Selective SGLT2 Inhibitor’s Estimation and Validation from Galenical Form by RP-HPLC Method

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i57B34029

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/78034

Received 06 October 2021
Accepted 12 December 2021
Published 14 December 2021

ABSTRACT

Aim: The recent analysis is required to do novel and simple, sensitive, precise, efficient, instant and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method for estimation of antidiabetic drug in the unit dosage form. Validation of this method is also planned to make it suitable for the actual use.

Study Design: Department of Pharmaceutical Chemistry, Datta Meghe Institute of Medical Sciences deemed to be university, Wardha in collaboration with Balkh university, Mazar-e-sharif, Afghanistan between August 2021 and December 2021.
**Methodology:** In that article develop the method and validate it by estimation of antidiabetic drugs in solid dosage form by RP-HPLC, by using System suitability test, Repeatability, Precision studies (Intra-day and Interday/Intermediate), Linearity/Calibration studies, Robustness, Force degradation, Specificity, Drug recovery/accuracy studies.

**Results:** as per ICH guidelines, the performance if system suitability in remogliflozin were achieved all guidelines; in that, tailing factor ($T$), separation factors ($\alpha$), theoretical plates ($N$), capacity factor ($k'$), resolution ($R$) and RSD (%). The validated stress degradation studies under thermal, oxidative, alkali and acid, in few degradation products for remogliflozin (REM).

**Conclusion:** From the results we conclude that, this novel technique which validated for exploration by reverse phase high performance liquid chromatography (RP-HPLC) should be used for routine quality control of remogliflozin (REM) prediction from developed formulation.

**Keywords:** Antidiabetic; remogliflozin; stress degradation; high performance liquid chromatography.

1. INTRODUCTION

According to previous research work, management of glycaemia control and reducing postprandial glucose excursions means change in concentration from before to after a meal can lower the risk of diabetic complications, e.g. Decrease probability of myocardial infarction means decreases or stops to the coronary artery of the heart, renal disease and retinopathy [1,2], the clinical management of T2DM is still difficult to manage, also with most of patients failing to reach and maintain their target of glycemic levels in practise [3]. Under normal physiological conditions when the glomerular filtrate reaches the proximal tubule, glucose is primarily reabsorbed through the active sodium dependent glucose transporter2 (SGLT2) located on the apical or luminal membrane of the epithelial cell in the S1 segment [4-6]. SGLT1 is a high-affinity, low-capacity glucose/galactose co-transporter primarily expressed in the intestine and in the kidney [7,8]. Together, SGLT1 and SGLT2 are responsible for the active reabsorption of glucose across the renal luminal membrane [9,10]. In humans, genetic alterations in SGLT2 increase renal glucose excretion (upto200g/day) with no apparent adverse effects on renal function or carbohydrate metabolism [11].

Remogliflozin etabonate is the ester prodrug of remogliflozin [12], which is the active entity that selectively inhibits SGLT2. Remogliflozin etabonate causes a concentration dependent increase in urinary glucose excretion in mice and rats [12, 13]. Unlike earlier SGLT inhibitors, such as phlorizin and T-1095, remogliflozin displays a high level of selectivity for SGLT2 over SGLT1 [14,15].

H3PO4 in fluid, also known as extraction buffer, has been the most frequently used solvent phase. A phosphate buffer’s pH can be easily adapted by using mono-, di-, or tribasic phosphate salts. When phosphate salts are used, however, To eliminate non-soluble molecules, solution was stirred through 0.22m filter paper. Other non-UV active acids or bases could be used to alter peak shape and accumulation [16].

High Performance Liquid Chromatography (HPLC) is more efficient as compared to gas chromatography (GC) since, it is not limited to volatile and thermally stable samples, and the choice of mobile and stationary phases is wider [17,18].

The pH of the solution is usually the deciding factor when selecting a buffer. The pH range for reversed phase on silica-based filling is typically 2 to 8. It is critical that the buffer has a pH that is close to the requested pH because buffers control pH best at about there pKa. A rule must select a buffer with a pKa value. [19-23].

Resolution, selectivity, and efficiency are all affected by the mobile phase. An aquatic buffer and a non-UV active water miscible organic solvent make up the mobile phases in reverse phase chromatography. The effects of the lipophilic and hydrophilic phases, as well as the

![Fig. A. Structure of remogliflozin etabonate](image-url)
amounts in which they are mixed, will influence the drug molecule's analysis. The synthetic nature of the analyte and the hydrophobicity of the analytes in the combination determine the mobile-phase and gradient parameters, respectively. The aqueous buffers are in a state of flux. The mobile phase protonates free silanols on the column at low pH, which lowers peak tailing and decreases peak tailing. Basic analytes are prepared by the reaction at low pH; when ionised, the particles become ionised. [24].

2. MATERIALS AND METHODS

The high-performance liquid chromatography (HPLC) of Shimadzu SCL-10AVP in built with binary pump(LC-10ATVP), UV detector(SPD-10AVP), Rheodyne20μl loop capacity manual injector(P/N77251) was used throughout the analysis. The LC-Solutions software was used to interpret the HPLC reports. Water-Symmetry® C18, 5μm; 150 x 4.6 mm ID., column purchased from(Newcastle-UK) was used throughout the analysis. Digital weighing balance (ME-204)purchased from Mettler Toledo(USA), ultrasonicator Labman® purchased from Ultra Chrom Ltd, India. Digital pH meter from Mettler-Toledo was purchased from(Mumbai- India). 50 μ micro- syringe was purchased from Hamilton USA. 0.20μ and 0.45μ nylon membrane filters were purchased from Phenomenex®Mumbai, India.

2.1 Standard Stock Solutions of REM

Standard stock solutions of REM (1 mg mL-1) were prepared differently by dissolving 10 mg of the drug in acetonitrile-water (2:1 v/v) using a 20 mL volumetric flask and completing the final volume by adjusting with either acetonitrile and water, based on their solubility in particular solvents. Furthermore, freshly A sonicator was used to sonicate the stock solution that had been obtained for 10-20 minutes and filtered through 0.20μ nylon filters. Required serial dilution was made for evaluating the validation studies.

2.2 Working Stock Solution

Working stock solution of REM (40 μg mL-1) was made by finishing to volume with the organic phase after samples were diluted of 4 mL of its sample solution in a volumetric flask and diluted.

2.3 Chromatography Condition

Separation was carried using an isocratic elution based on water-acetonitrile (40:60, v/v) as a mobile phase on a Water-Symmetry C18 column (150 mm 4.6mm, 5μm). At 230 nm, the ultraviolet detector was turned on. Leading up to use, the buffer mixture was filtered through such a 0.2 μm nylon membrane filter and sonicated in an ultrasonic bath for 10-20 minutes. The aqueous layer was pumped through the column at a rate of 1.1 mL.min-1. The column temperature was determined to 28 degrees Celsius, and the added dropwise was 20 litres.

2.4 Sample Preparation for Accuracy / drug Recovery Studies

10-20 Remogliflozin100mg tablets were independently weighed, grinded, and mixed in a mortar. An weighed accurately amount of finely powdered Remo Tablet® 100mg: sonicated until dissolved in 100mL of acetonitrile and water. For each laboratory activity, the solutions were filtered, accompanied by serial dilutions to the mandatory concentration levels using the solvent system and the calibration curve method.

2.5 Sample Preparation for Linearity / Calibration Studies

Accurately measured aliquots of stock solutions remogliflozin100mg, respectively were transferred separately into a series of 10mL volumetric flasks. The final volume was adjusted with same mobile phase, and then 20μL were injected into HPLC. A calibration curve (linearity graph) was plotted by calculating peak area against concentration.

2.6 Precision of the Three Methods

Three similar concentrations of the remogliflozin 100mg (250ppm) solution were analyzed three times, within the same day (intraday precision). (Table 5,6) Also the mentioned concentrations were analyzed on three successive days using the same procedure to determine the intermediate precision (Fig 2).

2.7 Robustness for the Chromatographic Method

To analyze the influence of the flow velocity, the solvent system flow rate was changed by 2 decimal points from 1.1 mL.min-1 to 1.3 mL.min-1 and to 0.9 mL.min-1; similarly, the variation of organic modifier used as acetonitrile was changed by 2% from 60% to 62 percent and 58 percent to monitor the peak area as well as retention time. Finally, the effect of wavelength was studied by varying the wavelength from 230
to 223nm and testing and evaluating distinctions in standard calibration characteristics such as retention time, peak tailing, capacity factor, resolution, and theoretical plates [24].

3. RESULTS

It is the first-time estimation of remogliflozin (REM) was attempted on Water-Symmetry® C18, 5µ column; 150 x 4.6 mm exhibited the good peak symmetry and height. Moreover, it improved the capacity factor (k’) and theoretical plates (T) at 230 nm UV detection. Importantly, no any article has been published yet to report the analysis of remogliflozin on C18 column. Therefore, this novel analytical method, proved effectively the estimation of remogliflozin with acceptable all criteria given by ICH guidelines and US-FDA Chromatography.

Parameter-Analutes: Remogliflozin (250ppm), Column: Water-Symmetry® C18, 5µ, 150X4.6mm. ID. Mobile Phase: Water-Acetonitrile; 40:60v/v ,Flow rate:1.1mL.min-1, Elution mode: Isocratic elution mode, Wavelength selected: 230nm, Temperature: Room temperature, Run time: 10minutes Retention time: remogliflozin (4.90min).( Fig.1.).

Fig. 1. Chromatograph of remogliflozin (4,90min) at flow rate 1.1 ml min-1

Table 1. Method development parameters of remogliflozin

| Peak Ret. Time | Area 48103 | Height 3643 | Area% 0.5599 | T Plate 256.416 | Resolution 0 | k’ 1.507 | Tailing F. 0 | Separation |
|----------------|-----------|------------|--------------|----------------|--------------|---------|------------|------------|
| 1              | 1.336     |            |              |                |              |         |            |            |
| 2              | 4.449     | 8542803    | 770826       | 99.4401        | 3901.801     | 10.066  | 2.331      | 1.054      |            |

Table 2. System suitability data of remogliflozin

| System suitability parameters | Remogliflozin (REM) | Acceptable Values |
|------------------------------|---------------------|-------------------|
| Theoretical plates (N)       | 3874                | > 2000            |
| Capacity Factor (K’)         | 2.766               | > 1.5 - <10       |
| Resolution (R)               | ---                 | ≥ 2               |
| Selectivity/Separation factor (α) | 0.00              | >k’               |
| Asymmetry/Tailing factor (T) | 1.03                | > 2               |
| Retention time (tR)          | 4.903 min.          | >k’               |
| Wavelength of Detection (nm) | 230 nm              | > 200 nm          |
| Repeatability (%RSD)         | 0.20                | < 2               |
| Intra-Day Precision (%RSD)   | 0.20 – 0.84         | < 2               |
| Inter-Day Precision (%RSD)   | 0.07 – 0.88         | < 2               |
| Linearity range              | 15.65 – 250 µg.ml-1 | NA                |
| Regression equation          | Y= 35958x - 43049   | NA                |
| SE of intercept (Se)         | 92429.58            | NA                |
| SD of intercept (Sa)         | 41335.76            | NA                |
| Correlation Coefficient (r2) | 0.998               | NA                |
| LOQa (µg.mL−1)               | 3.79 µg.ml-1        | NA                |
| LODa (µg.mL−1)               | 11.49 µg.ml-1       | NA                |
3.1 System Suitability Tests for Remogliflozin

System suitability test reveals the factors such as, the theoretical plate (N), capacity factor (k’), resolution (R), separation factor (α), tailing factor (T), Mean ±SD and RSD % which should in acceptable range for at least 6 successive injections of same analytic. Table No.2 represents the systems suitability studies for remogliflozin.

3.2 Method Validation

3.2.1 Repeatability

The freshly prepared stock solution of remogliflozin of same concentrations (250 μg.mL−1), were evaluated for six injections within the same day. The % RSD was calculated and found it is less than 2%; shown in (Table 3).

Table 3. Repeatability data of REM

| S. No. | Cons. (ppm) | Area | STDEV | %RSD |
|--------|------------|------|-------|------|
| 1      | 250 ppm    | 8054477 | 21528.49 | 0.26 |
| 2      | 250 ppm    | 8022523 |       |      |
| 3      | 250 ppm    | 8056201 |       |      |
| 4      | 250 ppm    | 8066521 |       |      |
| 5      | 250 ppm    | 8052996 |       |      |
| Mean   | 8050543 | 16537.25 |       |      |
| RSD (%)| 0.20      |       |       |      |

3.2.2 Intradaay precision

The freshly prepared stock solution of REM of three replicate soother same concentrations;250 ppm were tested and evaluated within the same day (intra-day precision). The %RSD was calculated and found less than 2%; shown in (Table 4).

3.2.3 Interday (intermediate) precision

The stock solution of REM of three replicates of three different concentrations; 250 ppm, were tested and evaluated in three successive days (interday/intermediate precision). The %RSD was calculated and found less than 2%; shown in (Table 5).

The above-mentioned concentrations were analyzed on three successive days using, the procedure mentioned under section. The % RSD was calculated and the results are shown in (Table 2).

3.2.4. Linearity

Under linearity or calibration studies, a line relationship between are under peak values and selected drug concentration (μg.mL.min−1) was plotted for five chosen concentrations of each drug. The linearity of the calibration curves was validated by the high value of correlation coefficient, acceptable values of regression coefficient, standard deviation of the slope and standard deviation of the intercept; shown in (Table 6).

Limit of detection (LOD) which represents the concentration of analyte at S/N ratio of 3.3 and limit of quantification (LOQ) at which S/N is10 were determined and results are given in (Table 6). Low values of LOD and LOQ indicate sensitivity of the applied method for determination of the mentioned drugs in tablets.

Table 4. Intraday precision data of remogliflozin

| S. No. | Concentration (ppm) | Area | STDEV | %RSD |
|--------|---------------------|------|-------|------|
| 1      | 250 ppm             | 8071715 |       |      |
| 2      | 250 ppm             | 8029231 |       |      |
| 3      | 250 ppm             | 8044410 | 21528.49 | 0.26 |
| 4      | 250 ppm             | 8151020 |       |      |
| 5      | 250 ppm             | 8261006 |       |      |
| 2      | 250 ppm             | 8278611 | 69145.16 | 0.84 |
| 3      | 250 ppm             | 8164001 |       |      |
| 4      | 250 ppm             | 8135719 |       |      |
| 5      | 250 ppm             | 8134523 | 16684.59 | 0.20 |
| Mean   | 8050543 | 16537.25 |       |      |
| RSD (%)| 0.20 – 0.84 |       |       |      |
Table 5. Interday (intermediate) Precision data of remogliflozin

| S. No. | Concentration (ppm) | Area      | Mean ± SD | %RSD |
|--------|---------------------|-----------|-----------|------|
| DAY 1  | 250 ppm             | 8194200   | 15201.58  | 0.18 |
| DAY 1  | 250 ppm             | 8185512   |           |      |
| DAY 2  | 250 ppm             | 814624    | 6104.97   | 0.07 |
| DAY 2  | 250 ppm             | 8051316   |           |      |
| DAY 3  | 250 ppm             | 813021    | 72354     | 0.88 |
| DAY 3  | 250 ppm             | 8231003   |           |      |

Range of %RSD

0.07–0.88

Table 6. Linearity data of remogliflozin

| S. No. | Concentration (µg.mL⁻¹) | Area      | Average (Mean) |
|--------|-------------------------|-----------|----------------|
| 1      | 250 PPM                 | 8962176   | 8955948        |
| 2      | 125 PPM                 | 4411430   | 4494852        |
| 3      | 62.5 PPM                | 4578275   | 4494852        |
| 4      | 31.25 PPM               | 2060779   | 2092642        |
| 5      | 15.62 PPM               | 1006792   | 969040         |
| 6      | 15.62 PPM               | 682023    | 689029         |

Regression Equation

Y = 35958x - 43049

Correlation coefficient (R²)

0.998

Std. Error of intercept

92429.58

Std. Dev. of intercept

41335.76

LOQ

3.79 µg.ml⁻¹

LOD

11.49 µg.ml⁻¹
3.2.5 Robustness for the chromatographic method

From all above studies, after making deliberated changes in flow rate (± 0.2mL.min⁻¹), organic modifier concentration; acetonitrile (±2%) and wavelength (±2nm) have not made any significant changes in resolution, capacity factor and tailing factor. None the less, it seems minute changes in robustness studies makes significant changes in the cortical plate counts. Robustness studies for REM displayed in Table 8. Finally, the wavelength was changed by ±2nm wavelength and results were reported in Table 7.

3.2.6 Accuracy

Accuracy of the results was calculated by % recovery of 5 different concentrations of each drug. The results including the mean of the recovery and standard deviation are shown in (Table 2).

4. DISCUSSION

From all above results and discussion, it has been concluded that the developed analytical method for the estimation of remogliflozin (REM) in both bulk and tablet formulation has obliged the ICH guidelines. As per the ICH guidelines, the developed method has complied the linearity range (calibration data), accuracy/drug recovery studies (%), repeatability, precision studies (intraday and interday/intermediate), and robustness. Moreover, as per the ICH guidelines, the system suitability test performed for remogliflozin has achieved all guidelines; including, tailing factor (T), separation factors (α), the cortical plates (N), capacity factor (k'), resolution (R) and RSD (%). The validated stress degradation studies under thermal, oxidative, alkali and acid as certain few degradation products for remogliflozin (REM).

Calibration Data of Remogliflozin

![Calibration curve of remogliflozin](image)

Fig. 3. Calibration curve of remogliflozin

| Content          | F. (-0.2 ml.mL⁻¹) | F (+0.2 ml.mL⁻¹) | A (-2 ml) | A (+2 ml) | WL (-2 nm) | WL (+2 nm) |
|------------------|-------------------|------------------|-----------|-----------|------------|------------|
| Resolution       | ----              | ----             |          |           |            |            |
| Tailing factor   | 1.05              | 0.97             | 1.01      | 0.99      | 1.01       | 1.01       |
| Capacity factor  | 2.32              | 3.31             | 2.77      | 2.75      | 2.76       | 2.75       |
| Theoretical plates | 3834             | 4259             | 3835      | 4174      | 3999       | 4027       |
### Table 8. Effect of flow rate on remogliflozin

| Peak# | Ret. Time | Area | Area% | T.Plate# | Resolution k' | Tailing F. | Separation |
|-------|-----------|------|-------|----------|---------------|------------|------------|
| 1     | 1.337     | 43155| 3225  | 0.5115   | 208.989       | 0          | 1.487      |
| 2     | 4.452     | 8393031 | 751322 | 99.4885 | 3834.979     | 9.473      | 2.329      |

### Table 9. Effect of organic modifier (-2%) on Remogliflozin

| Peak# | Ret. Time | Area | Area% | T.Plate# | Resolution k' | Tailing F. | Separation |
|-------|-----------|------|-------|----------|---------------|------------|------------|
| 1     | 1.596     | 24385| 2410  | 0.2314   | 479.882       | 0          | --         |
| 2     | 1.783     | 4543 | 928   | 0.0431   | 582.844       | 0.117      | --         |
| 3     | 6.02      | 10507628 | 706789 | 99.7255 | 3835.829     | 12.384     | 2.772      |

Fig. 4. Chromatograph of REM (4.45 min) at flow rate 0.9 mL min-1

Fig. 5. Robustness studies for REM
Table 10. Accuracy data of REM

| Conc. (%) | S. No. | S. amt. (µg.mL⁻¹) | D. added (µg.mL⁻¹) | Amt. rec. (µg.mL⁻¹) | % recovery | Mean±SD | % RSD |
|-----------|--------|-------------------|-------------------|---------------------|------------|--------|------|
| 80%       | 1      | 100               | 80                | 169.45              | 94.14      |         |      |
|           | 2      | 100               | 80                | 177.99              | 98.89      |         |      |
|           | 3      | 100               | 80                | 171.14              | 95.32      | 97.11±2.47 | 2.57 |
|           | 1      | 100               | 100               | 187.33              | 93.67      |         |      |
|           | 2      | 100               | 100               | 187.89              | 93.94      |         |      |
| 100%      | 3      | 100               | 100               | 192.41              | 96.20      | 94.60±1.38 | 1.46 |
|           | 1      | 100               | 120               | 214.63              | 97.56      |         |      |
|           | 2      | 100               | 120               | 211.56              | 96.16      |         |      |
| 120%      | 3      | 100               | 120               | 214.99              | 97.72      | 97.14±0.85 | 0.88 |

Table 11. Force degradation studies

| Conditions | % Area Std | % degradation | No. of degradants |
|------------|------------|---------------|-------------------|
| Acid (0.1N/M HCl) + 60°C + 12 Hrs. | 56% | 44% | 7 |
| Base (0.1N/M NaOH) + 60°C + 12 Hrs. | 69% | 30% | 1 |
| Thermal (60°C) + 12 Hrs. | 87% | 13% | 7 |
| Oxidation (3-6% H2O2) + Room Temp. | 10% | 90% | 1 at t0 |
5. CONCLUSION

Above all results we, may conclude that, this developed and validated method for investigation by reverse phase high performance liquid chromatography (RP-HPLC) can be used for routine analysis of estimation of remogliflozin (REM) from marketed formulation.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.
ACKNOWLEDGEMENTS

I am thankful to Principal, Guide, family members and colleagues for providing technical support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/78034