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ORIGINAL ARTICLE

A Novel Analytical Method for the Simultaneous Estimation of Remogliflozin and Metformin Hydrochloride by UPLC/PDA in Bulk and Formulation Application to the Estimation of Product Traces

Bulk ve Formülasyon Uygulamalarında Eser Ürünlerin Kestirimi için UPLC/PDA ile Remogliflozin ve Metformin Hidroklorürü Eşzamanlı Belirleyebilen Yeni Bir Analitik Yöntem

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ABSTRACT

Objectives: A selective and novel method has been optimized for the evaluation of remogliflozin and metformin hydrochloride in bulk and in the formulation and cleaning of samples by UPLC-PDA in bulk and formulation and product traces.

Materials and Methods: The principle analytes were eluted with phosphate buffer (pH: 4.5): acetonitrile (60:40%, v/v) as the mobile phase using the Spherisorb C18, 5 µm, 4.6 mm x 150 mm analytical column with a 1.0 mL/min flow rate and a 10 µL sample volume at 245 nm in a photodiode array detector.

Results: The retention times of remogliflozin and metformin hydrochloride were 3.017 min and 5.011 min with a total run time of 8 min. The curve indicates that the correlation coefficient (r^2) was superior with a value of 1.000 in the linear range of 10 ng/mL-100.0 ng/mL for remogliflozin and 50 ng/mL-500.0 ng/mL for metformin hydrochloride. The correlation coefficient (r^2) for metformin hydrochloride was found to be 1.000. The lower limits of quantification and detection for remogliflozin and metformin hydrochloride were found to be 10 ng/mL and 50 ng/mL, and 5 ng/mL and 10 ng/mL, respectively.

Conclusion: The developed method was validated and applied to the bulk drug estimation and drug formulation and cleaning samples. All the results obtained with this method was accurate and precise.

Key words: Remogliflozin, metformin hydrochloride, bulk drug, formulation, cleaning samples, UPLC-PDA

ÖZ

Amaç: UPLC-PDA ile yığında ve formülasyon uygulamalarında remogliflozin ve metformin hidroklorürün eş zamanlı tayini ve örnek temizliğinin belirlenmesi için seçici ve yeni bir yöntem optimize edilmiştir.

Gereç ve Yöntemler: Temel analitler, hareketli faz olarak fosfat tamponu (pH: 4,5): asetonitril (60: 40%, v/v) ile, Spherisorb C18, 5 µm, 4,6 mm x 150 mm analitik kolon kullanılarak, 1,0 mL/dk akış hızında fotodiyot array dedektörü ile 245 nm'de 10 uL örnek hacmi ile elüe edilmiştir.

Bulgular: Remogliflozin ve metformin hidroklorürün alıkonma süreleri sırasıyla 3,017 dakika ve 5,011 dakikaydı ve toplam çalışma süresi 8 dakikaydı. Eğri, korelasyon katsayısının (r^2), remogliflozin için 10 ng/mL-100,0 ng/mL ve metformin hidroklorür için 50 ng/mL-500,0 ng/mL doğrusal aralıkta 1,000 değeriyle üstün olduğunu göstermektedir. Metformin hidroklorür için korelasyon katsayısı (r^2) 1,000 olarak bulundu. Remogliflozin ve metformin hidroklorür için alt kantifikasyon ve saptama sınırları sırasıyla 10 ng/mL ile 50 ng/mL ve 5 ng/mL ile 10 ng/mL olarak bulunmuştur.

Sonuç: Geliştirilen yöntem valide edilmiş ve yığında, ilaç formülasyonunda ve temizleme numunelerinde ilaç belirlenmesi için uygulanmıştır. Bu yöntemle elde edilen tüm sonuçlar doğru ve kesindir.

Anahtar kelimeler: Remogliflozin, metformin hidroklorür, yığın ilaç, formülasyon, temizleme numuneleri, UPLC-PDA

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INTRODUCTION

Remogliflozin etabonate [5-methyl-4-(4-(1-methylethoxy) benzyl)-1-(1-methylethyl)-1H-pyrazol-3-yl 6-O-(ethoxycarbonyl)-β-D-glucopyranoside] is a pro-drug of remogliflozin. It belongs to the glifozin class of drugs. This drug is primarily used in cases of non-alcoholic steatohepatitis and type-2 diabetes. Remogliflozin inhibits the sodium-glucose transport proteins, which are responsible for glucose reabsorption in the kidney. Metformin (N,N-dimethylimido dicarbonimidicdiamide) is used to lower blood sugar in those with type 2 diabetes. It is also used to treat polycystic ovary syndrome. Metformin is a dimethyl biguanide that reduces elevated blood glucose levels primarily by reducing hepatic glucose production and improving peripheral tissue sensitivity to insulin.[1]

Based on a literature survey, there are no existing analytical methods for this new formulation, i.e., remogliflozin and metformin hydrochloride. Several methods have been developed for other gliflozin drugs, such as dapagliflozin, empagliflozin, and canagliflozin, with other combination of gliptins such as saxagliptin and linagliptin and with biguanides such as metformin.[2-20] For the remogliflozin and metformin hydrochloride combination, there was a lack of sensitive analytical methods for the identification and quantification in bulk and in formulations. Moreover, there was no sensitive analytical method with the 10 ng/mL sensitivity necessary to quantify the product traces left in manufacturing areas after a product changeover.

MATERIALS AND METHODS

Remogliflozin (Figure 1), metformin hydrochloride (Figure 2), and high-purity acetonitrile were from (J.T. Baker, Phillipsburg, NJ, USA); water was from a (Milli-Q system, Millipore, Bedford, MA, USA); potassium dihydrogen phosphate and sodium dihydrogen phosphate were from (Merck Pvt. Ltd, Worli, Mumbai); and ortho phosphoric acid was from (Merck Pvt. Ltd, Worli, Mumbai). The formulation was provided by the Yountus Life Sciences, Andhra Pradesh, India.

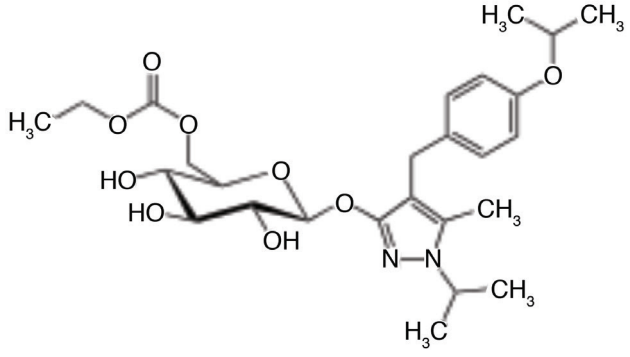

Figure 1. Remogliflozin

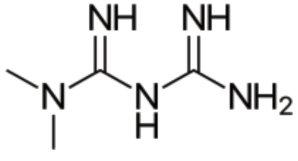

Figure 2. Metformin

Preparation of standard solutions

Metformin hydrochloride and remogliflozin etabonate standard stock solutions were prepared by placing 25.38 mg and 126.92 mg, respectively in 25 mL volumetric flasks and then adding 10 mL diluent and sonicating for 3 minutes. Then, the volume was adjusted to 25 mL with diluent. From the stock 25 mL, 1 mL was removed to a 1000 mL volumetric flask and the volume adjusted to 25 mL with diluent. From this 1000 mL, 1 mL was removed to a 10 mL volumetric flask and the volume adjusted to the mark with diluent to obtain a 100 ng/mL solution of remogliflozin and a 500 ng/mL solution of metformin hydrochloride.

Preparation of buffer (pH 4.5)

Potassium dihydrogen phosphate (13.9 g) and disodium hydrogen phosphate (35.04 g) were weighed precisely and added to a 1000 mL beaker. Water (500 mL) was added and stirred with a glass rod to completely dissolve the salts, and then the volume was adjusted to 1000 mL with water. The prepared buffer solution was adjusted to pH to 4.5 with dilute ortho phosphoric acid.

Preparation of the mobile phase

From the 1000 mL buffer, 600 mL buffer was removed and added to a 1000 mL mobile-phase bottle. Acetonitrile (400 mL) was added to the buffer and the buffer degassed to prepare 1000 mL of mobile phase.

Preparation of diluent

The diluent was prepared by adding 2000 mL of water to a 4000 mL mobile-phase bottle and then adding 2000 mL of methanol and degassing to obtain 4000 mL of diluent.

Optimization of chromatographic conditions

After a series of trials, the final chromatographic conditions were determined as follows. The mobile phase was a buffer with pH 4.5 and acetonitrile (60:40% v/v), and the stationary phase was a Spherisorb C_{18} column with dimensions 5 µm, 4.6 mm x 150 mm to obtain the best peak shape. The separation of remogliflozin and metformin hydrochloride was good at 245 nm with a column temperature of 25°C, a sample compartment temperature of 10°C, a flow rate of 1.0 mL/min, and a sample volume of 10 µL.

Assay sample preparation

One tablet (REMO-M) containing remogliflozin 100 mg and metformin 500 mg was added to a 1000 mL volumetric flask, dissolved in diluent, and the volume adjusted to 1000 mL. This preparation was considered as the stock solution. From the stock solution, 1 mL was removed and added to a 1000 mL volumetric flask and the volume adjusted to the mark with

diluent to obtain 100 ng/mL of remogliflozin and 500 ng/mL of metformin hydrochloride.

Validation of the analytical method

Validation was performed for the developed method within stringent limits to test the efficiency of this method.[1,2]

To verify that the system produced consistent results with the optimized method, the standard was injected 6 times with the criteria of % relative standard deviation (RSD) for retention time (RT) and area not more than (NMT) 2.0%, the oretical plates not less than (NLT) 3000 plates, tailing factor NMT 1.5, and resolution NLT 4.

Selectivity

To verify the method validation in terms of selectivity and exactness, triplicate preparations of 100% concentration, i.e., 100 ng/mL of remogliflozin and 500 ng/mL of metformin hydrochloride, were injected. Then, one blank was also injected to test for carryover. The limit of specificity is that it should pass the system suitability criteria, and there should not be an RT shift for any of the three preparations.

Precision

After passing the specificity and system suitability criteria, the method was verified for system precision and method precision with the limit of % RSD for the RT and area NMT 2%. The intermediate precision was verified on the next day with another column by setting the limit as % RSD for the RT and NMT 2% for the area.

Accuracy and recovery

To verify the method accuracy, triplicate preparations were prepared at 80%, 100%, and 120% of the 100% concentrations (100 ng/mL for remogliflozin and 500 ng/mL for metformin hydrochloride) by spiking the standard into the diluent. The percent recovery was calculated with acceptance criteria of 95%-105%.

Linearity

The method linearity was verified with 5 dilutions of the 100% concentration: 10 ng/mL, 20 ng/mL, 50 ng/mL, 75 ng/mL, and 100 ng/mL for remogliflozin and 50 ng/mL, 100 ng/mL, 250 ng/mL, 375 ng/mL, and 500 ng/mL for metformin hydrochloride. The acceptance criterion of the regression coefficient (R^2) was NLT 0.99.

Robustness

To verify the method efficiency when minor changes occurred in optimized method parameters such as mobile-phase composition, column temperature and flow, and buffer pH, these parameters were tested with the criteria that they should pass the system suitability criteria.

Lower level of quantification (LOQ)

By considering the 10% concentration of the target concentration, the sample was injected into the system with the acceptance criteria S/N ratio NLT 10. From the lower LOQ, preparations of different concentrations were injected to identify the detectability with the acceptance criteria 3:1, and the minimum detectability was five times out of six injections from the same concentration.

Lower level of quantification precision

LOQ precision was verified with the limit NMT 2.0% for the RT and area.

Assessment of stability of the standard and mobile phase

The prepared mobile phase and standard preparations were verified for stability up to 72 hours.

Degradation behavior

To test the developed method for stability indicating method the formulation sample was subjected to acid and base, and thermal, photo, and peroxide degradation were carried with the aim of detection of degradants in the chromatogram. Acid degradation was carried out by adding 20 mL of 0.1N HCL to the stock solution, and from that 1 mL was removed and added to a 1000 mL volumetric flask and the volume adjusted to the mark. In the same way, 2 mL 1N NaOH was added to test for base degradation. To test for thermal degradation, the sample was subjected to heat at 105°C for 3 hours and the sample prepared as per the assay procedure. For photo degradation, the sample was exposed to ultraviolet light with an intensity NLT 2000 lux power for 6 hours and the sample prepared as per the assay procedure. For peroxide degradation, 2 mL H_2O_2 were added to the stock 1000 mL volumetric flask, 1 mL was removed and added to a 1000 mL flask, the volume adjusted to the mark with the diluent, and the sample was injected.

Filter compatibility

To evaluate the impact of polyvinylidene fluoride (PVDF) and Nylon filters on the assay results, the samples were analyzed after passage through the filters.

Recovery of the Swabs from the stainless steel (SS) and glass and epoxy plate

Due to the high sensitivity (nanogram level) of the developed method, it can be used in cleaning method validation or for surface cleaning sample quantification at the time of product changeover in the manufacturing area. Hence, the method applicability for the quantification of surface cleaning samples in the manufacturing area was verified. Three surfaces (SS, glass, epoxy) were selected based on the manufacturing area designs as per the cGmp. Sterile swabs were taken and the recovery verified from the SS plate, glass plate, and epoxy plate with the acceptance criteria NLT 90% with the LOQ concentration (10 ng/mL remogliflozin and 50 ng/mL metformin). The recovery was calculated by pouring the 1 mL sample before the final concentration (after the first dilution in 1000 mL) of the standard preparation on the plates. After drying, the swab was added to a 10 mL volumetric flask and the volume adjusted to the mark with diluent.

Statistical analysis

The data were processed through the Q Sight software, and the results were calculated as mean and ± SD for the accuracy and

the RSD was calculated for the precision. The coefficient of regression was also calculated in the linearity parameter.

RESULTS

Clear separation and good resolution without any carryover was achieved with this method as shown in Figure 3-6. The system suitability acceptance criteria were also found to be satisfactory as shown in Table 1, 2. For the system precision parameters, the % RSD of RT and area for remogliflozin and metformin hydrochloride achieved 0.02% and 0.03%, and 0.01% and 0.03% as shown in Table 3 against the limit NMT 2.0%. For the method precision parameters, the %RSD of RT and area for remogliflozin and metformin hydrochloride achieved 0.03% and 0.02%, and 0.02% and 0.05% against the limit NMT 2.0% as shown in Table 4. The linearity parameter was quantified by peak area vs. concentration methodology. Different concentrations from 10 ng/mL to 100 ng/mL standard solutions for remogliflozin and from 50 ng/mL to 100 ng/mL were prepared and injected into the system. The recovery for 80%, 100%, and 120% was more than 99% against the acceptance criteria of 95%-105% as shown in Table 5 and Figure 7-9. The calculated regression coefficient for remogliflozin and metformin hydrochloride was 1.000 as shown in Figure 10, 11. To evaluate the method's capability of producing precise results with minor variations in flow, mobile-phase composition, pH, and column temperature variations, a test for robustness was performed. The results are shown in the Table 6. The results prove that the method was stable to produce consistent results with minor variations of the method parameters. The compatibility of the filters was verified with PVDF and Nylon filters. The assay for remogliflozin and metformin hydrochloride was more accurate (100.2% for remogliflozin and 99.7% for metformin hydrochloride) with the PVDF filter compared with the Nylon filter (99.8% for remogliflozin, 98.9% for metformin hydrochloride) as shown in Table 7. To demonstrate that the

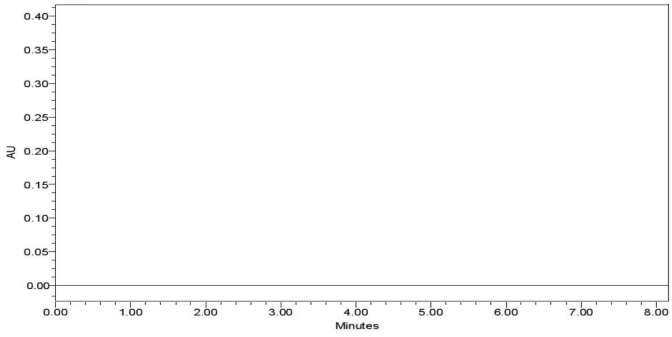

Figure 3. Blank chromatogram

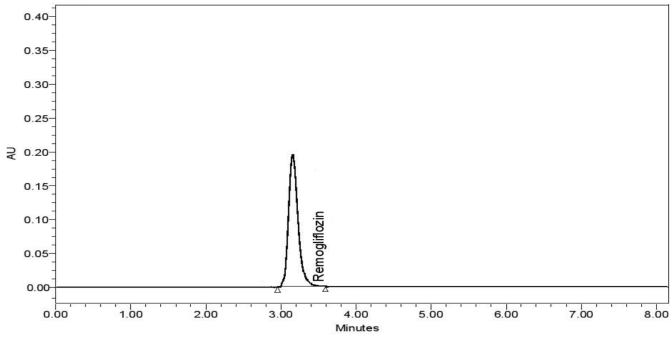

Figure 4. Specificity chromatogram of remogliflozin

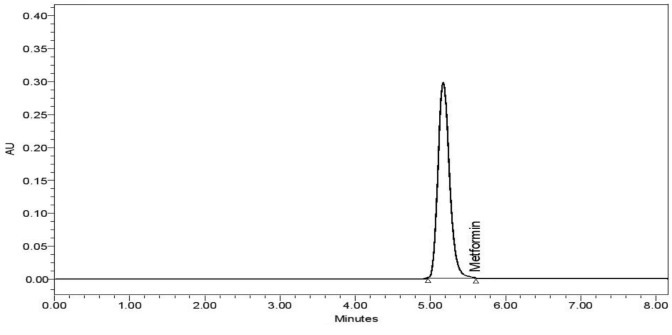

Figure 5. Specificity chromatogram of metformin

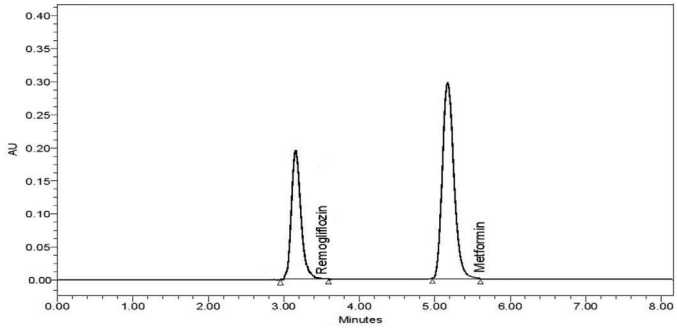

Figure 6. System suitability chromatogram of remogliflozin and metformin

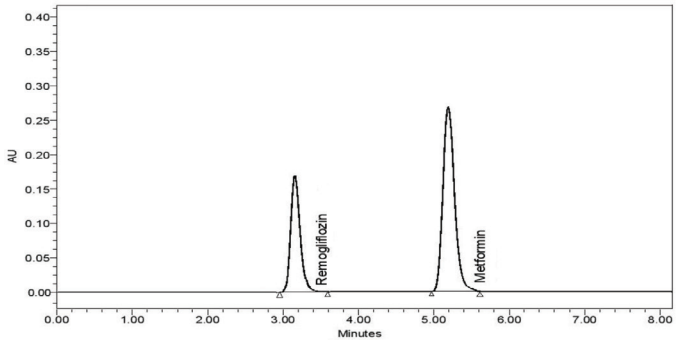

Figure 7. 80% accuracy level chromatogram of remogliflozin and metformin

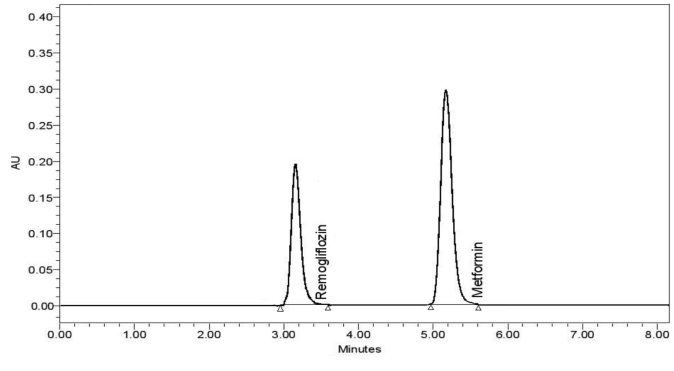

Figure 8. 100% accuracy level chromatogram of remogliflozin and metformin

Table 1. Specificity data

S. no	Injection	Remogliflozin	RT	Area	Metformin	RT	Area
01	Blank	Not detected	NA	NA	Not detected	NA	NA
02	01	Detected	3.018	983652	Detected	5.011	1215689
03	02	Detected	3.017	983259	Detected	5.012	1215697
04	03	Detected	3.018	983452	Detected	5.011	1215986

RT: Retention time

Table 2. System suitability data

Parameter	Remogliflozin	Metformin
Retention time	3.017	5.011
Area	983717	1216101
Asymmetry	0.8	1.1
Theoretical plates	6200	7800
Resolution	5.4	
% RSD of area	0.02	0.03

RSD: Relative standard deviation

Table 3. System precision data

Drug name	Remogliflozin		Metformin	
Injection	RT	Area	RT	Area
01	3.018	983251	5.011	1215641
02	3.017	983652	5.012	1216121
03	3.018	983569	5.011	1215624
04	3.018	983569	5.011	1215698
05	3.017	983957	5.012	1215564
06	3.018	983267	5.011	1216521
Average	3.018	983544	5.011	1215862
SD	0.0005	263.0821	0.0005	380.1435
% RSD	0.02	0.03	0.01	0.03

RSD: Relative standard deviation, SD: Standard deviation, RT: Retention time

method was stable, acid degradation was carried out, and the degradants were identified at 4.019 min and 6.017 min as shown in Figure 12. In base degradation, the degradants were detected at 4.516 min and 5.802 min and 7.224 min as shown in Figure 13. In light degradation, the degradants were detected at 3.681 min and 5.844 min and 6.192 min as shown in Figure 14. In thermal degradation, the degradants were detected in 3.841 min and 4.412 min and 5.942 min and 6.454 min as shown in the Figure 15. In the peroxide stress condition, the degradants occurred at 3.642 min and 4.235 min and 6.94 min and 7.421 min as shown in Figure 16. The LOQ for remogliflozin was 10 ng/mL and 50 ng/mL with S/N ratios of 11.8 and 10.8 as shown in Table 8. The LOQ precision was also performed to evaluate the repeatability at the lower end of the quantification range. The obtained % RSD of the area for remogliflozin and metformin hydrochloride

Table 4. Method precision data

Drug name	Remogliflozin		Metformin	
Injection	RT	Area	RT	Area
01	3.016	983958	5.012	1215632
02	3.015	983587	5.011	1216985
03	3.017	983695	5.012	1215896
04	3.016	983895	5.013	1215348
05	3.015	983958	5.012	1215835
06	3.017	983689	5.011	1215798
Average	3.016	983797	5.012	1215916
SD	0.0009	159.7586	0.0008	559.8081
% RSD	0.03	0.02	0.02	0.05

RSD: Relative standard deviation, SD: Standard deviation, RT: Retention time

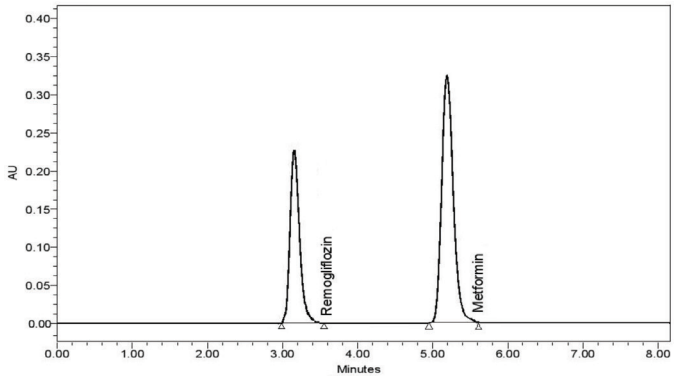

Figure 9. 120% accuracy level chromatogram of remogliflozin and metformin

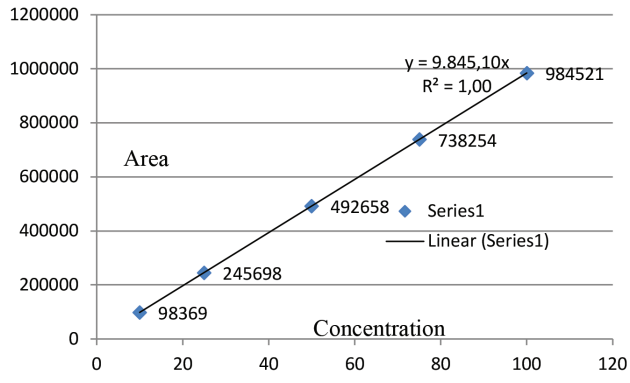

Figure 10. Linearity graph of remogliflozin

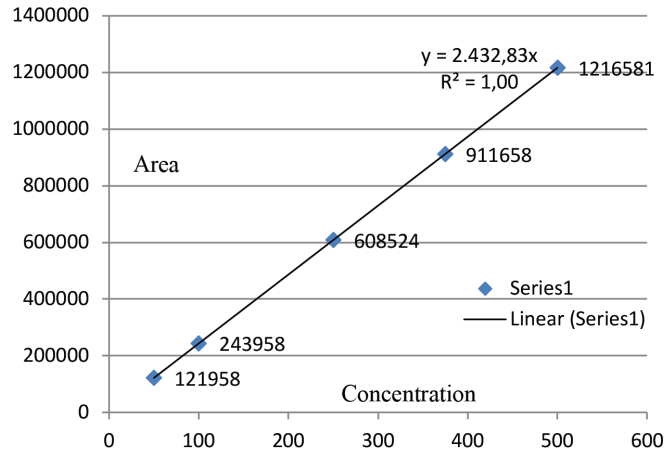

Figure 11. Linearity graph of metformin

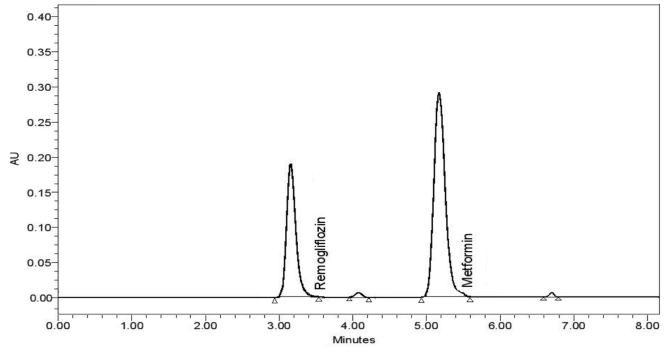

Figure 12. Acid degradation chromatogram

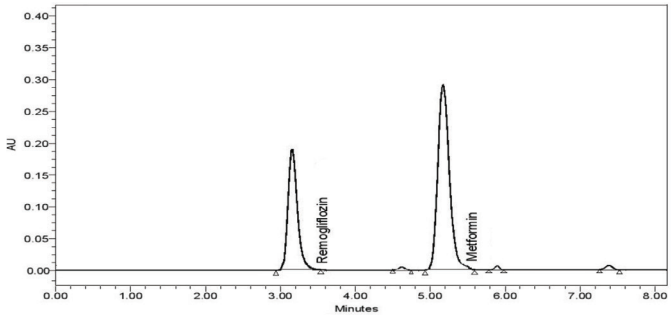

Figure 13. Base degradation chromatogram

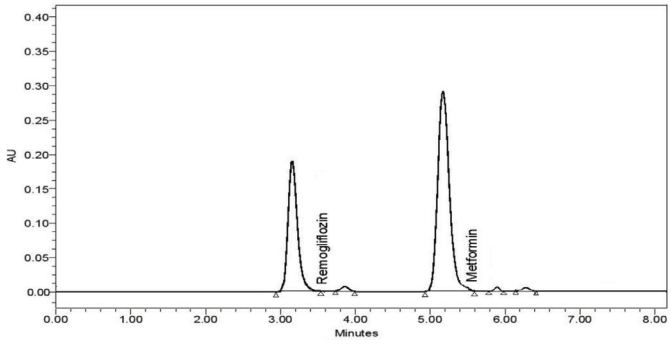

Figure 14. Photo degradation chromatogram

was 0.03 and 0.18% as shown in Table 9. The lower limit of detection (LOD) for remogliflozin was 5 ng/mL and 10.0 ng/mL with an S/N ratio of 3.8 and 3.5 as shown in Table 10, and clear detection is shown in Figure 17. For the intermediate precision parameter, the % RSD of area for remogliflozin and metformin hydrochloride achieved on day-1 was 0.03% and 0.02 and on the next day 0.06% and 0.02% against the limit NMT 2.0% as shown in Table 11. Solution and mobile-phase stability were established, and it was confirmed that the solution and mobile phase were stable for 72 hours as per the data furnished in Table 12. The purity angle and purity threshold were good as shown in Table 13. From these results, we can conclude that the method was stable. The method was verified for robustness as well as interday and intraday precision. The LOQ and LOD were identified by injecting the lower concentrations with the S/N ratio criteria, and the drugs were detected six times out of six injections. The obtained % RSD showing the capability of

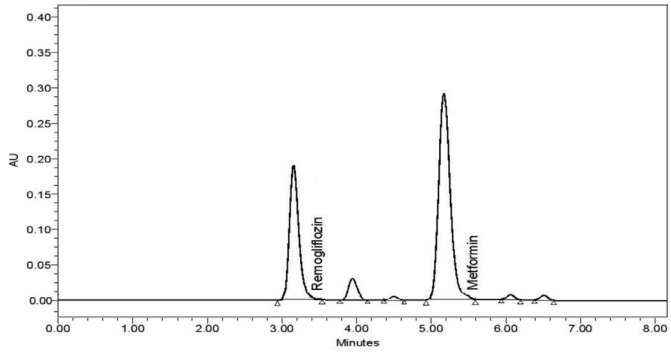

Figure 15. Thermal degradation chromatogram

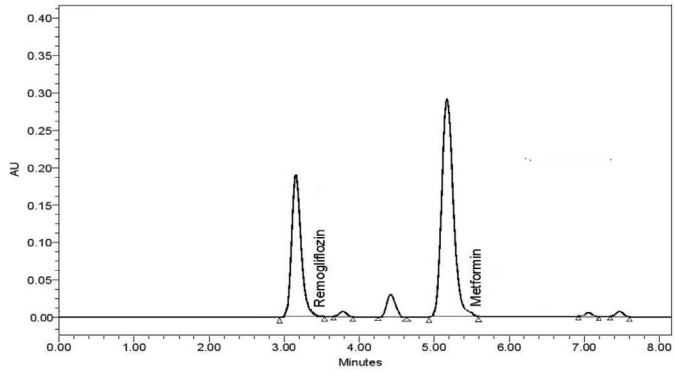

Figure 16. Peroxide degradation chromatogram

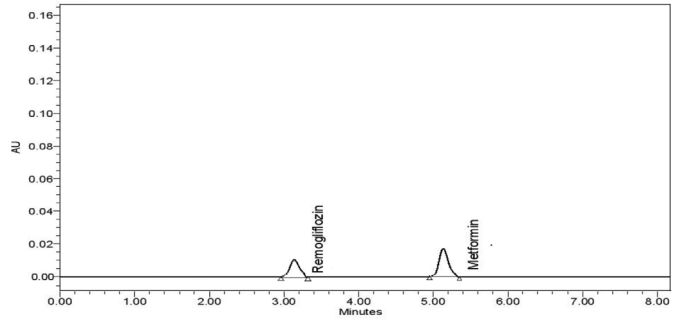

Figure 17. Limit of detection chromatogram

also quantifying the activities at lower concentrations. Then, the method was applied for recovery on a SS plate, a glass plate and an epoxy plate with the aim of recovery NLT 90% to prove its utility in cleaning method validation. The obtained average recovery for remogliflozin and metformin hydrochloride was above 94% as shown in Table 14-16.

DISCUSSION

During method optimization, organic solvents were initially used as the mobile phase with water in varying composition. However, neither compound was detected. Then, buffer was used with organic solvent such as acetonitrile in different ratios and at varying pH with the Spherisorb C_{18}, 5 µm,

Table 5. Accuracy and recovery data

S. no	Drug name	% Level spiking	Spiked amount (ng)	Area	Recovered amount (ng)	% Recovery	% CV
01	Remogliflozin	80	80.06	786851	80.00	99.9	0.10
			80.14	785695	79.88	99.7	
			80.22	785968	80.22	99.6	
02		100	100.08	983561	100.00	99.9	0.04
			99.90	982564	99.90	100.0	
			100.00	983651	100.01	100.0	
03		120	120.01	1178952	119.86	99.9	0.19
			120.17	1176951	119.66	99.6	
			120.09	1175689	119.53	99.5	
01	Metformin	80	403.32	974258	402.73	99.9	0.03
			403.40	975121	403.08	99.9	
			403.32	974568	402.85	99.9	
02		100	504.25	1216495	502.86	99.7	0.13
			504.21	1219585	504.14	100.0	
			504.25	1220214	504.40	100.0	
03		120	605.02	1459889	603.47	99.7	0.10
			605.09	1462315	604.47	99.9	
			605.17	1454898	601.41	99.7	

Table 6. Robustness data

Condition	Value	Remogliflozin				Metformin		
		RT	Area	Asymmetry	Resolution	RT	Area	Asymmetry
Flow	0.8 mL/min	3.112	984526	0.84	5.2	5.112	1218987	1.12
	1.0 mL/min	3.018	983625	0.81	5.4	5.011	1214658	1.10
	1.2 mL/min	2.997	982652	0.80	5.1	4.998	1214236	1.14
Mobile phase composition (buffer:acetonitrile)	55/35 v/v	3.201	984265	0.82	5.3	5.042	1210565	1.13
	60/40 v/v	3.016	983584	0.81	5.4	5.012	1215987	1.11
	65/45 v/v	2.895	982674	0.84	5.3	5.001	1201985	1.19
pH	4.0	2.965	984652	0.82	5.5	5.125	1219875	1.13
	4.5	3.017	983875	0.80	5.4	5.012	1215897	1.10
	5.0	2.912	982159	0.83	5.3	4.958	1219837	1.17
Column temperature	23	3.124	983121	0.82	5.3	5.064	1219856	1.12
	25	3.016	983898	0.80	5.4	5.011	1215648	1.10
	27	2.986	983687	0.85	5.3	4.985	1219765	1.15

RT: Retention time

Table 7. Filter compatibility

Drug name	0.2 µm PVDF filter assay	0.2 µm Nylon filter assay
Remogliflozin	100.2%	99.8
Metformin	99.7%	98.9
Difference	0.4% for remogliflozin, 0.8% for metformin	
Suitability	PVDF 0.2 µM filter	

PVDF: Polyvinylidene fluoride

Table 8. Limit of quantitation

Drug name	Area	LOQ	S/N ratio
Remogliflozin	98526	10 ng/mL	11.8
Metformin	122652	50 ng/mL	10.8

LOQ: Limit of quantitation, S/N: Signal to noise

Table 9. Limit of quantitation precision

Drug name	Remogliflozin		Metformin	
Injection	RT	Area	RT	Area
01	3.017	98537	5.013	122561
02	3.015	98579	5.012	122565
03	3.018	98567	5.012	122869
04	3.017	98521	5.011	122875
05	3.017	98585	5.011	122856
06	3.018	98596	5.011	122359
Average	3.017	98564	5.012	122681
SD	0.0011	29.24665	0.0008	216.8616
% RSD	0.04	0.03	0.02	0.18

RT: Retention time, RSD: Relative standard deviation, SD: Standard deviation

Table 10. Limit of detection

Drug name	Area	LOD	S/N ratio
Remogliflozin	49263	5 ng/mL	3.8
Metformin	24530	10 ng/mL	3.5

LOD: Limit of detection, S/N: Signal to noise

4.6 mm x 150 column. Finally, the method was found to be optimized with the conditions of mobile phase [buffer pH 4.5 and acetonitrile (60:40% v/v), wavelength 245 nm, flow rate of 1.0 mL/min, column temperature of 25˚C, sample compartment temperature of 10˚C, and sample volume of 10 µL]. With this method, both active compounds, i.e., remogliflozin and metformin hydrochloride eluted at 3.017 min and 5.011 min with good resolution and symmetry. Following method optimization, the method was validated as per ICH guidelines. As per the results obtained in the method validation, there was no interference of the blank or carryover problem, even at the LOQ. Both the LOQ and LOD of this method were verified practically in the instrument with S/N ratio criteria. The results were found to be satisfactory. The method was applied to degraded samples to verify its usefulness within the shelf-life period (stability indicating nature). The method detected degradants successfully in all the degradation conditions. As the method was highly sensitive, it was applied to the quantification of cleaning samples of manufacturing area surfaces with the criteria of recovery NLT 90%. Based on the results of recovery from SS, glass, and epoxy plates, this method has proven its capability to analyze cleaning validation samples at the time of products changeover in the manufacturing area.

CONCLUSION

Based on the results obtained in the current study, the developed method was very sensitive, accurate, linear, and economical. Due to the short duration of the chromatographic program, more samples can be analyzed within a short period, which will be helpful in the industry at a time when multiple products are manufactured continuously. The method met all the predefined acceptance criteria. With this method, the sample of bulk and formulation samples and surface cleaning samples can be analyzed. As the method is capable of detecting degradant formulations, bulk shelf-life samples can also be analyzed by using this method.

Table 11. Ruggedness data

Drug name	Injection	Day-1	Day-2	Drug name	Day-1	Day-2
Remogliflozin	01	983562	983256	Metformin	1216525	1215698
	02	984452	983265		1216956	1215669
	03	983652	983598		1215985	1215985
	04	983598	983645		1215152	1215678
	05	983675	983759		1214985	1215345
	06	983656	983458		1216256	1215985
Average		983766	983497		1215977	1215727
Standard deviation		338.7662	206.9661		774.7693	239.0219
% RSD		0.03	0.02		0.06	0.02

RSD: Relative standard deviation

Table 12. Standard and mobile-phase stability

Drug name	Remogliflozin		Metformin	
Injection	RT	Area	RT	Area
Initial	3.018	983251	5.011	1215641
12	3.015	983165	5.011	1215591
24	3.017	982991	5.012	1215232
36	3.017	982854	5.012	1214985
48	3.016	982718	5.012	1214568
72	3.015	982568	5.011	1214121
Average	3.016	982925	5.012	1215023
SD	0.0012	262.1334	0.0005	595.0297
% RSD	0.04	0.03	0.01	0.05

RT: Retention time, RSD: Relative standard deviation, SD: Standard deviation

Table 13. Degradation study on drug product data

Drug name	Condition	Peak area	% Recovery	% Degradation	Purity angle	Purity threshold
Remogliflozin	Undegraded	985652	100.2	-	-	-
	Acid	980125	99.6	0.6	0.211	1.221
	Base	981002	99.7	0.5	0.201	1.212
	Photo	979758	99.6	0.6	0.214	1.298
	Thermal	935654	95.1	5.1	0.944	1.720
	Peroxide	925452	94.1	6.1	0.984	1.611
Metformin	Undegraded	1209568	100.0	-	-	-
	Acid	1206521	99.7	0.3	0.116	1.141
	Base	1195681	98.9	1.1	0.311	1.351
	Photo	1195282	98.8	1.2	0.329	1.324
	Thermal	1186525	98.1	1.9	0.365	1.285
	Peroxide	1176521	97.3	2.7	0.485	1.261

Table 14. Recovery on stainless steel plate of 100% spiking

Drug name	Amount spiked (ng/mL)	Recovery (ng/mL)	% Recovery
Remogliflozin	10.17	9.66	95.0
Metformin	50.48	48.83	96.7

Table 15. Recovery on glass plate of 100% spiking

Drug name	Amount spiked (ng/mL)	Recovery (ng/mL)	% Recovery
Remogliflozin	10.32	9.77	94.6
Metformin	50.88	48.71	95.7

Table 16. Recovery on epoxy plate of 100% spiking

Drug name	Amount spiked (ng/mL)	Recovery (ng/mL)	% Recovery
Remogliflozin	10.13	9.71	95.9
Metformin	50.40	48.16	95.5

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