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 hidroksitürün eş zamanlı tayini ve örnek temizliğinin belirlenmesi için seçici ve yeni bir yöntemi 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 hidroksitürün alınma 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 hidroksitür için 50 ng/mL-500.0 ng/mL doğrusal aralığa 1,000 değerine üstün olduğunu göstermekteidir. Metformin hidroksitür için korelasyon katsayısı (r^2) 1,000 olarak bulundu. Remogliflozin ve metformin hidroksitür 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 yöntemi elde edilen tüm sonuçlar doğru ve kesindir.

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

Remogliflozin etabonate [5-methyl-4-(4-(1-methylethoxy)benzyl)-1-(1-methylethyl)-1H-pyrazol-3-yl 6-O-(ethoxycarbonyl)-\(\beta\)-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-dimethylimidodicarbonimidicdiamide) 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 glifozin 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.

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 \(\mu\)m, 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 \(\mu\)L.

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

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**Figure 1. Remogliflozin**

**Figure 2. Metformin**
Validation of the analytical method

Validation was performed for the developed method within stringent limits to test the efficiency of this method.\(^2\)

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\(_2\)O\(_2\) 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
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 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

| 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

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

RSD: Relative standard deviation

| 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     | 0.02          | 0.03      | 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                             |
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
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      |       |    |      |           |            |    |      |           |
| Flow      | 0.8 mL/min | 3.12 | 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 | 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
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 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

### Table 11. Ruggedness data

| Drug name | Injection | Day-1 | Day-2 | Drug name | Day-1 | Day-2 |
|-----------|-----------|------|------|-----------|------|------|
| Remogliflozin | 01 | 983562 | 983256 | 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
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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 | 98.2 | - | - | - |
| | 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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