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Authentication of a Rapid RP-HPLC Technique for Quantification of Remogliflozin and Metformin in Medicinal Formulations

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Abstract

A new, precise, and stability-assessing advanced high-performance liquid chromatography technique for simultaneous quantification of Remogliflozin and Metformin in fixed-dose pharmaceutical formulations was designed and verified. Optimization of the technique was carried out on flowing phase composed of ethanenitrile and buffer, under isocratic conditions, the resolution of peak being sharp with retention times of 3.24 min and 4.89 min for Remogliflozin and Metformin, respectively. Technology suitability was confirmed by the proportionality across a broad concentration span with correlation coefficient (R^2) < 0.999. As per ICH rules, the criteria for validation, including accuracy, precision, robustness, and system suitability, were found as good %RSD Involvement in robustness studies confirmed the reliability of the method under very small deliberate changes in the analytical conditions. Results of multiple marketed formulations had to meet pharmacopeial limits, proving the method well applicable in routine quality control. Developed high-performance liquid chromatography method possesses rapid, sensitive, and cost-effective concurrent assessment of Remogliflozin and Metformin for bulk and formulation, which may serve as a useful tool for pharmaceutical analysis.

Keywords: Remogliflozin, Metformin, HPLC, method validation, pharmaceutical analysis

INTRODUCTION

The high blood glucose levels are referred to as diabetes mellitus. The type of diabetes to be described is that of Type 2 diabetes, which is way too common, occurring in large numbers almost everywhere in the world [1]. Hence, the administration of Type 2 diabetes mellitus is usually a case of a combination of drugs acting by different mechanisms for blood sugar control. Remogliflozin Etabonate and metformin are the two such medications that are commonly prescribed. It is an SGLT-2 inhibitor that inhibits glucose reabsorption in the kidneys and binds to glucose without the requirement of insulin [2]. A drug of the biguanide class, it is a first-line therapy of Type 2 diabetes, and reduces liver glucose production as well as enhances insulin responsiveness, making it a first-line therapy for Type 2 diabetes [3]. With the increased usage of the combination therapies, it is necessary to develop an effective and reliable approach for concurrent measurement of these medications as a combination [4]. Reverse phase–high-performance liquid chromatography (RP-HPLC) is one that is

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commonly recognized to be a good analytical technique because of its speed, precision, and powerful ability of separation and quantitation of complex mixtures of compounds [5]. A robust method is required to estimate Remogliflozin and Metformin simultaneously, and this method should be able to resolve the explained drug with high accuracy and precision. Aims and objectives of the current research are formulated to create and confirm a concurrent rapid RP-HPLC approach for determination of Remogliflozin and Metformin in raw medication substance and tablet formulations [6]. The method will then be used to validate suitability to be used as an efficient method of quality control, stability studies, and formulation monitoring of pharmaceutical formulations containing these drugs after validation of the method using regulatory guidelines [7].

MATERIALS AND METHODS

Materials

Remogliflozin and Metformin reference standards were received from AKUMS Drugs Pharmaceutical Ltd., Haridwar, Uttarakhand, India, generously. These drugs are included in pharmaceutical tablets purchased from a local market in Moradabad, Uttar Pradesh, India. All the solvents and reagents were of the highest quality. Acetonitrile and water for HPLC use were procured from Merck® India Ltd, Mumbai, India, and ammonium formate ((NH_4HCO_2) was purchased from Rankem Laboratory Chemicals. The buffer solution was prepared using ammonium formate, and the pH was adjusted with formic acid.

Chromatographic Conditions

Remogliflozin and Metformin were separated by chromatography using various Hypersil ODS C18 columns (250 × 4.6 mm inner diameter, 2.5 µm). The HPLC system was Shimadzu (Japan) Corporation, HPLC system including two LC-20AD pumps, SPD-M20A Diode Array Detector (DAD), CBM-20A system regulator, controlled by LC Control Software [8]. Analysis was done in the mobile phase of acetonitrile and 20 mM ammonium formate solution buffer (pH 3.5) (v/v: 40:60). The 1.0 ml per min was kept constant. The analytes were detected by using DAD at 243 nm. The operation was at ambient temperature and an injection volume of 20 µL for each sample [9].

Mobile Phase Preparation

Combined acetonitrile and 20 mM ammonium formate solution (pH 3.5) is used as moving phase in the proportion of 45:55 (v/v). The mixture was strained for any particulates through a 0.45 µm nylon filter membrane. The air bubble in the demulating phase was removed by degassing of mobile phase in an ultrasonic bath before analysis to avoid column blockage problems [10].

Preparation of Buffer Solution

The ammonium formate was blended in 1 L of HPLC-grade water to make the buffer solution, and the final concentration was 1.26 g. Formic acid was subsequently employed to adjust the pH to 3.5. When the pH was adjusted, the solution was strained through a 0.45 µm membrane filter and degassed in an ultrasonic bath to ensure the smooth operation and no air contamination in the HPLC system [11].

Preparation of Standard Solution

It was brought to standard preparation after dissolution of 10 mg Remogliflozin and 50 mg Metformin in 80 ml methanol. Make the volume of the final volume 100 ml with methanol, and then sonicate for 10 minutes so that it completely dissolves. To make the Remogliflozin and Metformin stock solutions in the amount ranges of 2.5–25 µg/ml for Remogliflozin and 12.5–125 µg/ml for Metformin, 10 ml of the same solvent was made up in 10 ml. After, the solutions were strained through a 0.45 µm Millipore membrane filtration to remove any solid particles [12, 13].

Sample Solution Preparation

20 tablets of Remogliflozin and Metformin were measured, finely ground. Finally, Remogliflozin equal to 10 mg and Metformin equivalent to 50 mg were diluted from a 100 ml volumetric flask. Sonicating the solution ensured complete dissolution of the powder into the mobile phase. The volume

was adjusted to 100 ml after dissolution using the mobile phase, and the filtrate was filtered to remove any particulates through use of a 0.45 µm membrane strain [14].

Chromatographic Examination

The prepared mobile phase was then used for the chromatographic analysis under isocratic conditions. 1.0 ml per minute flow rate and 20 µl injection volume was employed. The DAD at 243 nm was used for the detection of the analytes. During the analysis, the system was set to ambient temperature to ensure the stability of the samples [15].

RESULTS AND DISCUSSION

Method Creation and Optimization

Concurrent determination of Remogliflozin and Metformin in their raw form and pharmaceutical formulation to get high resolution and reproducibility was achieved through a successful development and optimization of the RP-HPLC approach. Therefore, the best chromatographic parameters included the use of a Hypersil ODS-C18 column (250 × 4.6 mm, inner diameter, 2.5 µm) and a moving phase consisting of a 45:55 (v/v) combination between acetonitrile and 20 mM ammonium formate solution (pH 3.5). This assay came out at 243 nm with a velocity rate of 1 ml/min, and detection was done with retention times of Remogliflozin, as well as of 3.24 and 4.89 mins are shown in Figure 1.

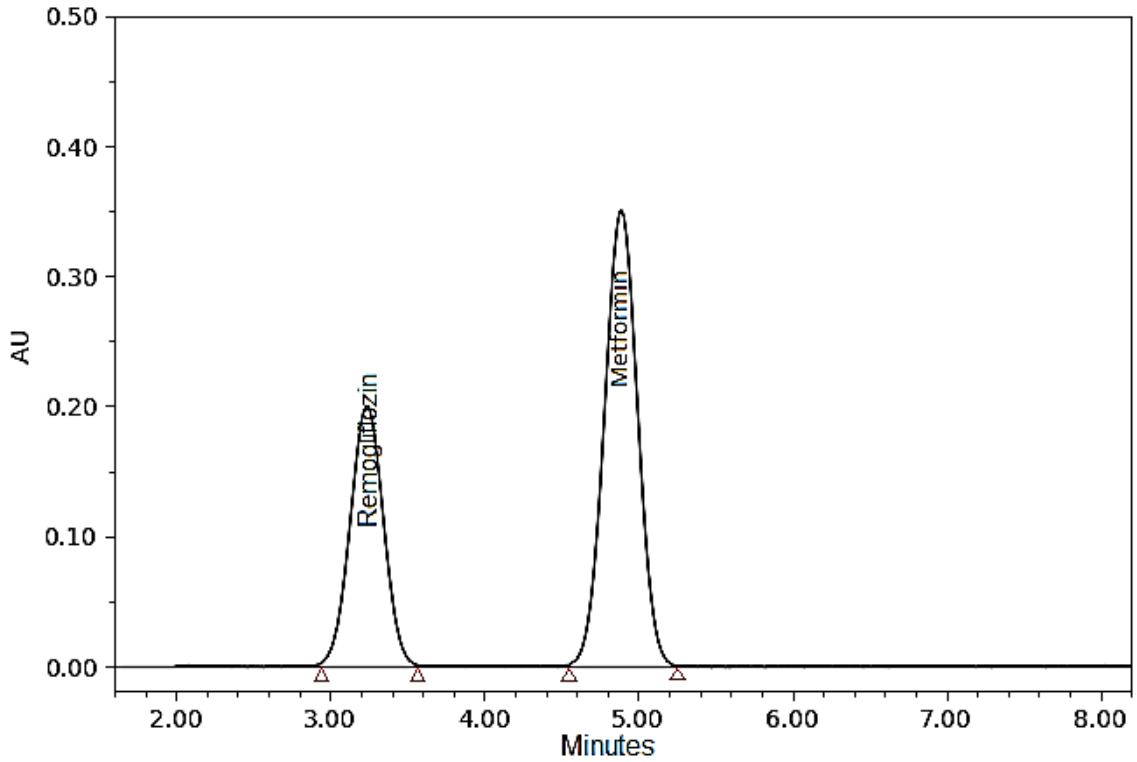


Figure 1. Chromatogram showing retention peak.

System Appropriateness Parameters

The RP-HPLC technique, in the retention times of Remogliflozin as well as Metformin, is consistent in multiple injections, indicating excellent stability and reproducibility. The %RSD values for peak area variations were 0.90% for Remogliflozin and 0.13% for Metformin, which showed excellent precision of the method. Furthermore, the two drugs demonstrated excellent peak symmetry with USP tailing factors of 1.29 for Remogliflozin and 0.97 for Metformin and were within acceptable peak boundary conditions (≤2). In fact, the high plate count values of 5380 and 6580 were recorded for Remogliflozin and Metformin, with the column confirmed to be efficient, offering good resolution and clear separation

of the two drugs. Evaluation of the system suitability parameters was done to confirm the reliability and efficacy of the chromatographic system. It was found that the system is working well, since all the parameters satisfied the specified ones, and their results are given in Table 1.

Table 1. System suitability parameters.

Injection No.	Retention Duration	Peak Region	Retention Time	Peak Region	USP Tailing Factor	USP Plate Count	USP Tailing Factor	USP Plate Count
	Remogliflozin		Metformin		Remogliflozin		Metformin	
1.	3.240	361580	4.890	1624800	1.29	5380	0.97	6580
2.	3.258	359742	4.895	1619840	1.28	5370	0.96	6570
3.	3.220	355672	4.910	1631284	1.30	5365	0.96	6560
4.	3.230	358926	4.900	1623935	1.29	5390	0.97	6590
5.	3.212	363852	4.870	1617621	1.28	5400	0.96	6600
6.	3.250	364003	4.920	1621065	1.29	5375	0.97	6575
Mean	3.240	360768	4.895	1620537.5	1.29	5380	0.97	6580
SD	0.016	3250.12	0.018	2104.56				
%RSD	0.49	0.90	0.37	0.13				

Accuracy and Recovery

To confirm the amount of quantification of Remogliflozin and Metformin, the developed RP-HPLC method was assessed by recovery studies at 80%, 100%, and 120% spiking levels. The results from Table 2 of the method were validated up to 99.80–101.48%, making it highly accurate. The mean values at 80%, 100%, and 120% spiking levels of Remogliflozin were 1.01.48%, 0.99.80% and 1.00.43% %RSD, and showed exceptionally good precision. Similarly, the recovery values for Metformin were from 100.24% to 100.49% with less than 0.4% %RSD, indicating method precision to validate. Routinely analyzing pharmaceutical formulations is shown to infer that the recovery values are within the acceptable range (98%−102%), and the minimal standard deviation values for different spiking levels mean that the technique is robust as well as reproducible. The proposed technique can be applied reliably for measurable estimation of Remogliflozin and Metformin in bulk as well as in tablet dosage form.

Table 2. Accuracy and retrieval data (n = 6).

Drug	% Spiking Grade	Medication in Tablet (µg)	Standard Drug Added (µg)	Overall Drug (µg)	Overall Recovered (Mean ± SD) (µg)	%RSD	Retrieval %
Remogliflozin	80%	4.5	3.6	8.1	8.22 ± 0.102	1.24	101.48
	100%	5.0	5.0	10.0	9.98 ± 0.180	1.80	99.80
	120%	5.5	6.2	11.7	11.75 ± 0.115	0.98	100.43
Metformin	80%	24.5	19.8	44.3	44.52 ± 0.154	0.34	100.49
	100%	25.0	25.0	50.0	50.12 ± 0.148	0.29	100.24
	120%	25.5	30.5	56.0	56.18 ± 0.205	0.37	100.32

PRECISION

System precision and method precision parameters were used for evaluating precision of the RP-HPLC method. L distilled water containing Metformin or Remogliflozin 8 mg/ml was injected three times on each of the four columns. The %RSD for Metformin was 1.42% (system precision) and 0.68% (method precision), and for Remogliflozin was 0.58% and 0.97%, respectively. The extent of repeatability and reproducibility of the method is shown by these values. In Table 3, the findings are summarized.

Intermediate Accuracy (Ruggedness)

This involved conducting precision studies at the intraday and interday level to assess intermediate precision of the developed RP-HPLC method; namely, repeatability and robustness of the method.

Similar to Remogliflozin and Metformin, this analysis was done in six replicates on the same day for %RSD of Remogliflozin and Metformin were 0.69 and 0.31, respectively, at concentrations of 10 µg/ml and 50 µg/ml. Interday precision was tested on Remogliflozin and Metformin in six samples that were analyzed on three different days, %RSDs were 1.18 for Remogliflozin and 1.07 for Metformin. The %RSD values are such low numbers that it confirms the high precision and reproductibility of the method for quality control analysis. This is reported in Table 4.

Table 3. System and method accuracy study data.

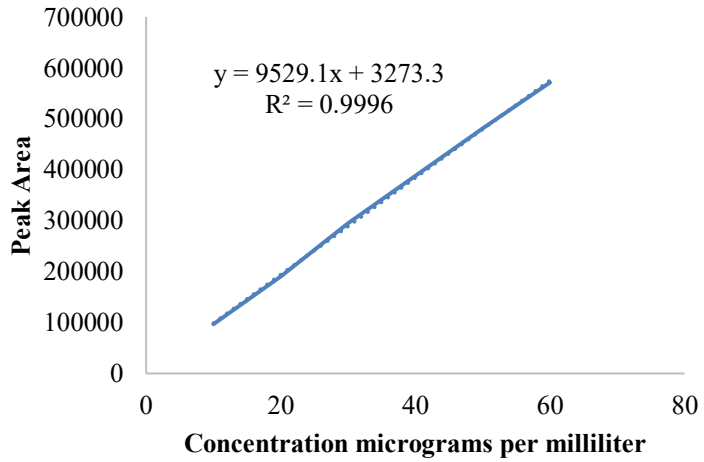
Injection Quantity	Peak Region of Remogliflozin	Peak Region of Metformin	% Assay of Remogliflozin	% Assay of Metformin
1.	355214	1601278	99.12	99.85
2.	352318	1589264	100.28	99.34
3.	348672	1587129	100.42	99.52
4.	345892	1579263	101.15	101.28
5.	357182	1597251	99.86	99.72
6.	343961	1575234	101.38	101.42
Mean	350373	1588236	100.37	100.19
SD (±)	4976.35	9653.42	0.68	0.97
RSD (%)	1.42	0.58	0.68	0.97
Acceptance criteria: %RSD value should be ≤ 2				
Concentration: Remogliflozin (10 µg/ml) and Metformin (50 µg/ml)				

Table 4. Intraday and interday precision data.

Concentration (µg/ml)	Intraday Accuracy (%RSD)	Interday Accuracy (%RSD)
Remogliflozin 8 & Metformin 40	0.69	1.18
Remogliflozin 10 & Metformin 50	0.31	1.07
Remogliflozin 12 & Metformin 60	1.02	1.38

Linearity and Range

Linearity of 2.5–25 µg/mlfor Remogliflozin as well as 12.5–125 µg/ml for Metformin was demonstrated by the technique. Results show a strong linear relationship between concentration and peak area, as correlation coefficients (R^2 values) of 0.9993 were observed for Remogliflozin and 0.9996 for Metformin are shown in Figure 2.



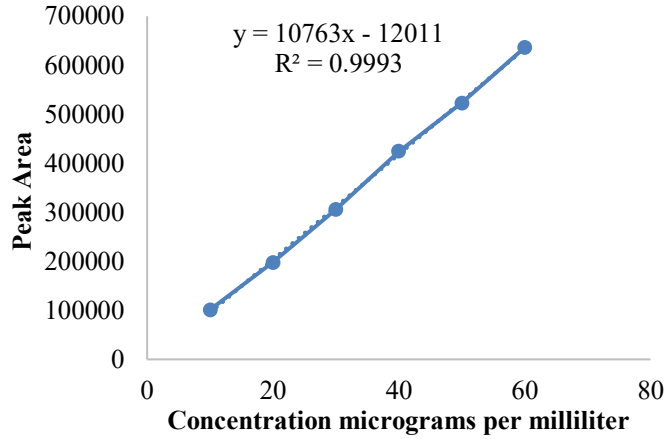


Figure 2. Calibration curve of remogliflozin and metformin.

Table 5. Robustness study data for Remogliflozin and Metformin under various analytical conditions.

Parameter	Level	%RSD	Tailing Factor	% Recovery	%RSD	Tailing Factor	% Recovery
		Remogliflozin			Metformin		
Flow rate (ml/min)	0.8	0.72	1.12	99.41	0.21	1.04	99.89
	1.0	0.55	1.07	100.15	0.32	1.19	99.52
	1.2	0.89	1.31	99.09	0.48	1.13	100.39
pH	3.5	0.11	1.02	99.76	0.57	1.22	99.26
	4.0	0.71	1.41	99.63	0.51	1.16	98.95
Wavelength (nm)	241	0.66	1.20	99.27	0.85	1.10	99.14
	243	0.09	1.04	99.48	0.54	1.08	99.74
	245	0.57	1.33	100.76	0.92	1.21	99.42
Mobile phase ratio (ACN: buffer)	30:70	0.95	1.38	101.42	0.24	1.09	101.21
	40:60	0.49	1.05	99.31	0.83	1.07	100.74
	50:50	0.85	1.06	99.38	0.61	1.18	100.71

Robustness Data

The analysis of robustness study further confirms that the developed RP-HPLC technique for simultaneous quantification of Remogliflozin as well as Metformin is highly stable and robust even under minor variations in analytical conditions. From Table 5, the %RSD values less than 2%, peak symmetry, and within the acceptable range of the drug recovery revealed the robustness of this method for the routine quality control/pharmaceutical analysis.

Detection and Quantitation Limits

The developed RP-HPLC technique was evaluated in terms of its sensitivity by determining the limit of detection (LOD) as well as limit of quantitation (LOQ). Remogliflozin had a LOD of 0.38 µg/ml and Metformin as 1.88 µg/ml. The method was found to be highly sensitive due to LOQ values (1.14 µg/ml for Remogliflozin and 5.72 µg/ml for Metformin), which suggest a potential capability of the detection and quantification of low concentrations of the two drugs. The results are summarized within Table 6.

Table 6. LOD and LOQ information.

Medication	LOD (Micrograms/ml)	LOQ (Micrograms/ml)
Remogliflozin	0.38	1.14
Metformin	1.88	5.72

Table 7. Assay data for marketed formulations.

Marketed Formulation	Drug	Retention Time (min)	Peak Area	% Assay
GLIREMO-M	Remogliflozin 100 mg	3.29	345892	99.45
	Metformin 500 mg	5.08	1587234	99.76
REMOGLIP-M	Remogliflozin 100 mg	3.31	351274	100.32
	Metformin 500 mg	5.11	1604876	100.28
METREMO-XR	Remogliflozin 100 mg	3.26	358291	101.23
	Metformin 500 mg	4.97	1617825	101.05

Assay of Marketed Formulations

This RP-HPLC method development was applied for two marketed formulations, GLIREMO-M, REMOGLIP-M, and METREMO-XR. From the result of Table 7, each of which contained 100 mg Remogliflozin and 500 mg Metformin. Retention times of Remogliflozin (3.26–3.31 min) and Metformin (4.97–5.11 min) were reproducible. The acceptable range of 90%–110% calculated for percentage assay values confirmed the accuracy and reliability of the method for routine quality control.

CONCLUSIONS

RP-HPLC method developed was successfully optimized and validated for the simultaneous determination of Remogliflozin and Metformin in bulk and marketed formulations. The method showed high precision, accuracy, linearity, robustness, and sensitivity, so it strongly quantified reliably. The retention times were sufficiently resolved to be applied to quantitative assay results for routine quality control. Because of this, it can be used for pharmaceutical analysis and applied to the quality assessment of fixed-dose combinations of Remogliflozin and Metformin.

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Conflict of Interest

The authors announce that there is no disagreement of interest associated with this research work.

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