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Original Article

Development of an RP-HPLC Method for Simultaneous Estimation of Remogliflozin, Metformin and Teneligliptin in Tablet Formulation

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ABSTRACT

Objectives: A cost-effective and reliable RP-HPLC method was developed and validated for the simultaneous estimation of Remogliflozin, Metformin and Teneligliptin in Pharmaceutical dosage form. **Materials and Methods:** Using an ECO-C18 (15 mm*4.6 mm*5 µ (particle size)) column with a mobile phase consisting of 0.6 M Phosphate Buffer pH 3.5: ACN (Acetonitrile) 40:60 v/v, analysis was conducted at 222 nm with a flow rate of 1.0 mL/min. **Results:** The validation method followed ICH guidelines, demonstrating linearity with LOQ values of 4.79 µg/mL for Remogliflozin, 24.85 µg/mL for Metformin and 0.56 µg/mL for Teneligliptin. LOD values were determined as 1.43 µg/mL for Remogliflozin, 7.53 µg/mL for Metformin and 0.17 µg/mL for Teneligliptin, with correlation coefficients of 0.99 for all compounds. % Recovery ranged from 99.73% to 100.86% for Remogliflozin, 98.59% to 100.90% for Metformin and 100.11 to 100.89% for Teneligliptin, while relative standard deviation values for Replication, inter-intraday precision were all below 2%. **Conclusion:** The proposed method exhibited specificity, sensitivity, precision, accuracy, and robustness, making it suitable for routine analysis.

Keywords: Validation, Remogliflozin, Metformin, Teneligliptin.

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INTRODUCTION

Remogliflozin, Metformin and Teneligliptin in combination used for the treatment of diabetes and Cholesterol.[1,2] Combination was approved by CDSCO at 12/01/2022. Diabetes particularly type 2 prevalent, increasing in most countries and contributing significantly to the disease burden.[3,4] Diabetes-related complications result in 4.2 million deaths annually. Type 1 diabetes mellitus is marked by inadequate insulin production, while Type 2 Diabetes Mellitus (T2DM) results from insulin resistance at the cellular level.[5] Treatment typically involves anti-diabetic drugs aimed at lowering blood glucose levels, crucial for managing T2DM symptoms like increased hunger, frequent urination, and excessive thirst, necessitating lifelong medication. The primary treatment objectives focus on achieving glycaemic control and minimizing diabetes-related cardiovascular risks, as hyperglycaemia correlates with reduced Lifespan and diminished standard of life owing to associated microcirculatory and macrovascular Difficulties.[6] In clinical practice, patients newly diagnosed with diabetes are often prescribed insulin sensitizers like Metformin.[7,8] Metformin is favoured for its low risk of hypoglycaemia, minimal drug interactions, and high safety profile, making it a preferred first-line therapy for early-stage T2DM management.[9,10] Given the multifactorial nature of T2DM, treatment typically involves a combination of drugs targeting different mechanisms to effectively control plasma glucose levels, recognizing the involvement of multiple organs in its etiology.[11,12]

Remogliflozin (Remo) (Figure 1 (A)) 5-Methyl-4-[4-(isopropoxy)benzyl]-1-isopropyl-1H-pyrazol-3-yl 6-O-(ethoxycarbonyl)-β-D-glucopyranoside. Recently, SGLT-2 (sodium-glucose co-transporter 2) inhibitors have risen to prominence as a significant category of oral medications for managing type 2 diabetes mellitus, particularly in individuals with cardiovascular or renal issues. These inhibitors are endorsed in all recent treatment guidelines, highlighting their increasing importance in diabetes management.[13] Their unique insulin-independent mode of action, which enhances urinary glucose excretion to lower blood glucose levels, confers benefits for individuals with compromised pancreatic function or insulin resistance.[14] SGLT2 inhibitors also help control blood sugar,

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support weight loss, reduce blood pressure, improve kidney function, and decrease cardiovascular risks, making them especially beneficial for patients with hypertension and a high risk of hypoglycemia.[15,16]

Metformin hydrochloride (MET) (Figure 2 (B)) is the chemical designation for Dimethylbiguanide hydrochloride.[17] It serves as a potent biguanide antidiabetic agent, extensively utilized for decades Maintain optimal blood glucose (T2D).[18,19] International guidelines have recognized Metformin as the primary treatment option. Its mechanism of action involves inhibition of hepatic gluconeogenesis through mitochondrial inhibition and activation of AMPK. Additionally, Metformin enhances skeletal muscle sensitivity to insulin, both directly and indirectly.[20] Metformin holds official recognition in various pharmacopeia's, including the official Pharmacopoeia like British Pharmacopoeia, Indian Pharmacopoeia, European Pharmacopoeia.[21,22]

Teneligliptin (Tene) (Figure 3 (C)) chemically identified as [(2S, 4S)-4-[4-(5-methyl-2phenylpyrazol-3-yl) piperazin-1-yl] pyrrolidin-2-yl] -(1,3-thiazolidin-3-yl),[23] stands as a groundbreaking oral medication classified as a dipeptidyl peptidase-4 inhibitor.[6] It targets type 2 diabetes mellitus (T2DM by virtue of its distinct molecular structure, characterized by five consecutive rings, enabling it to yield potent and enduring effects. Currently, teneligliptin is prescribed in scenarios where conventional approaches such as dietary adjustments, physical activity[24-26] or a combination of these with oral hypoglycemic medications like Biguanides and Sulphonylureas prove inadequate in achieving satisfactory glycemic control.[27,28]

To ensure Quality assurance, it is Necessary for establish Evaluation methods for combined formulations. Various sophisticated techniques, such as UV spectrometry, HPLC, and stability-indicating HPLC methods have been reported in the literature for analysing Remogliflozin, Metformin, and Teneligliptin, both individually and in combination.[5,10,29-36] or in combination with other[37-44] medications. However, there is currently a lack of a stability-indicating RP-HPLC chromatographic procedure specifically designed to evaluate Remogliflozin, Metformin and Teneligliptin in the dosage form. Hence, a specific, precise, and sensitive chromatographic technique was Formulated and confirmed following the ICH Guideline Q2 (R1).[45]

MATERIALS AND METHODS

The reference standards for Remogliflozin, Metformin and Teneligliptin were generously provided by Glenmark Pharmaceuticals Limited, situated in Ahmedabad. Solvents and reagents utilized in this research included formic acid, water, methanol, acetonitrile with HPLC grade and Laboratory-grade reagent orthophosphoric acid, all bought from Finar Mumbai. Furthermore, a commercial tablet formulation, Zucor-500, containing Remogliflozin (100 mg), Metformin (500 mg) and Teneligliptin (10 mg), manufactured by Glenmark Pharma Pvt. Ltd., was obtained from the local market for analysis.

Instrumentation

Different instruments were employed during the method development process, such as the Analytical LC-20 AT HPLC chromatographic system, a Shimadzu ATV 226 digital weighing balance, an Agilent pH meter, a Frontline Ultrasonic Cleaner ultrasonicator, a hot air oven from India, and an analytical-lab unit from Mumbai. Filtration was performed using a 0.45 µ Millipore filter.

Selection and Detection of Wavelength

The selectivity of an HPLC method using UV detection relies heavily on choosing the appropriate detection wavelength. The optimal wavelength should provide a strong response for the target drug while minimizing solvent interference. Estimation of Remogliflozin, Metformin, and Teneligliptin, a wavelength of 222 nm in methanol was found to be ideal. At this wavelength, the drugs exhibited maximum absorbance and produced clear peaks without solvent interference, as illustrated in Figure 2.

Chromatographic Condition

Remogliflozin, Metformin and Teneligliptin were effectively separated using an ECO-C_{18} 5µ column (15 mm*4.6 mm*5 µ particle size). The mobile phase comprised Phosphate saline solution pH 3.5: CAN in a proportion of 40:60 v/v, velocity of flow 1 mL/min. A 20.0 µL injection volume was utilized and Identification was carried out at a wavelength (λ_{max}) of 222 nm over a 15-min duration.

Mobile Phase Preparation

Phosphate Buffer (Monopotassium Phosphate) 40 mL and the pH were adjusted to 3.5 using hydrogen phosphate solution. The pH of the prepared buffer was checked with an ultrasonic pH meter and 60 mL of acetonitrile.

Standard Solution Preparation

Standard stock solution of Remogliflozin

Weighed accurately about 100 mg of Remogliflozin, 500 mg of Metformin and 10 mg of Teneligliptin, placed into a 100 mL volumetric flask and the volume was adjusted to 100 mL with solvent to achieve a final concentration of 1000 mcg/mL of Remogliflozin., 5000 mcg/mL of Metformin and 100 mcg/mL of Teneligliptin. From above solution pipette out 2 mL and placed into 50 mL of volumetric flask and diluted up to 50 mL with solvent to get final concentration of working standard solution is 40 mcg/mL, 200 mcg/mL and 4 mcg/mL Respectively

Sample Solution preparation from Marketed Formulation

Stock solution

Approximately 10 tablets of Zucor-500 weighed, and the mean weight of the tablets was calculated. The tablets were then micronized using a mortar-pestle. Equivalent to 100 mg of Remogliflozin, 500 mg of Metformin, and 10 mg of Teneligliptin powder was weighed and Placed in a 100 mL volumetric flask. The contents were then diluted with the mobile phase up to the mark to obtain concentrations of 1000 µg/mL of Remogliflozin, 5000 µg/mL of Metformin, and 100 µg/mL of Teneligliptin.

Working solution

Pipette out 2 mL from stock solution and displaced into 50 mL of flask and volume make up to the mark with solvent to get 40 mcg/mL of Remogliflozin and 200 mcg/mL of Metformin and 4 mcg/mL of Teneligliptin.

Chromatographic Separation

Standard solutions of Remogliflozin, Metformin, and Teneligliptin were introduced into the column with a 20 µL micro-syringe to start the chromatographic analysis. Detection occurred at a wavelength of 222 nm throughout the required runtime, and the chromatogram concluded upon achieving full separation. Lab-solution software was employed to record data on resolution, retention time, and peak characteristics, including height and area.

Method Validation

The proposed method was validated according to ICH guidelines Q2 (R1), focusing on accuracy, precision, linearity, LOD, LOQ, and robustness.

Linearity

The linear relationship of an analytical method is evaluated by assessing calibration curve, which depicts response versus concentration, conforms to a straight line. In this study, a calibration curve was generated using a primary stock solution containing 1000 µg/mL of Remogliflozin, 5000 µg/mL of Metformin, and 100 µg/mL of Teneligliptin. Aliquots of 1 mL, 1.5 mL, 2 mL, 2.5 mL, and 3 mL were dispensed into 50 mL volumetric flasks, sonicate for 10 min, and then volume make up to the 50 mL with a suitable diluent. Process yielded solutions with concentrations of 20, 30, 40, 50, and 60 µg/mL for Remogliflozin, 100, 150, 200, 250, and 300 µg/mL for Metformin, and 2, 3, 4, 5, and 6 µg/mL for Teneligliptin. Subsequently, a 20 µL aliquot from each prepared solution was loaded into the chromatography system according to the predefined operational parameters. Peak areas were plotted against concentrations to generate a calibration curve, and a regression equation was derived. Each data point on the plot represented the average of three measurements to ensure accuracy.

Precision

Repeatability

System reliability was assessed by introducing a standard solution into the system of Remogliflozin (40 µg/mL), Metformin (200 µg/mL), and Teneligliptin (4 µg/mL) six times. The chromatograms

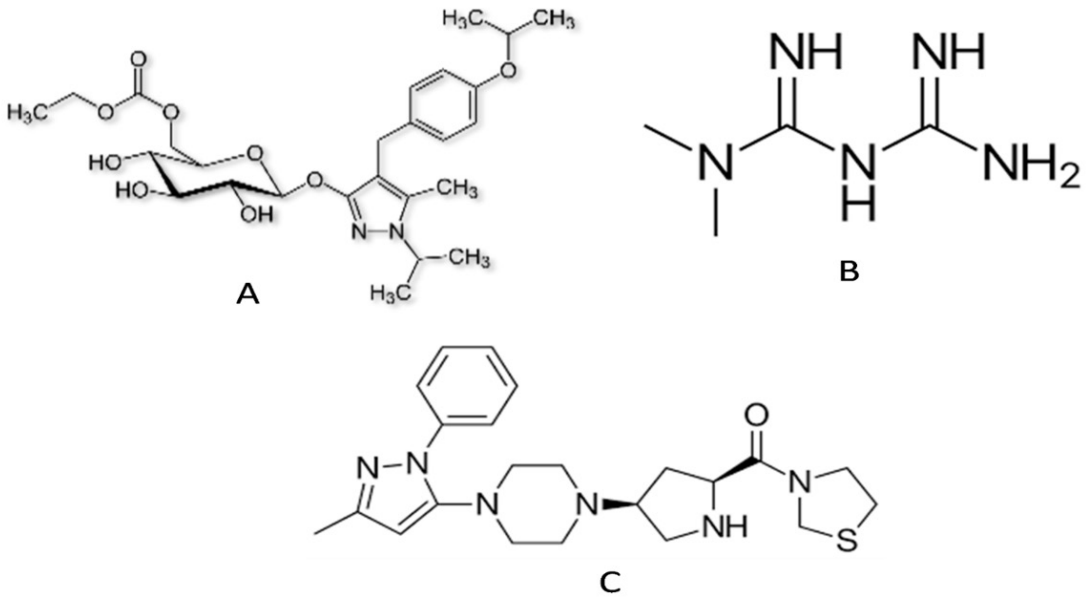

Figure 1: Structures of Active Pharmaceutical Ingredient. A. Structure of Remogliflozin, B. Structure of Metformin, C. Structure of Teneligliptin.

obtained were analysed, and peak areas were recorded to evaluate repeatability.

Interday Precision

For precision assessment, a standard solution with concentrations of Remogliflozin 40 µg/mL, Metformin 200 µg/mL, and Teneligliptin 4 µg/mL was utilized. These solutions were subjected to Interday precision analysis in three different days.

Intraday Precision

Intraday precision analysis was achieved by injecting 40 µg/mL of Remogliflozin, 200 µg/mL metformin and 4 µg/mL of Teneligliptin in multiple times on the same day itself. % RSD was then estimated from these tests to determine method precision.

LOD and LOQ

The Limits of Detection (LoD) and Limits of Quantification (LoQ) for the drugs were established using data collected from linearity studies. Afterwards, calculated using a specified Equation.

$$LoQ=10*SD/Slope$$

$$LoQ=3.3*SD/Slope$$

Accuracy

The correctness of the method for measuring Remogliflozin, Metformin, and Teneligliptin was tested through recovery studies conducted at 80%, 100%, and 120% of the test concentration, following ICH guidelines, with each level tested three times.

Robustness

The robustness study was conducted under chromatographic conditions to assess the impact of minor variations as outlined in the Chromatographic Conditions section. This study examined factors recognized as key sources of variability in the operating procedures. Specifically, adjustments included altering the mobile phase ratio by ±2 mL, pH of mobile phase ±0.2 and modifying the flow capacity of the carrier solvent by ±0.2 mL/min. Throughout these experiments, the composition of the mobile-phase components remained unchanged. The effects of these alterations were then evaluated in terms of their impact on the system suitability for standard preparation.

RESULTS

After conducting several trials, the optimal chromatographic conditions were established shown in Table 1 and chromatogram shown in Figure 3 A.

System Suitability Parameter

System performance criteria were evaluated using factors like theoretical plates, retention time, tailing factor and resolution to ensure the system's repeatability and resolution were appropriate for the evaluation. The operational parameter for Remogliflozin, Metformin, and Teneligliptin are listed in Table 1.

Specificity

Specificity was verified by checking the separation between the peaks of drug and nearby peaks, as well as among all other peaks. The method's specificity was confirmed by comparing the chromatograms of the blank, standard and test solutions to ensure no interference, as shown in Figures 3 (A. Standard Chromatogram B. Sample Chromatogram and C. Blank Chromatogram).

Linearity

The linearity of Remogliflozin, Metformin and Teneligliptin was assessed by analysing a combined standard solution within specified ranges: 20 to 60 µg/mL, 100 to 300 µg/mL, and 2 to 6 µg/mL, sequentially. The r-value for the calibration curve of each compound was found to be not less than 0.999. Figure 4 depict the calibration curves of Remogliflozin (A), Metformin (B) and Teneligliptin (C) respectively, while Table 1 displays the % RSD.

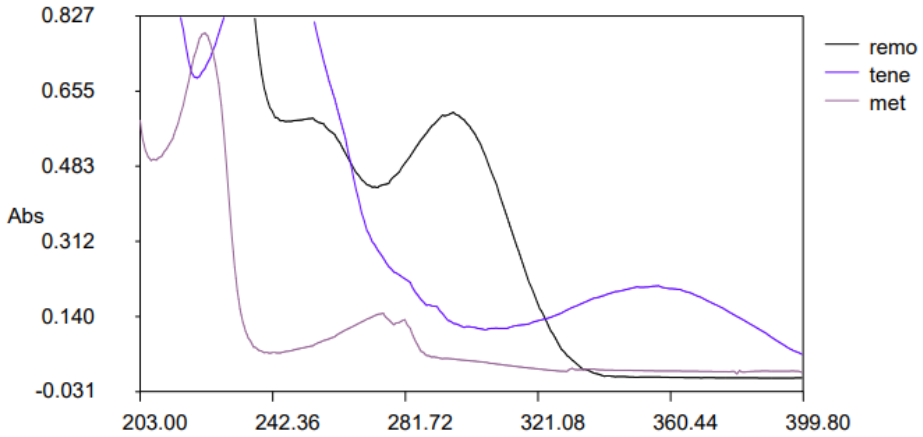

Figure 2: Selection of analytical wavelength.

Table 1: Optimized Chromatographic Condition.

Parameters	Conditions		
Mobile Phase	Phosphate Buffer pH 3.5: ACN (Acetonitrile) 40:60% v/v.		
Stationary Phase	ECO-C18 5 µ (15 mm*4.6 mm*5 µ (particle size)).		
Flow rate	1 mL/min		
Run time	15 min		
Volume of injection	20 µL		
Detection of wavelength	222 nm		
System Suitability Parameter	**Remogliflozin**	**Metformin**	**Teneligliptin**
Retention time	3.79	3.09	10.44
Theoretical plates	7019	6072	6022
Tailing factor	1.32	1.42	1.53
Resolution	-	4.15	18.46

Analytical Data of Linearity					
Remogliflozin		**Metformin**		**Teneligliptin**	
Concentration (µg/mL)	**Area±S.D**	**Concentration (µg/mL)**	**Area±S.D**	**Concentration (µg/mL)**	**Area±S.D**
20	88.73±0.19	100	891.34±0.42	2	151.02±0.68
30	134.61±0.44	150	1351.25±0.97	3	230.76±0.51
40	176.41±0.16	200	1769.06±0.85	4	302.46±0.54
50	217.84±0.56	250	2176.24±0.77	5	371.64±0.47
60	264.67±0.41	300	2648.49±1.00	6	452.46±0.76
SD	1.89	SD	19.80	SD	3.85
Correlation Coefficient (r)	0.99	Correlation Coefficient (r)	0.99	Correlation Coefficient (r)	0.99

Analytical Data for LOD and LOQ			
Parameter	**Remogliflozin**	**Metformin**	**Teneligliptin**
LOD	1.43	7.53	0.170
LOQ	4.79	24.85	0.56

Method Precision

Repeatability

Replication of peak area measurements of Remogliflozin, Metformin, and Teneligliptin was evaluated through 6 measurements of the similar Solution. The mean peak area values were 175.93 for Remogliflozin, 1768.90 for Metformin, and 300.89 for Teneligliptin, with % RSD of 0.89, 1.04, and 1.46 respectively. These % RSD values, as presented in Table 2, are well within the acceptance limit of Not More Than (NMT) 2%, indicating excellent repeatability.

Interday Precision

Interday precision was evaluated by analysing a standard solution containing Remogliflozin at concentration of 20, 40 and 60 mg/mL Metformin 100, 200 and 300 mg/mL and Teneligliptin at strength of 2, 4 and 6 mg/mL on three different days. The % RSD values were estimated. Table 2 shows results of interday precision.

Intraday Precision

The precision within the same day was evaluated by studying a standard solution containing Remogliflozin, of 20, 40, and 60 mg/mL, Metformin of 100, 200 and 300 and Teneligliptin at concentrations of 5, 10, and 15 mg/mL, three times on the same day. The % RSD were calculated, and the data are mentioned in Table 2.

LOD and LOQ

The Limits of Detection (LOD) and Limits of Quantitation (LOQ) for the drugs were estimated using the linearity data in Figure 4. The calibration curve was performed five times, and the standard deviation of the interceptions was determined. The LOD for Remogliflozin, Metformin and Teneligliptin was 1.43, 7.53 and 0.17 respectively. The LOQ for Remogliflozin, Metformin and Teneligliptin was 4.79, 24.85 and 0.56 respectively; the results are presented in Table 1.

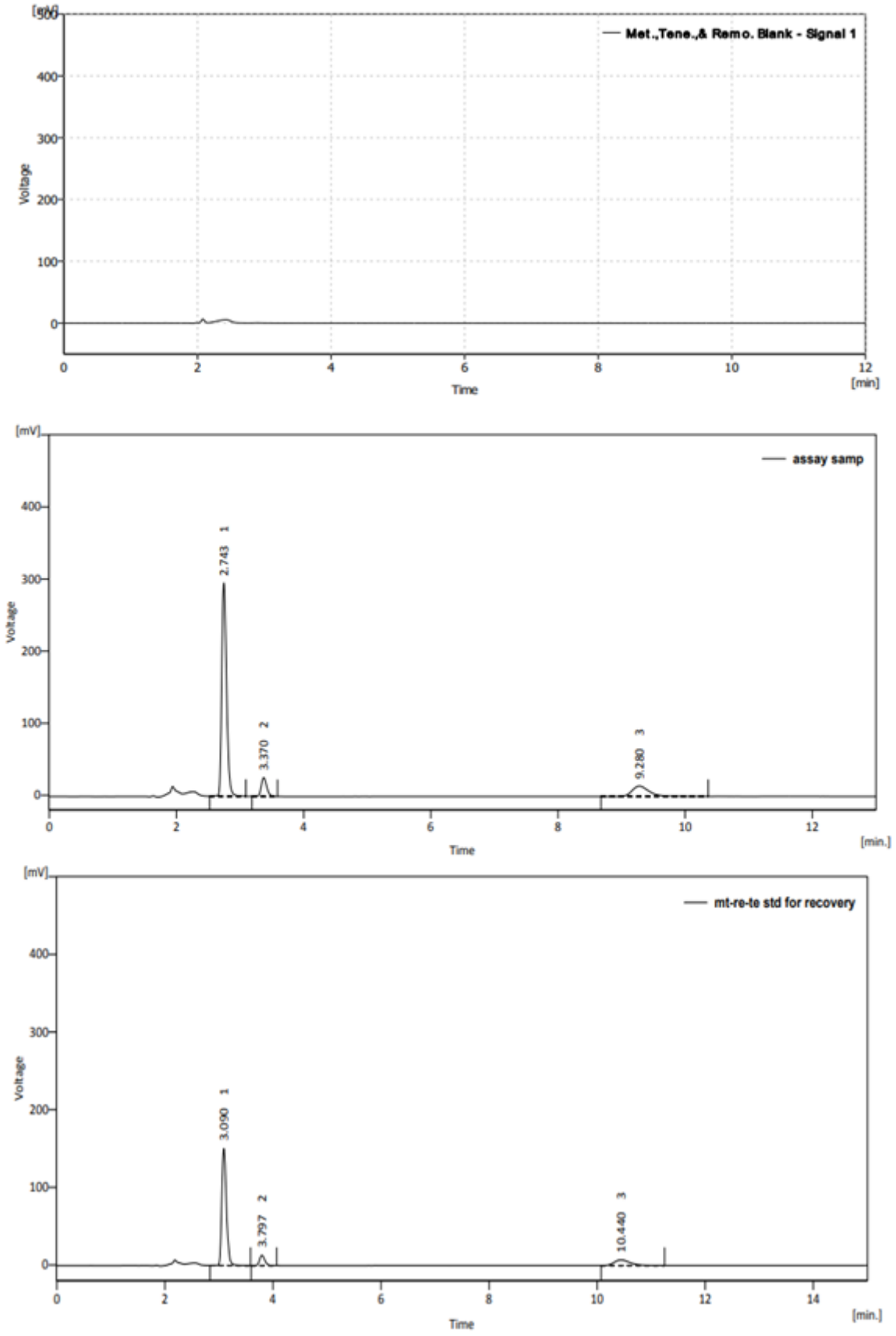

Figure 3: Chromatogram of Remogliflozin, Metformin and Teneliptin.A.Blank B. Sample C.Standard.

Table 2: Analytical Data of Repeatability, Intraday, Interday.

Analytical Data for Repeatability								
Remogliflozin			Metformin			Teneligliptin		
Sl. No.	Conc	Area	Sl. No.	Conc	Area	Sl. No.	Conc	Area
1	40 µg/mL	173.514	1	200 µg/mL	1741.171	1	4 µg/mL	297.345
2		175.815	2		1764.186	2		301.84
3		175.99	3		1765.918	3		302.162
4		178.468	4		1790.691	4		306.373
5		176.128	5		1788.51	5		303.503
6		175.695	6		1762.948	6		294.128
Mean±S. D (*n*=6)	175.93±1.57		Mean±S. D (*n*=6)	1768.90±18.41		Mean±S. D (*n*=6)	300.89±4.42	
% RSD	0.89		% RSD	1.04		% RSD	1.46	
Intraday Study								
Remogliflozin			Metformin			Teneligliptin		
Sl. No.	Conc	Area Mean±S.D (*n*=3)	% RSD	Sl. No.	Conc	Area Mean±S.D (*n*=3)	% RSD	Sl. No.	Conc	Area Mean±S.D (*n*=6)	% RSD
1	20	87.35±1.22	1.40	1	100	880.59±7.73	0.87	1	2	146.65±1.88	1.28
2	40	173.17±3.03	1.75	2	200	1749.79±7.00	0.40	2	4	298.36±3.75	1.25
3	60	23.69±2.90	1.10	3	300	2645.81±18.58	0.70	3	6	450.27±7.73	1.71
Interday Study											
Remogliflozin				Metformin				Teneligliptin			
Sl. No.	Conc µg/mL	Area Mean±S.D (*n*=3)	% RSD	Sl. No.	Conc µg/mL	Area Mean±S.D (*n*=3)	% RSD	Sl. No.	Conc µg/mL	Area Mean±S.D (*n*=6)	% RSD
1	20	86.84±1.37	1.58	1	100	882.78±3.08	0.34	1	2	146.50±1.38	0.94
2	40	172.70±1.40	0.81	2	200	1740.32±9.90	0.56	2	4	294.39±5.67	1.92
3	60	261.03±2.25	0.86	3	300	2629.17±8.49	0.32	3	6	450.27±7.73	1.7

Accuracy

To evaluate the precision of the proposed technique for quantifying Remogliflozin, Metformin, and Teneligliptin, recovery tests were performed at the Limit of Quantification (LoQ4 levels of 80%, 100%, and 120% of the test concentration, in accordance with ICH standards. The method's precision was validated by conducting a recovery test using a commercial formulation at three different addition levels. The recovery percentages were found to be between 99.73% and 100.86% for Remogliflozin, 100.11% to 100.89% for Metformin, and 98.59% to 100.90% for Teneligliptin. The results are presented in Table 3.

Robustness

The robustness study, which involved deliberately altering chromatographic conditions by ±2 mL in the mobile phase ratio, ±0.2 mL/min in the flow rate and ±0.2 pH. Demonstrated that these adjustments did not significantly impact on the system's suitability for preparing standard samples, with % RSD values remaining below the 2% standard limit as shown in Table 3. Summary of validation data shown in Table 4.

Analysis of Marketed Formulation

A 20 µL portion of the sample solution which contained 40 mg of Remogliflozin, 200 mg of Metformin and 4 mg of Teneligliptin was administered into the chromatographic system, and the peak area was measured to calculate the % assay using a regression equation. The response was an average of six determinations, and the outcomes are presented in Table 3.

Table 3: Analytical data for Accuracy, Robustness and Marketed Formulation.

Analytical data for Accuracy						
Drug	**% Level**	**Amount of sample taken (mg)**	**Mean area SD**	**Amount of standard recovery**	**% Recovery**	**%RSD**
Remogliflozin	80	16	71.93±0.84	16.29	100.86%	0.82
	100	20	88.06±1.70	19.94	99.73%	1.71
	120	24	106.30±1.07	24.07	100.32%	1.07
Metformin	80	80	715.94±0.81	80.71	100.89%	0.81
	100	100	88.05±0.94	100.15	100.11%	0.94
	120	120	1066.38±1.04	120.22	100.18%	1.04
Teneligliptin	80	4	117.04±0.64	3.94	98.59%	0.65
	100	5	147.97±1.20	4.97	99.71%	1.20
	120	6	179.70±1.16	6.05	100.90%	1.15

Analytical Data of Robustness				
Drug	**Variation**		**Mean area±SD**	**%RSD**
Remogliflozin	Flow rate	1.2 mL	157.77±1.48	0.93
		0.8 mL	190.33±1.83	0.96
	Mobile Phase	62:38% v/v	162.50±2.65	1.63
		58:42% v/v	188.88±2.62	1.38
	pH	3.7	184.49±2.89	1.56
		3.3	163.55±1.65	1.009
Metformin	Flow rate	1.2 mL	1586.41±6.39	0.40
		0.8 mL	1923.37±13.40	0.69
	Mobile Phase	62:38% v/v	1623.37±16.12	0.98
		58:42% v/v	1902.25±5.79	0.30
	pH	3.7	1874.21±4.03	0.21
		3.3	1650.948±9.68	0.58
Teneligliptin	Flow rate	1.2 mL	270.12±2.82	1.04
		0.8 mL	324.060±5.45	1.68
	Mobile Phase	62:38% v/v	278.96±3.65	1.30
		58:42% v/v	323.39±4.11	1.27
	pH	3.7	316.1±3.02	0.95
		3.3	280.85±2.91	1.03

Analysis of Marketed Formulation											
Remogliflozin				**Metformin**				**Teneligliptin**			
Standard area- 175.64				**Standard area- 1762.53**				**Standard area-301.56**			
Sl. No.	**Conc.**	**Area**	**%Assay**	**Sl. No**	**Conc.**	**Area**	**%Assay**	**Sl. No.**	**Conc.**	**Area**	**%Assay**
1	100	173.283	98.65	1	500	1740.74	98.76	1	10	298.19	98.88
2	100	171.02	97.36	2	500	1739.45	98.69	2	10	294.39	97.62
3	100	172.54	98.23	3	500	1723.43	97.41	3	10	295.23	98.13
Mean			98.083	Mean			98.41	Mean			98.13
SD			0.65	SD			0.54	SD			0.66
%RSD			0.66	%RSD			0.55	%RSD			0.67

Table 4: Summary of Validation Parameter.

Parameter	Remogliflozin	Metformin	Teneligliptin
Linearity (Regression Value)	20-60 µg/mL (0.994)	100-300 µg/mL (0.994)	2-6 µg/mL (0.992)
% Recovery	99.73%-100.86%	100.11%-100.89%	98.59%-100.90%
Repeatability (%RSD, *n*=6)	0.89	1.040	1.46
Precision (RSD)			
Intra-day (*n*=3)	1.40-1.130	0.40-0.87	1.25-1.71
Inter-day (*n*=3)	0.86-1.58	0.32-0.56	0.94-1.92
Limit of Detection	1.43	7.53	0.17
Limit of Quantification	4.79	24.85	0.56
Robustness	Robust	Robust	Robust

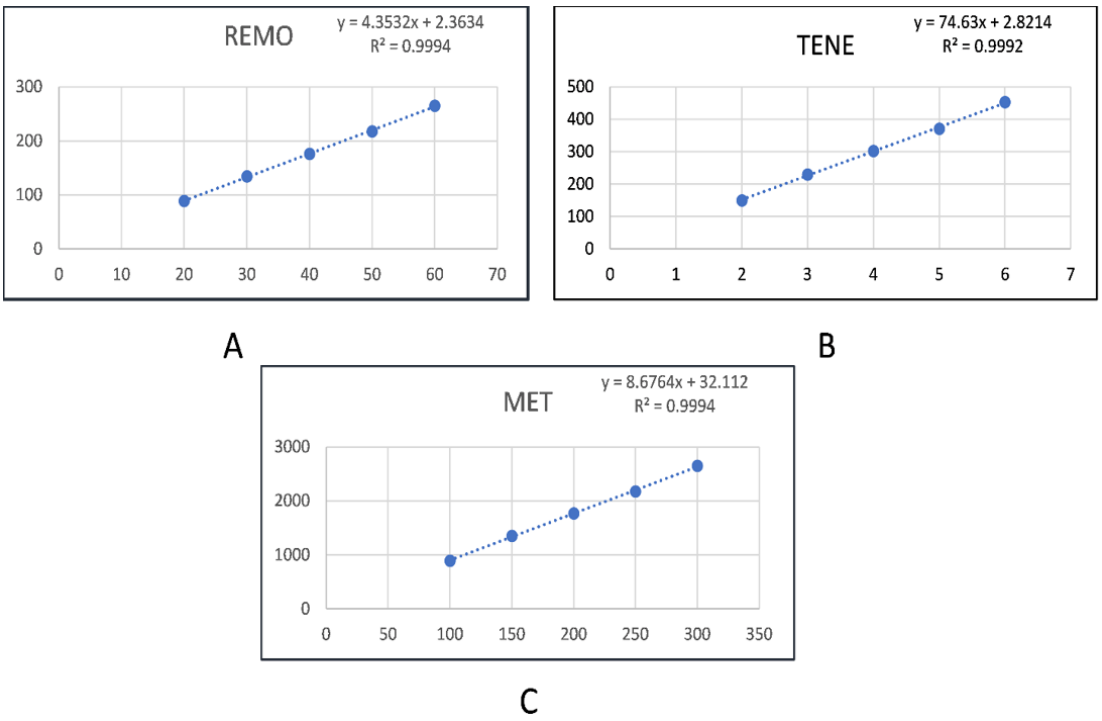

Figure 4: Linearity Graphs (A. Calibration curve of Remogliflozin. B. Calibration curve of Metformin. C. Calibration curve of Teneligliptin).

DISCUSSION

Reverse Phase-HPLC technique was formulated and verified for the concurrent quantification of Remogliflozin, Metformin, and Teneligliptin in a pharmaceutical formulation. The analysis used an ECO-C18 column (15 mm×4.6 mm, 5 µm particle size) with a mobile phase of 0.6 M phosphate saline solution at pH 3.5 and acetonitrile in a 40:60 v/v ratio. During trials with different mobile phases using acetonitrile and methanol with different ratio and several issues were encountered, such as, either no peaks were detected for all three drugs, or when peaks did appear, they lacked proper resolution. Other factors, like tailing and peak shape, did not meet acceptable criteria. After considering these issues, various pH levels were tested, and pH 3.5 yielded optimal peaks, resolution, and met all required criteria. Hence, this mobile phase was selected, producing retention times for Remogliflozin, Metformin, and Teneligliptin of 3.79 min, 3.09 min, and 10.44 min, respectively. Theoretical plate counts were 7019, 6072, and 6022, with tailing factors of 1.32, 1.42, and 1.53, respectively. The method was conducted with a flow rate of 1.0 mL/min and at 222 nm of wavelength.

Regarding validation, for Remogliflozin, Metformin, and Teneligliptin, the correlation coefficients for the linearity between concentration and area were 0.994, 0.9945, and 0.992, respectively, indicating an acceptable linear relationship across the investigated range. The mean percentage recovery rates were 100.30% for Remogliflozin, 100.39% for Metformin, and 99.73% for Teneligliptin, confirming the method's accuracy. The % RSD was within acceptable limits, demonstrating precision. The percentage assays for these drugs fell within the 90.0-110.0% range, and robustness testing confirmed the method's resilience, with % RSD not exceeding 2.0%.

CONCLUSION

No previous analytical studies have been found in the literature regarding the RP-HPLC method for analysing Remogliflozin, Metformin, and Teneligliptin. There is a lack of detailed information on the behaviour of these drugs under chromatographic conditions and their other relevant analytical characteristics. This research introduces a new approach to validate and develop an RP-HPLC method, advancing this field of study. The suggested RP-HPLC technique is efficient, highly sensitive, and straightforward, allowing for the simultaneous determination of Remogliflozin, Metformin, and Teneligliptin with excellent chromatographic resolution and peak separation. The mobile phase used consisted of buffer solution pH 3.5 and ACN (40:60% v/v). Recovery results from all formulations aligned with their labelled amounts, confirming that there was no interference from excipients during the analysis. The analysis was successfully verified in correspondence with ICH guidelines, ensuring its specificity, precision, linearity, accuracy and robustness. Thus, the suggested method is appropriate for regular analysis. To estimate Remogliflozin, Metformin, and Teneligliptin in combined dosage forms by RP-HPLC.

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ABBREVIATIONS

RPHPLC: Reverse Phase High Performance Liquid Chromatography; **LOD:** Limit of Detection; **LOQ:** Limit of Detection; **T2DM:** Type 2 diabetes mellitus; **ICH:** International Conference of Harmonization; **Remo:** Remogliflozin; **Met:** Metformin; **Tene:** Teneligliptin; **ACN:** Acetonitrile; **RSD:** Relative Standard Deviation; **mcg:** Microgram; **mL:** Millilitre.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

In Pharmaceutical analysis, it is important for a chromatography user to develop and validate specific, accurate, precise and robust method with short run time. In this study we have developed a short run time using RPHPLC compatible method of analysis for quantitative estimation of all possible way using marketed formulation of Remogliflozin, Metformin and Teneligliptin in pharmaceutical dosage form.

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