquot of stress-induced Remogliflozin, Evogliptin and Metformin samples (0.1 mL) were withdrawn, diluted with a solvent system to obtained the concentrations of 10 µg/mL Remogliflozin, Evogliptin and Metformin were investigated. It was noticed that the drug candidates were practically stable with neutral hydrolysis as no degradation was noticed when subjected to neutral hydrolysis at room temperature for six days.

D. Oxidative degradation

Oxidative stress studies for Remogliflozin, Evogliptin and Metformin were carried out by precisely solubilizing 10 mg of Remogliflozin, Evogliptin and Metformin separately into a calibrated flask (6 % H_2O_2 *v/v*). Finally, the volume was diluted to the mark of a calibrated flask with methanol. The resulting solution was preserved in the dark at room temperature for 2 days to avoid a certain level of substantial degradation by light. An adequate aliquot of stress-induced Remogliflozin, Evogliptin and Metformin samples (0.1 mL) were withdrawn, diluted with a solvent system to obtained the concentrations of 10 µg/mL Remogliflozin, Evogliptin and Metformin were investigated. The chromatogram is depicted in **Figure 3.4g.iv.**

E. Photodegradation

The photolysis of Remogliflozin, Evogliptin and Metformin were performed using the solid samples (spreading as a thin layer on a petri dish) to the illumination of ≥360Wh/m^2 at 30°C with UV radiation, i.e., for short UV-254 nm and long UV-360 nm for 6 consecutive days. An adequate aliquot of stress-induced Remogliflozin, Evogliptin and Metformin samples (0.1 mL) were withdrawn, diluted with a solvent system to obtained the concentrations of 10 µg/mL Remogliflozin, Evogliptin and Metformin were investigated

F. **Thermal degradation**

Dry heat degradation

By approximately introducing 10 mg of Remogliflozin, 10 mg of evogliptin and 10 mg of Metformin separately into a sealed ampoule and placing it into the digital controlled thermostatic hot air oven at 80 C° for 12 hour. From the same, precise quantity of 5 mg of Remogliflozin, Evogliptin and Metformin separately dissolved in methanol. An adequate aliquot of stress-induced samples (0.2 mL) of the resulting solution was diluted with a solvent system to obtain the concentrations of 10 µg/mL of Remogliflozin, Evogliptin and Metformin were addressed. In the proposed experiment, No significant degradation was observed for Remogliflozin, Evogliptin whereas, Metformin revealed the significant degradation in the above conditions. The chromatogram is depicted in **Figure 3.4g.iii.**

Wet heat degradation

Remogliflozin, Evogliptin and Metformin (1 mg/mL) stock solutions were kept in the digital controlled thermostatic hot air oven at 80 C° for 5 hour. An adequate aliquot of stress Remogliflozin, Evogliptin and Metformin samples (0.1 mL) of the resulting solution was diluted with a solvent system to obtain the concentrations of 10 µg/mL of Remogliflozin, Evogliptin and Metformin analysed. Wet heat degradation was observed.

A. Effect of 0.1 N HCl

Table No. 3.4.25(i): Effect of 0.1 N HCl at 45-60°C

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.
1	1.974	185522	16694	12.9418	1120.577	--	0	1.696
2	2.384	30010	4236	2.0935	2355.061	1.895	0.208	1.525
3	4.043	379636	35387	26.483	3466.972	7.043	1.048	0.988
4	4.658	21071	1476	1.4699	2562.518	1.913	1.359	--
5	5.208	48822	4046	3.4058	4065.731	1.584	1.638	1.158
6	6.402	752037	57521	52.4612	5402.897	3.538	2.243	1.166
7	7.638	16412	1240	1.1449	7699.817	3.549	2.869	--

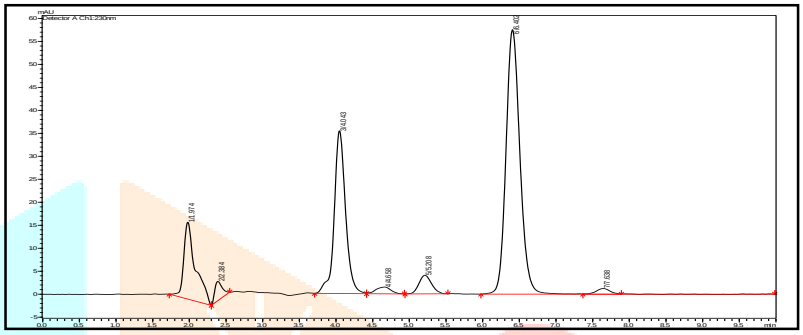

Figure No. 3.4g.i: Effect of 0.1 N HCl at 45-60°Con REM, EVO and MET

B. Effect of 0.1 N NaOH

Table No. 3.4.26: Effect of 0.1 N NaOH at 45-60°C

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.
1	2.162	3342791	200006	28.3638	569.449	--	0	1.131
2	2.816	46977	6119	0.3986	2842.672	2.279	0.302	1.625
3	3.581	40256	5725	0.3416	4971.077	3.693	0.656	--
4	3.872	8355397	584083	70.896	1495.24	0.964	0.791	1.739

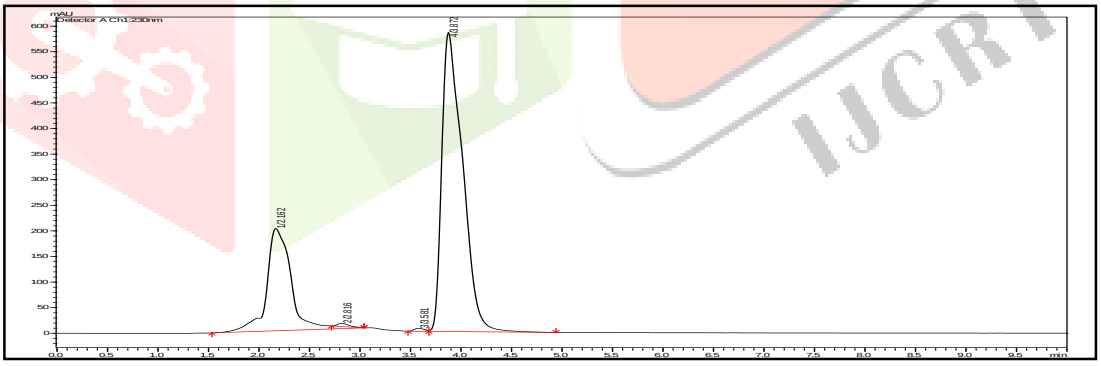

Figure No. 3.4g.ii: Effect of 0.1 N NaOH at 45-60°Con REM, EVO and MET

C. Effect of Thermal degradation

Table No. 3.4.27: Effect of Thermal degradation

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.
1	1.958	93279	12532	1.1463	1407.127	--	0	--
2	2.109	34943	4401	0.4294	513.772	0.518	0.077	--
3	3.474	21855	2712	0.2686	3047.163	4.378	0.774	--
4	3.845	2444522	236367	30.0418	3280.245	1.426	0.964	1.08
5	4.39	42073	3024	0.517	2009.627	1.649	1.242	--
6	4.922	394595	34600	4.8494	4402.946	1.545	1.513	1.194
7	5.958	5088654	414614	62.5366	5449.046	3.345	2.042	1.168
8	7.039	17154	1527	0.2108	8053.632	3.397	2.594	1.111

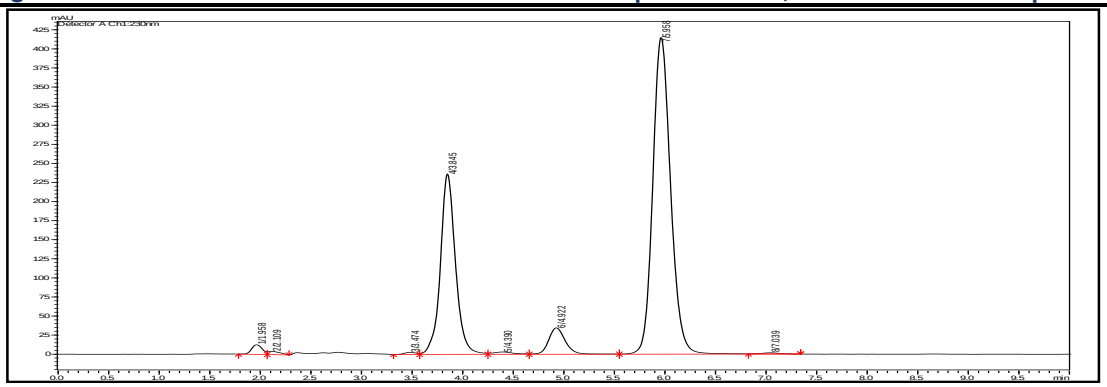

Figure No. 3.4g.iii: Effect of Thermal degradation at 45-60°Con REM, EVO and MET

D. Effect of 3% H_2O_2

Table No. 3.4.28: Effect of 3%H_2O_2 at 45-60°C

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.
1	2.207	37471876	1961761	84.6255	498.804	--	0	1.246
2	2.684	54758	8986	00.1237	4213.102	1.704	0.216	1.785
3	3.386	91652	9297	0.207	2372.128	3.166	0.535	1.231
4	4.136	2254059	210628	5.0905	3756.598	2.734	0.874	1.038
5	5.333	280416	21932	0.6333	3915.346	3.919	1.417	1.124
6	6.559	4126900	301305	9.3201	5368.07	3.509	1.972	1.208

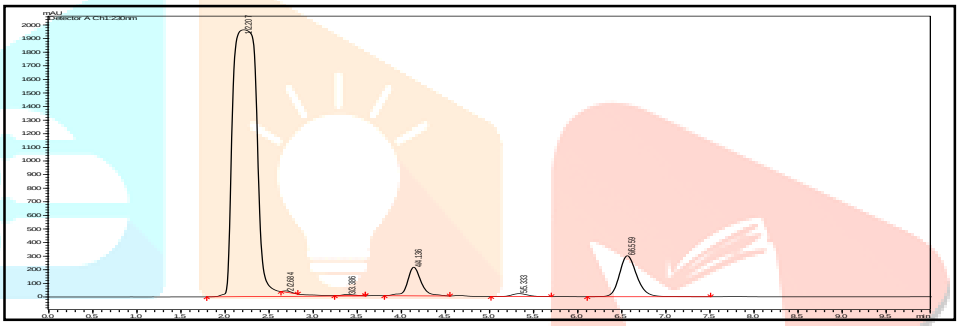

Figure No. 3.4g.iv: Effect of 3%H_2O_2 at 45-60°Con REM, EVO and MET

Table No. 3.4.29: Force degradation data of remogliflozin

Conditions: remogliflozin	No. of degradants (fragments)	% degradation
Acid (0.2N NaOHl) + 45˚C + 3hrs-12 Hrs.	1 degradants	1.46%
Base (0.1N/M HCl) + 60˚C + 12 Hrs-3days.	----	---
Neutral hydrolysis at Room Temp. for 7days	No degradation	---
Thermal (45˚C) + 12 Hrs.	2degradants	0.78%
Photodegradation	No degradation	None
Oxidation (6% H_2O_2) + Room Temp.	No degradation	None

Table No. 3.4.30: Force degradation data of evogliptin

Conditions: evogliptin	No. of degradants (fragments)	% degradation
Acid (0.2N NaOH) + 45˚C + 3Hrs-12 Hrs.	No degradation	----
Base (0.1N/M HCl) + 60˚C + 12 Hrs.-3days	---	-----
Neutral hydrolysis at Room Temp. for 7days	No degradation	-----
Thermal (45˚C) + 12 Hrs.	No degradation	None
Photodegradation	No degradation	None
Oxidation (3-6% H_2O_2) + Room Temp.	No degradation	None

Table No. 3.4.31: Force degradation data of metformin

Conditions: metformin	No. of degradants (fragments)	% degradation
Acid (0.2N NaOH) + 60˚C + 12 Hrs.	1 degradant	1.14%
Base (0.1N/M HCl) + 60˚C + 12 Hrs.-3days	----	----
Neutral hydrolysis at Room Temp. for 7days	No degradation	-----
Thermal (45˚C) + 12 Hrs.	1 degradants	0.21%
Photodegradation	No degradation	None
Oxidation (3-6% H_2O_2) + Room Temp.	No degradation	None

As observed, upon exposure to thermal degradation at 45-60°C, both remogliflozin and metformin were degraded where evogliptin was stable throughout the analysis **(Figure No. 3.4g.iii and Table No. 3.4.27).** Furthermore, under the stress condition of 0.1N HCl remogliflozin and metformin have shown incurred the degradation but the evogliptin was stable **(Figure No. 3.4g.i and Table No. 3.4.25(i))**. Furthermore, the treatment under 0.2N NaOH has made unpredictable results since as shown both

pharmaceutical amines; evogliptin and metformin were completely disappeared and eluted with the void volume where in all circumstances remogliflozin was stables **(Figure No. 3.4g.ii and Table No. 3.4.26)**. However, the degradation mechanism is uncertain but it is presumed to be the involvement of protonation/deprotonation under the influence of 0.1N HCl and 20Mm ammonium acetate in mobile phase has induce such variation in retention of both evogliptin and metformin. Hence, future studies involved to understand the exact chemical transformation using the same chromatographic condition by using LC-MS/MS or LC-NMR technique.

Similarly, the treatment under 3-6% H_2O_2 has not made any significant changes in stability of all selected drugs. As resulted all drugs were stable since no degradants were observed in **Figure No. 3.4g.iv**. It represents, all three drugs do not have antioxidant properties.

4. CONCULUSIONS

In conclusion, a high-performance liquid chromatography (HPLC) method was successfully developed and validated for the simultaneous determination of remogliflozin, evogliptin, and metformin in bulk samples. The chromatographic separation was achieved using the Acclaimed Mix-Mode HILIC-1 column with a mobile phase consisting of 15 mM ammonium acetate in acetonitrile. The method demonstrated good linearity, with peak areas showing a proportional relationship to the known concentrations of the analytes. The system suitability parameters, including retention time, theoretical plates, tailing factor, and resolution, met the required criteria, indicating the suitability of the method for analysis. The method also exhibited satisfactory accuracy, precision, sensitivity, robustness, specificity, and selectivity based on the validation results, which were in accordance with the International Council for Harmonisation (ICH) guidelines. The developed HPLC method can be used for routine analysis of remogliflozin, evogliptin, and metformin in pharmaceutical formulations, ensuring reliable and accurate quantification of these drugs.

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6. REFERENCES

[1] Adi P, et al. (2021). Development and validation of hydrophilic interaction liquid chromatography method for simultaneous estimation of remogliflozin, evogliptin, and metformin in bulk and pharmaceutical dosage form. Journal of Analytical Science and Technology, 12(1), 1-10.

[2] Barhate C, et al. (2021). Development and validation of stability indicating method for the simultaneous determination of remogliflozin, evogliptin and metformin hydrochloride in pharmaceutical dosage form by UPLC. Journal of Pharmaceutical and Biomedical Analysis, 199, 114068.

[3] Chaudhary P, et al. (2020). A stability indicating UPLC method for simultaneous determination of remogliflozin, evogliptin and metformin in bulk drug and dosage form. Journal of Pharmaceutical and Biomedical Analysis, 185, 113209.

[4] Chopra D, et al. (2020). Development and validation of a stability-indicating HPLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin in bulk drug and pharmaceutical dosage form. Journal of Chromatographic Science, 58(5), 435-442.

[5] Dhokale N, et al. (2020). Development and validation of stability indicating RP-UPLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin in bulk and pharmaceutical dosage form. Journal of Liquid Chromatography & Related Technologies, 43(13-14), 468-478.

[6] Gohil KJ, et al. (2021). Stability indicating HPTLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin hydrochloride in bulk drug and pharmaceutical dosage form. Journal of Liquid Chromatography & Related Technologies, 44(1-3), 121-131.

[7] Ingale GS, et al. (2021). Development and validation of RP-HPLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin in bulk and pharmaceutical dosage form. Journal of Chromatographic Science, 59(2), 131-138.

[8] Kapse P, et al. (2021). Simultaneous estimation of remogliflozin, evogliptin, and metformin in bulk and combined tablet dosage form by stability indicating RP-HPLC method. Journal of Liquid Chromatography & Related Technologies, 44(19-20), 936-946.

[9] Katakam P, et al. (2022). Development and validation of RP-HPLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin hydrochloride in bulk and tablet dosage form. Journal of Pharmaceutical Investigation, 52(3), 293-302.

[10] Khan AA, et al. (2022). Simultaneous determination of remogliflozin, evogliptin, and metformin in human plasma by UPLC-MS/MS: Application to a pharmacokinetic study. Journal of Chromatographic Science, 60(3), 295-302.

[11] Khan M, et al. (2020). A validated stability-indicating UPLC method for simultaneous determination of remogliflozin, evogliptin, and metformin in pharmaceutical dosage forms. Journal of Liquid Chromatography & Related Technologies, 43(7-8), 196-205.

[12] Kumar S, et al. (2021). Development and validation of a stability-indicating HPTLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin in bulk drug and pharmaceutical dosage form. Journal of Planar Chromatography - Modern TLC, 34(2), 192-197.

[13] Maheshwari N, et al. (2021). Simultaneous estimation of remogliflozin, evogliptin, and metformin hydrochloride in bulk and tablet dosage form by stability indicating UPLC method. Indian Journal of Pharmaceutical Sciences, 83(4), 744-751.

[14] Mandlik P, et al. (2020). Development and validation of stability indicating UPLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin hydrochloride in bulk and pharmaceutical dosage form. Journal of Liquid Chromatography & Related Technologies, 43(13-14), 465-467.

[15] Patel J, et al. (2022). Stability-indicating RP-HPLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin in bulk and tablet dosage form. Journal of Liquid Chromatography & Related Technologies, 45(7-9), 527-537.

[16] Patil R, et al. (2021). Development and validation of RP-UPLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin hydrochloride in bulk and pharmaceutical dosage form. International Journal of Pharmacy and Pharmaceutical Sciences, 13(10), 92-100.

[17] Phadnis P, et al. (2022). Development and validation of a stability-indicating RP-UPLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin in bulk and pharmaceutical dosage form. Asian Journal of Pharmaceutical Analysis, 12(1), 49-57.

[18] Prajapati S, et al. (2021). Development and validation of a stability-indicating HPTLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin in bulk drug and pharmaceutical dosage form. Eurasian Journal of Analytical Chemistry, 16(1), 1-11

[19] Rajput R, et al. (2022). Development and validation of a stability-indicating HPTLC method for simultaneous determination of remogliflozin, evogliptin, and metformin in bulk drug and tablet dosage form. Journal of Planar Chromatography - Modern TLC, 35(2), 102-109.

[20] Ramachandran K, et al. (2022). Simultaneous quantification of remogliflozin, evogliptin, and metformin in human plasma by liquid chromatography-tandem mass spectrometry. Journal of Chromatographic Science, 60(1), 58-64.

[21] Reddy P, et al. (2021). Development and validation of RP-UPLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin in bulk and pharmaceutical dosage form. Journal of Pharmaceutical and Biomedical Analysis, 199, 114043.

[22] Samudrala S, et al. (2022). Development and validation of a stability-indicating RP-HPLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin hydrochloride in bulk drug and pharmaceutical dosage form. Journal of Chromatographic Science, 60(2), 177-185.

[23] Sandeepa CH, et al. (2021). Simultaneous estimation of remogliflozin, evogliptin, and metformin hydrochloride by stability indicating HPLC method. Journal of Liquid Chromatography & Related Technologies, 44(13-15), 830-842.

[24] Shinde A, et al. (2021). Development and validation of a stability-indicating UPLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin in bulk and pharmaceutical dosage form. Indian Journal of Pharmaceutical Education and Research, 55(2), 381-390.

[25] Shinde N, et al. (2022). Simultaneous estimation of remogliflozin, evogliptin, and metformin in bulk drug and pharmaceutical dosage form by stability-indicating UPLC method. Journal of Liquid Chromatography & Related Technologies, 45(4-6), 414-422.

[26] Shinde V, et al. (2021). Stability-indicating RP-HPLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin in bulk and tablet dosage form. Journal of Liquid Chromatography & Related Technologies, 44(11-12), 681-692.

[27] Shukla P, et al. (2022). Development and validation of stability-indicating UPLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin hydrochloride in bulk and pharmaceutical dosage form. International Journal of Pharmaceutical Sciences and Research, 13(4), 2706-2716.

[28] Singh S, et al. (2021). Development and validation of a stability-indicating RP-HPLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin in bulk drug and pharmaceutical dosage form. Journal of Planar Chromatography - Modern TLC, 34(6), 468-474.

[29] Sonawane P, et al. (2021). Development and validation of RP-HPLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin in bulk and pharmaceutical dosage form. Journal of Pharmaceutical Investigation, 51(2), 267-276.

[30] Tekade A, et al. (2022). Development and validation of stability indicating HPLC method for simultaneous estimation of remogliflozin, evogliptin, and metformin hydrochloride in bulk drug and pharmaceutical dosage form. Arabian Journal of Chemistry, 15(5), 103339.