# VALIDATED HPLC METHOD FOR ANALYSIS OF REMOGLIFLOZIN ETABONATE IN TABLETS

**MUBEEN G*[1], NISAR AHMED[1], TASMIYA SULTANA[1], LALITHA N, V SHOBHA, VYSHNAVI A**

**[1]Department of Quality Assurance, Al-Ameen college of Pharmacy, Bangalore, India.**

***Corresponding Author: mubeenghani2@gmail.com.**

**ABSTRACT:**

A RP-HPLC method has been developed and validated for determination of Remogliflozin etabonate in bulk and pharmaceutical formulation. It was performed on Phenomenex Hyper clone column 5µm BDS C18-250 ×4.6 mm using Acetonitrile and water in the ratio of 80:20 as mobile phase at a flow rate of 1ml/min and analytes were monitored at 228 nm. The retention time for Remogliflozin etabonate was found to be 4.88 min. The peak obtained was symmetrical with tailing factor less than 1.5 and theoretical plates more than 2000. The developed method was validated in accordance to ICH guidelines and the results of all parameters was found to be within the acceptable limits. The linearity was found in the concentration range of 1-500µg/mL, LOD and LOQ was found to be 0.694 and 2.313µg/mL for Remogliflozin etabonate. The percentage Mean recovery at three different levels (80%, 100% and 120%) was found to be 100.71 %w/w and the % Assay for Remogliflozin etabonate in tablets was found to be 99.6 %w/w. The developed method has been found suitable for routine analysis of Remogliflozin etabonate in bulk and formulation (tablet).

**Key Words:** Remogliflozin etabonate, RP-HPLC, ICH Guidelines

## INTRODUCTION:

Remogliflozin etabonate is an orally bioavailable prodrug of Remogliflozin, a hypoglycemic agent, which acts by inhibiting SGLT2, an enzyme accountable for reabsorption of sugar in the kidneys, thereby increasing the elimination of excess sugars in the urine. Apart from glycemic control, SGLT2 inhibitors possess many beneficial effects that include lowering of body weight, reduction of systolic blood pressure, and lowering hemoglobin A1C levels. Remogliflozin etabonate is more helpful when administered to diabetic patients, particularly with cardiac and renal diseases, who require further reduction of hemoglobin A1C level. Few methods reported in the literature for determination of Remogliflozin etabonate individually and in combination with other drugs. This project work describes the development and Validation of RP-HPLC method for Remogliflozin Etabonate in tablets.

## EXPERIMENTAL:

## INSTRUMENTATION:

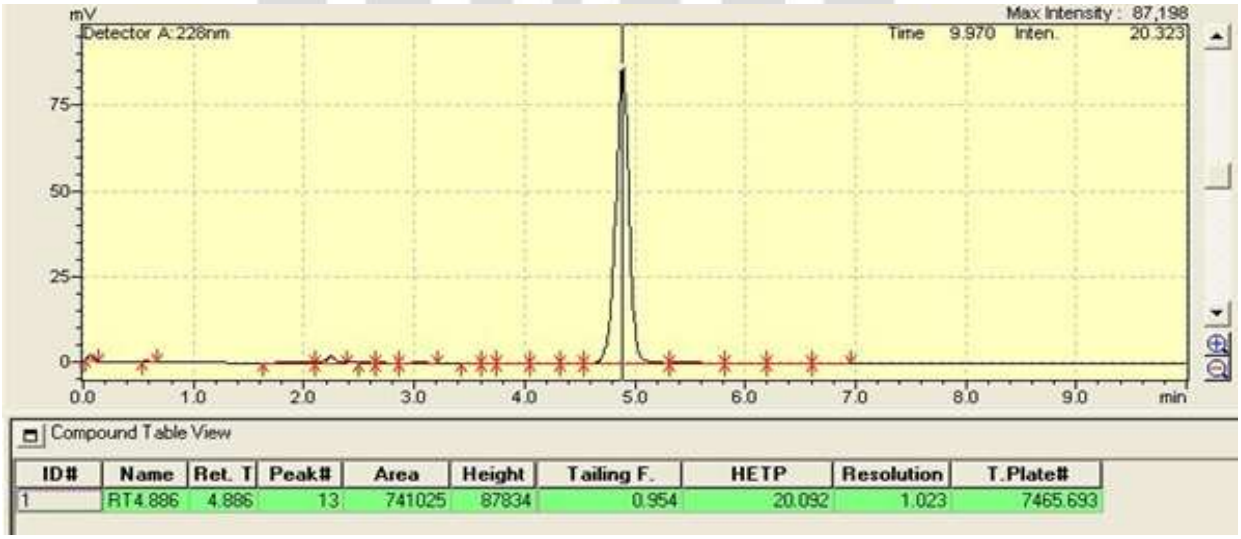
HPLC Analysis was performed with a Shimadzu chromatograph equipped with an LC-10 AT solvent-deliver module, an UV-Visible detector Shimadzu SPD- 10A and a Rheodyne -7725i injector with a fixed capacity loop of 20µL. The equipment was controlled by a LC Solution Software. Electronic weighing balance (Sartorius-TE214 S), Ultra-sonicater bath (RC System-MU 1700), Rotary vacuum pump, Supor200 Membrane filter (0.2µm), Analytical column - Phenomenon BDS C18 (250mm * 4.6mm, 5µm) were used throughout the studies.

## CHEMICAL AND REAGENTS:

Remogliflozin etabonate (Reference Standard). Remogliflozin etabonate Tablets (Remo 100mg, Glenmark Pharmaceuticals Ltd.) was procured from a local pharmacy. HPLC grade of Acetonitrile and Water were used throughout the analysis.

## CHROMATOGRAPHIC CONDITIONS:

Reversed-phase chromatography was performed using Phenomenex BDS C18 column (250mm, 4.6mm, 5µm), as a stationary phase and the mixture of acetonitrile and water in the ratio of 80:20 (v/v) as a mobile phase. The mobile phase was filtered through 0.2µ nylon membrane filter paper and degassed by sonication before use. The flow rate of mobile phase was maintained at 1.0mL/min. after equilibration of column with the mobile phase indicated by a stable baseline, aliquots of sample (20 µl) were injected and the total run time was kept for 20min and analyte was monitored at 228nm wavelength. The chromatogram is presented in Fig 1




| ID# | Name | Ret. T | Peak# | Area | Height | Tailing F. | HETP | Resolution | T.Plate# |
|---|---|---|---|---|---|---|---|---|---|
| 1 | RT4.886 | 4.886 | 13 | 741025 | 87834 | 0.954 | 20.092 | 1.023 | 7465.693 |

**Fig 1**: Chromatogram for REM (10µg/ml) in ACN and Water (80:20v/v) at 228nm

## PREPARTION OF MOBILE PHASE:

Take 80 ml of Acetonitrile and 20ml of Water in a 100ml measuring cylinder and mix (or) take it in 100ml of volumetric flask. The mobile phase was filtered through a membrane filter (0.2µm) and sonicated for 10 minutes before use.

**PREPARATION OF STANDARD REMOGLIFLOZIN ETABONATE SOLUTION:**

Accurately weighed 10mg of REM standard was transferred separately into a clean and dry 10mL of volumetric flask, 3-5mL of mobile phase was added and sonicated 2min to dissolve it fully. Make up the volume with mobile phase to get concentration 1000µg/mL labelled as Standard Stock REM solution, further 0.1mL was pipetted out into a 10mL volumetric flask and the volume was made up with the mobile phase to obtain the concentration of 10µg/mL of Working Standard REM solution.

**PREPARATION OF SERIAL STANDARD SOLUTIONS FOR CALIBRATION CURVE:**

From the Working Standard REM solution 1,2,3,5 mL aliquot was pipetted out separately in a series of 10mL volumetric flasks and from Standard stock REM solution 0.1, 0.2,0.3,0.5,1,2,3,5 mL aliquot was pipetted out separately in a series of 10mL volumetric flasks. The volume was made up with the mobile phase to obtain the concentration of 1,2,3,5,10,20,30,50,100,200,300,500 µg/mL of REM.

**PREPARATION OF SAMPLE SOLUTION:**

10 tablets containing REM were weighed and average weight of one tablet was calculated. The tablets were finely triturated and accurately weighed, a quantity of powder containing 0.01 gm of REM was transferred to 10 ml volumetric flask, solubilized with solvent and volume was made up to 10 ml mark, was filtered using Whitman filter paper & labelled as SAMPLE STOCK REM SOLUTION. Further 0.1 ml was diluted to 10ml with mobile phase to get 10µg/ml solution of REM.

**VALIDATION OF HPLC METHOD:**

The proposed method RP-HPLC for Remogliflozin etabonate was validated as per the recommendations of ICH guidelines (Q2R1) for the parameters like specificity, linearity and range, accuracy, precision, LOD, LOQ, robustness, and system suitability parameters. The data obtained for all the validation parameters are presented in **Table 1.**

**SPECIFICITY:**

The peak purity of REM was assessed by comparing the chromatogram of standard, sample and mobile phase(blank). The chromatogram is presented in Fig 2

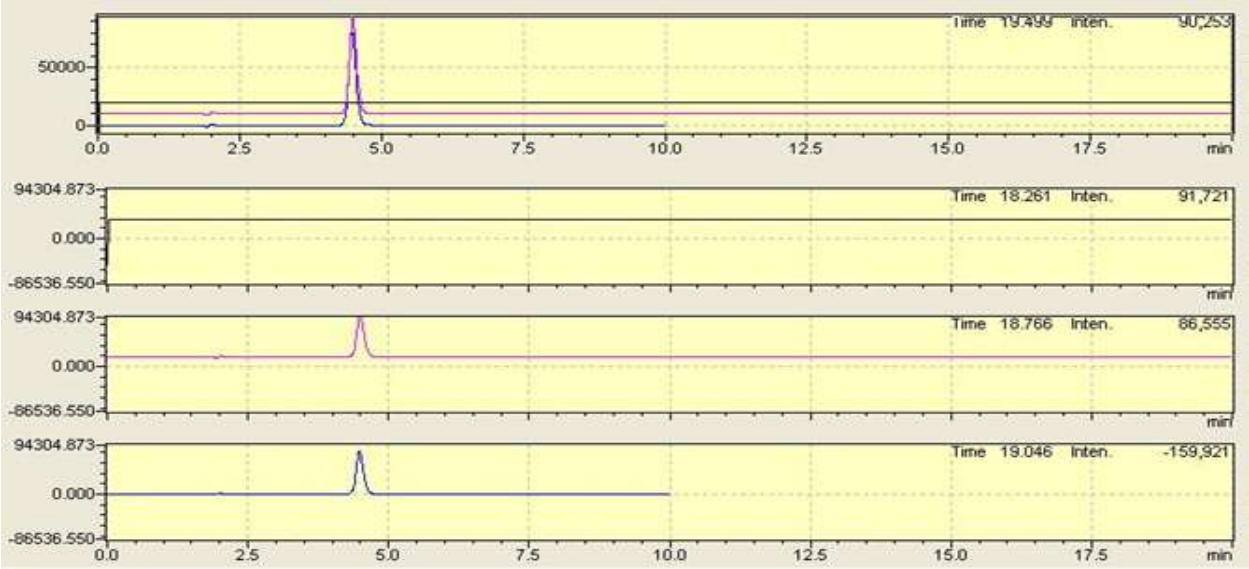



**Fig 2: Chromatogram for Specificity studies of REM**

## LINEARITY AND RANGE:

Serial dilutions of REM (1-500µg/mL) were injected and linearity was determined by plotting area under curve (AUC) vs concentration whereas range was determined by plotting response (AUC/Concentration) vs log concentration. The data obtained is presented in **Table 2 & Fig 3**

**Table 2: Linearity data for Remogliflozin Etabonate**

| Conc (µg/ml) | AUC |
|---|---|
| 1 | 135600 |
| 2 | 189858 |
| 3 | 268625 |
| 5 | 445686 |
| 10 | 765619 |
| 20 | 1296241 |
| 30 | 1612511 |
| 50 | 2192962 |
| 100 | 3890972 |
| 200 | 7825987 |
| 300 | 12003536 |
| 500 | 19293946 |

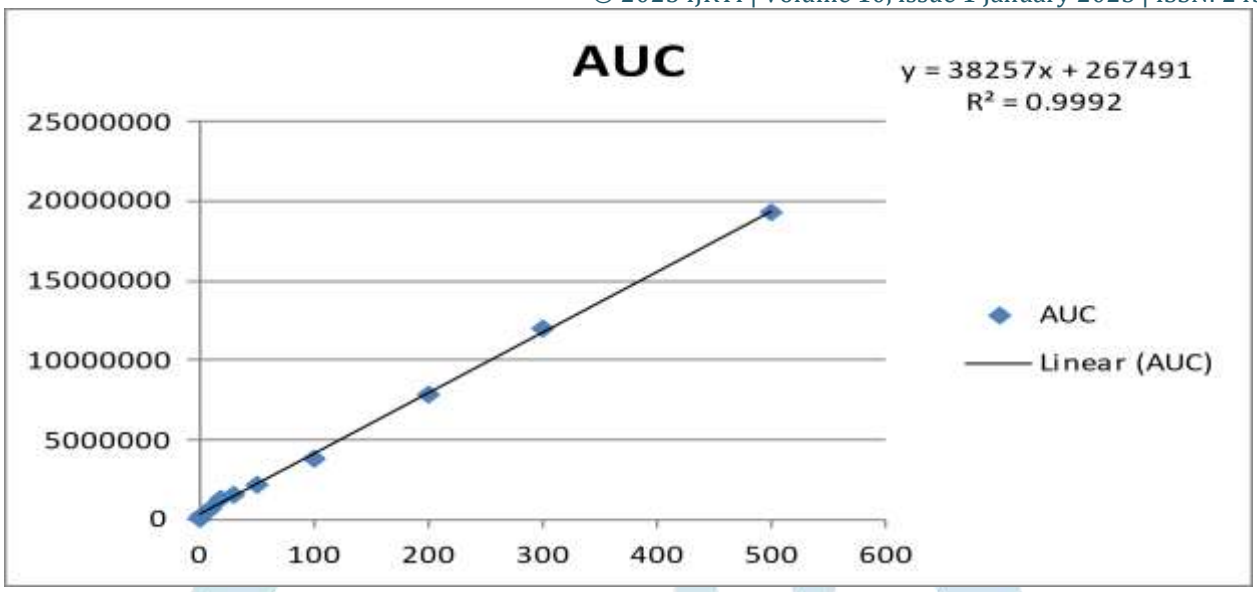



**Fig 3: Calibration curve for REM (1-500µg/ml)**

**PRECISION:**

Precision commonly expressed as the standard deviation or relative standard deviation was performed. The data obtained for all precision studies are presented in Table 1

**INTRA-DAY PRECISION-** Intraday precision was evaluated by analysing standard solution (n=3) of the concentration 10µg/mL at three different time intervals (0,1,2hrs) under the same experimental conditions on the same day.

**INTER-DAY PRECISION –** Inter day precision was determined by analysing standard solution (n=3) of the concentration 10µg/mL on three consecutive days.

**REPEATABILITY:**

Repeatability was evaluated by analysing the standard solution of the concentration range of 10µg/mL of REM six times on the same day at same time. The retention time and peak areas were recorded and %RSD was calculated.

**REPRODUCIBILITY-** Reproducibility was evaluated by analysing the standard solution of the concentration range of 10µg/mL of REM prepared by two different analysts. The t and F-test were performed to determine significant variation between the results and precision obtained by two analysts.

**SENSITIVITY-** Sensitivity of the method was determined by means of the detection limit (LOD) and quantification limit (LOQ). Calculations for LOD and LOQ were based on the standard deviation of the intercept from the calibration curve (σ) and the mean slope of curve (S), using the equation LOD =3.3×σ/S and the equation LOQ =10×σ/S.

**ROBUSTNESS:**

Robustness was evaluated on the basis of system suitability parameters to assess whether the system suitability parameters change with small but deliberate variations. The system suitability parameters for standard solution of REM were studied with variation in flow rate, pH and organic phase ratio.

**ACCURACY/RECOVERY STUDIES-** Recovery studies were carried out with standard addition method at three different levels (80%,100%,120%). A known amount of REM was added to pre-analysed tablet solution, analysed and percent recoveries were calculated. The results are presented in Table 3.

**Table 3: % Recovery for Accuracy studies of REM at 3 levels**

| Drug | Volume of Std solution (µg/ml) | Volume of Sample solution (µg/ml) | Total conc. (Std+ Sample) (µg/ml) | Amount* recovered for Std graph (µg/ml) | Recovery of Std (µg/ml) | %Recovery of Std (% W/W) |
|---|---|---|---|---|---|---|
| **REM** | 0.8ml (8µg/ml) | 0.1ml (10 µg/ml) | 18 µg/ml | 18.06 | 8.06µg/ml | 101.75% |
| | 0.1ml (10µg/ml) | 0.1ml (10 µg/ml) | 20 µg/ml | 19.99 | 9.99µg/ml | 99.9% |
| | 0.12ml (12µg/ml) | 0.1ml (10 µg/ml) | 22 µg/ml | 22.18 | 12.18µg/ml | 101.5% |
| **Mean % Recovery = 100.71%w/w** | | | | | | |

***Average of three readings**

**ASSAY OF REMOGLIFLOZIN ETABONATE TABLETS:**

**TABLET NAME: Remo*100**

**LABEL CLAIM: Remogliflozin Etabonate- 100mg (Glenmark Company)**

**10 tablets containing REM were weighed and average weight of one tablet was calculated. The tablets were finely triturated and accurately weighed, a quantity of powder containing 0.01 gm of REM was transferred to 10 ml volumetric flask, solubilized with solvent and volume was made up to 10 ml mark, was filtered using Whitman filter paper, further 0.1 ml was diluted to 10ml with mobile phase to get 10µg/ml solution of REM. The solutions of standard REM (10µg/ml) and sample REM solutions were filtered through 0.45µm Millipore filter using syringe and injected into the Rheodyne injector (50µl) of HPLC system and their chromatograms were recorded under the finalised chromatographic conditions after getting a stable baseline. Peak areas were recorded and the amount of REM present in the formulation was calculated by the following formulae.**

$$\% \text{ Assay} = \frac{\text{AUC of sample} \times \text{concentration of standard} \times \text{dilution factor} \times 100}{\text{AUC of standard} \times \text{weight of sample(gm)} \times \text{label claim}}$$

**Table 1: Data for HPLC Method Validation studies for REM**

| **Parameters** | **Remogliflozin Etabonate** |
|---|---|
| Retention time (min) | 4.88 |
| Absorption Maxima- wavelength | 228nm |
| Linearity (µg/ml) | 1-500 |
| Slope | 38257 |
| Intercept | 267491 |
| Co-relation Coefficient ($R^2$) | 0.9992 |
| LOD (µg/ml) | 0.694 |
| LOQ (µg/ml) | 2.313 |
| Accuracy(n=3) (%w/w)<br>Recovery at 80% level)<br>Recovery at 100% level)<br>Recovery at 120% level) | <br>101.75<br>99.9<br>101.5 |
| Precision --Intra-day (%RSD)<br>Inter-day (%RSD) | 0.5944<br>0.8127 |
| Repeatability(%RSD) | 0.512 |
| Reproducibility(%RSD) | A1=0.54<br>A2=0.72 |
| Analysis of Tablets (%Assay) %w/w | 99.6% |

**Table 4: Assay results of REM in tablet formulation**

| REM | RT (minutes) | Peak Area* | Conc (µg/ml) | Assay (%w/w) |
|---|---|---|---|---|
| **Standard** | 4.887 | 765619 | 10 µg/ml | 99.6% |
| **Sample** | 4.886 | 762686 | 9.96 µg/ml | |

***Average of 3 readings**

**Conclusion: A new HPLC method was developed and validated successfully for determination of REMO in bulk and tablets. The data obtained for all the validation parameters as per Q2(R1) indicated that the proposed method is simple, accurate, precise and less time consuming and can be useful for routine determination of Remogliflozin in tablets.**

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