BIO ANALYTICAL METHOD FOR THE ESTIMATION OF REMOGLIFLOZIN USING LC-MS

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Abstract

An easy, quick, precise, active and reproducible LC-MS/MS technique was developed for the bio analytical method of Remogliflozin using D6 Remogliflozin as internal standard. This article summarizes the recent progress on bioanalytical LC-MS/MS methods using waters x-bridge C18 column (150x4.6 mm, 3.5µ) column and organic mobile phase of sodium dihydrogen phosphate and Acetonitrile in 70:30 ratio. The calibration curve was linear in the concentration of 350ng/ml for Remogliflozin. Accuracy, precision, recovery, matrix effect and stability results were found to be within the suitable limits. Simple and efficient method was developed and utilized in pharmacokinetic studies to see the investigated analyte in body fluids. The application denotes all the parameters of system suitability, specificity, linearity and accuracy are in good agreement with USFDA guidelines and applied effectively for the investigation of pharmacokinetic studies in rat.

Key words: Remogliflozin, LCMS, method development, validation, USFDA Guidelines.

1. Introduction

Remogliflozin etabonate (INN/USAN)[1,2] is a drug of the gliflozin[3,4] class for the treatment of non-alcoholicsteatohepatitis ("NASH")[5] and type 2 diabetes. Remogliflozin was commercially launched first in India by Glenmark in May 2019.Remogliflozin etabonate was shown to enhance urinary glucose excretion in rodents and humans. Remogliflozin etabonate has been studied at doses up to 1000 mg. A pair of 12-week phase 2b randomized clinical trials of diabetics published in 2015, found reductions in glycatedhemoglobin[6] and that it was generally well tolerated. Remogliflozin etabonate isa pro-drug of remogliflozin. Remogliflozin inhibits the sodium-glucose transport proteins (SGLT),[7] which are responsible for glucose reabsorption in the kidney. Blocking this transporter causes blood glucose to be eliminated through the urine. Remogliflozinis selective for SGLT2. Some common side effects of Remogliflozin etabonate include nausea,[8] frequent urge to urinate, low blood sugar levels, fungal infection in the genital area, and increased thirst. These side effects are not familiar to everyone and vary individually. Remogliflozin etabonate may interact with antibiotics[9,10] (Fluconazole[11,12]), drugs treating impotence[13] (Sildenafi)[14,15], antidiabetics[16,17] (Glimepiride)[18,19], and anticonvulsants[20,21] (Pregabalin)[22,23].

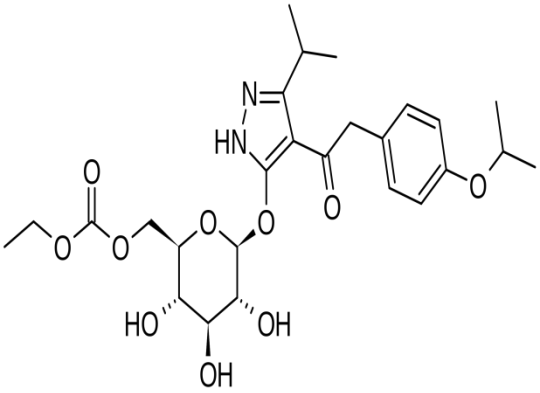


Fig 1: Chemical structure of Remogliflozin

2. Experimental Study

Chemicals and reagents

Acetonitrile and Hexane sulphonic acid, water (HPLC grade) were purchased from Merck (India) Ltd, Wroli, Mumbai, India. All APIs of Remogliflozin as reference standards were procured from Zydus Cadila , Ahamdabad.

Equipment

An HPLC system (waters alliance e2695 model) connected with mass spectrometer QTRAP 5500 triple quadrupole instrument(sciex) was used. By the Empower 2.0 software operation was performed.

Selection of animals

In the following study, 6 healthy white New Zealand rats (250 gms) were obtained from Biological E Limited, Hyderabad, India. The protocol of animal study was approved by institute of animal ethics committee (Reg.No:1074/PO/Re/S/05/CPCSEA).

Chromatographic conditions

Chromatographic separation, using x- bridge C18 (150 x 4.6 mm, 3.5 micron) columns, was administered in isocratic mode at room temperature. As a mobile phase, a mix of sodium dihydrogen phosphate and acetonitrile at 70:30 v/v with a flow of 1.0 ml/min was used. 10µl was the injection rate and the run time was 8 minutes.

Preparation of standard and internal control samples

Preparation of standard stock solution

Take 15 mg of Remogliflozin working standards is taken into a 10ml volumetric flask and add small amount of Acetonitrile and small amount of buffer, then sonicate for 10minutes to dissolve the contents completely and make up to the mark with equal amounts of acetonitrile and buffer. Further dilution by taking 2.63 ml into 10 ml volumetric flask and make upto the mark with HPLC water. From the above solution 0.2ml of the solution is taken into the 10ml volumetric flask and make up to the mark with the HPLC water.

Preparation of internal standard

Take 15 mg internal standard of Remogliflozin i.e. D6 Remogliflozin into a 10ml volumetric flask and make up to the mark with 5ml of Acetonitrile and 5 ml of Hexane sulphonic acid and sonicate for ten minutes to dissolve the contents completely. From this solution take 2.63ml of solution into 10ml volumetric flask and made upto the mark with HPLC water. From the above solution 0.2ml is taken into the 10ml volumetric flask and make up to the mark with the HPLC water.

Preparation of standard solution

For standard preparation 200µl of plasma was taken and 200µl of ACN into a 2ml centrifuge tube and vortex[24] for 5 minutes. Then 100µl of standard stock solutions and 100µl of IS and 900 µl of diluents were added and vortexed for 10 min. These samples further subjected for centrifuge at 2000rpm for 20 min. Collect the solution and filter through 0.45µ nylon syringe filter and the clear solution was transferred into vial and injected into a system.

Bio analytical Method validation

The method was validated in selective, sensitive, linearity, accuracy and precise, matrix condition, recovery study, re-injection reproducibility and stability.

Selectivity

By analyzing the six different rat's plasma samples and to check interference at the retention time selectivity was conducted.

Matrix effect

By comparing the height area ratio from the six various drug free plasma samples for Remogliflozin to get matrix effect. Experiments were performed at MQC levels in triplicate with six different plasma lots with the suitable precision of $\leq$ 15 %.

Precision and accuracy

It was determined by replicate analysis of internal control samples at a lower limit of quantification (LLOQ), low quality control (LQC), medium quality control (MQC), top quality control (HQC) levels. The half of CV should be less than 15 % and accuracy should be within 15% except LLOQ where 20%.

Recovery

The analysis of six samples reproduce at each internal control concentration is by extracting the Remogliflozin. By comparing the height areas of extracted standards to the height areas of unextracted standards, recovery is evaluated.

Carry over

Carry over deals with the analyte retained by the chromatographic system during the matrix with an analyte concentration ULOQC and above the diluting this sample with blank matrix.

Dilution integrity

By spiking the matrix with an analyte concentration above the ULOQC and diluting this sample with blank matrix, the dilution integrity should be explained.

Stability

By comparing the act of stock solution stability under the stability sample with the sample from the fresh stock sample preparation. Sample Stability studies in plasma were performed at the LQC and HQC concentration levels using six replicates at each level. Analyte was considered stable if the change is smaller amount than 15 % as per US FDA guidelines. The perfectness of spiked rat plasma stored at room temperature was evaluated for twenty four hrs. The stability of spiked rat plasma stored at RT in auto sampler was evaluated for twenty four hrs. The auto sampler stability(LQC,MQC and HQC) was evaluated by comparing the extract plasma samples that were injected immediately, with the samples that were re-injected after storing with wet extract stability at room temperature after 12 h and 18 h at 2-8^{o}C . The reinjection reproducibility was evaluated by comparing the extracted plasma samples that were injected immediately, with the samples that were re injected after storing in the dry extract stability at room temperature after 12 h and 18 h at -20^{o}± 3^{o}C. The freeze thaw stability was conducted by comparing the steadiness samples that had been frozen at -31^{o}C and thawed 3 times, with freshly spiked internal control samples. The short term stability was conducted 7 days at 7^{o}C. For long term stability evalution the concentrations obtained after 24 h were compared with initial concentration.

The plasma for the above experiment has been extracted from Albino rat[25] i.e., shown in figure 2.

Fig. 2 : Albino rat

3. Results and Discussion

The maximum response on air pressure chemical ionization mode selected in this method is by having the electro spray ionization. The mobile phase flow of 10 µl/min Remogliflozin are highly responsive in the positive ion mode to offer sensitivity and signal stability with continuous flow to electro spray ion.

Specificity

The specificity of the method to research Remogliflozin is proved. The chromatograms of blank and standard as shown in figure 3, 4, 5. The chromatograms of blank rat plasma[26-28] and standard having no interference peaks were observed.

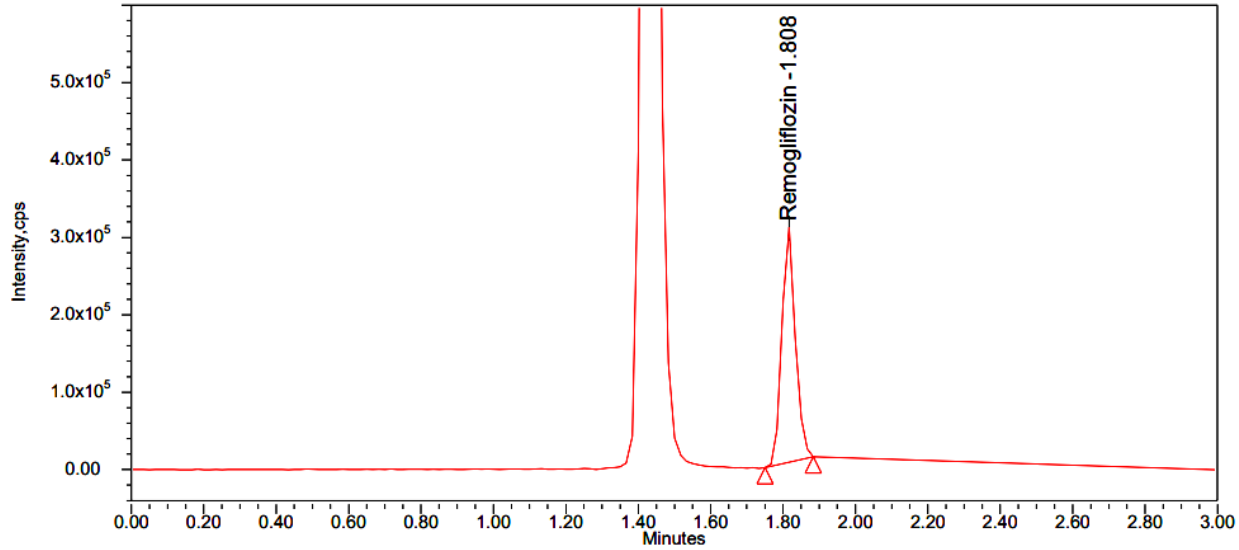


Fig 3. Chromatogram of Standard

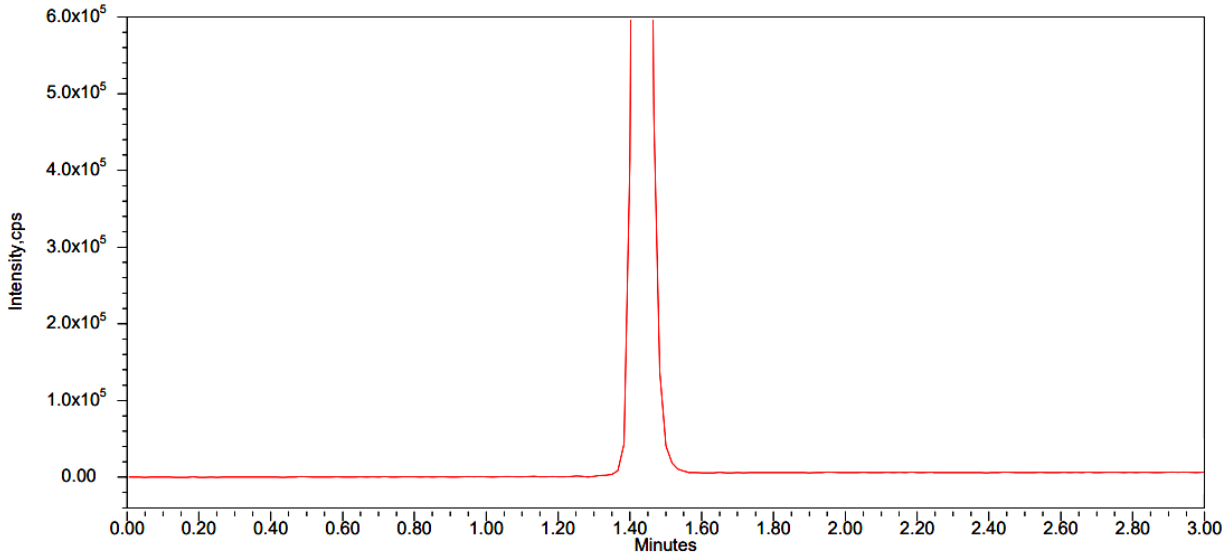


Fig 4. Chromatogram of blank

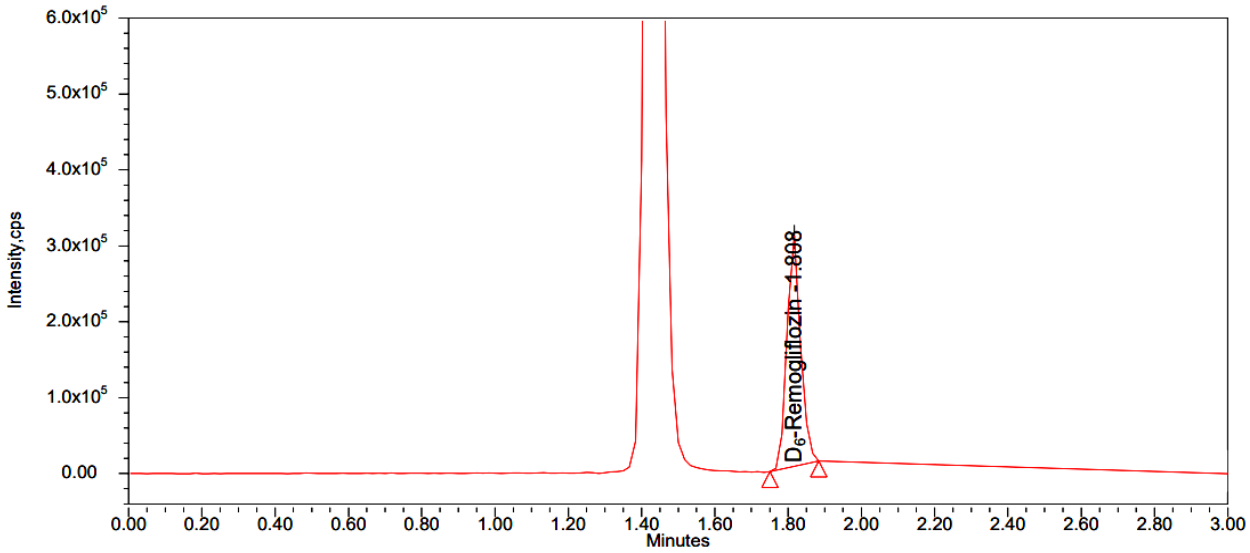


Fig 5. Chromatogram of blank plasma spiked with internal standard

Matrix Effect

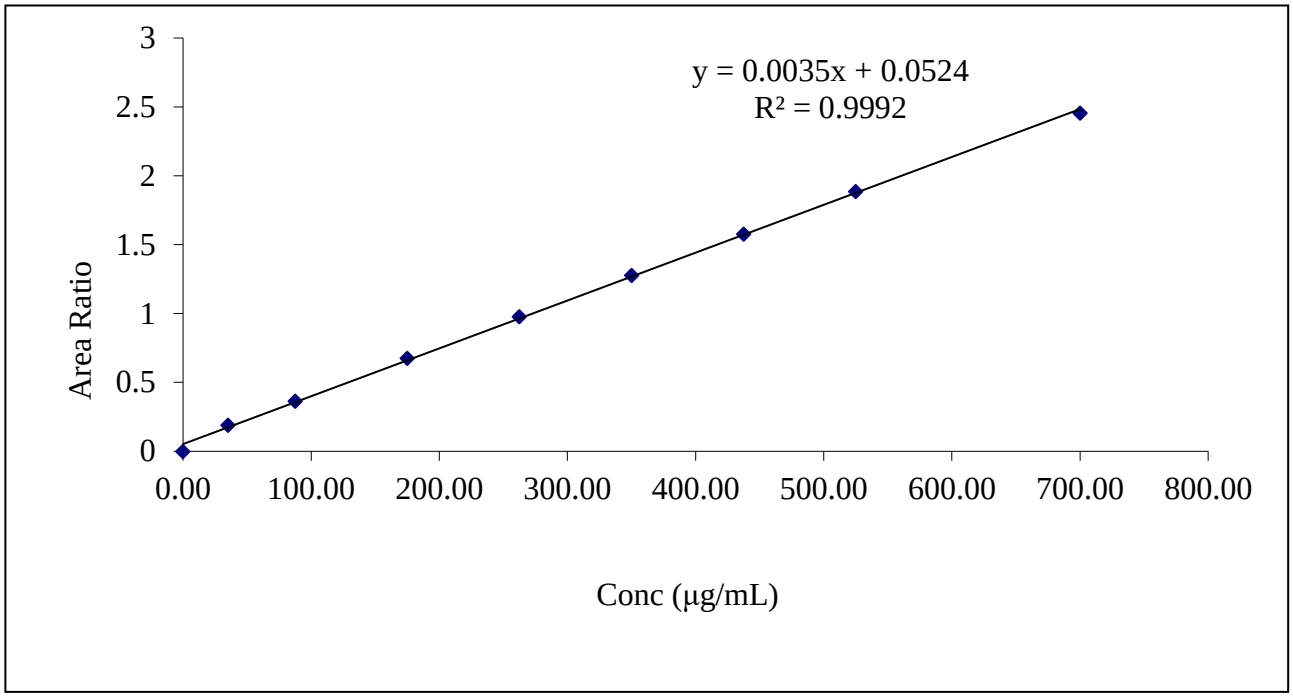
Percent RSD for within the signal, ion suppression/enhancement was observed as 0.175 per cent for Remogliflozin in LC-MS/MS, suggesting that under these circumstances the matrix effect on analyte ionization is within an acceptable range of ionization. In matrix effect LQC and HQC of Remogliflozin were 107.2% and 100.29%. %CV of Remogliflozin at LQC level was 0.21 and HQC level is 0.14 respectively. It indicates that the matrix effect on the ionization of the analyte is within the suitable limit.

Linearity

The peak area ratio of calibration standards was proportional to the concentration. The concentration range of Remogliflozin is 88-700ng/ml. Linearity results of Remogliflozin was shown in following table 1 [29-32] and their calibration plots were shown in figure 6. The calibration curves were appeared linear and coefficient of correlation was found to be 0.999 for Remogliflozin.

Table 1. Linearity results of Remogliflozin

Linearity	Remogliflozin		
	Conc. ng/ml	Peak response	Area Ratio
1	35	0.642	0.252
2	87.5	1.333	0.517
3	175	1.986	0.779
4	262.5	2.591	1.023
5	350	3.174	1.238
6	437.5	3.756	1.446
7	525	5.057	1.968
8	700	0.642	2.456
Slope	0.0231		
Intercept	0.00087		
CC	0.99967		



The intra-assay precision and accuracy were estimated by analyzing six replicates containing Remogliflozin at six different QC levels. The inter-assay precision was determined by analyzing the four levels QC samples on four different runs. The criteria for acceptability of the data include, accuracy within 85–115% from the actual values and a precision of within ±15% relative standard deviation (RSD) except for LLQC, where it should be within 80–120% for accuracy and <20% of RSD.

Table 2. Precision and accuracy of Remogliflozin

Quality control sample	Spiked concentration (ng/ml)	Mean (ng/ml)	SD	Accuracy (%)	RSD (%)
		Intra-day			
LLOQ	$0.2351x10^5$	0.2352	0.0013	91.58	0.82
LQC	$1.3654x10^5$	1.3674	0.0244	106.37	0.16
MQC	$2.5729x10^5$	2.5729	0.0987	100.22	0.33
HQC	$3.8461x10^5$	3.8421	0.0375	99.88	0.08
		Inter-day			
LLOQ	$0.2262x10^5$	0.2234	0.0049	88.11	0.39
LQC	$1.3351x10^5$	1.3318	0.0315	104.01	0.27
MQC	$2.5487x10^5$	2.5435	0.0632	99.28	0.18
HQC	$3.8263x10^5$	3.8254	0.0174	99.36	0.15

Recovery

The recovery of drug and IS was evaluated at three concentration levels namely low, medium and high quality control. Recovery was calculated by comparing its response in replicate samples with that of neat standard solution responses. Analyte recovery from a sample matrix (extraction efficiency) is a comparison of analytical response from an amount of analyte added to that determined from sample matrix. Because of basic properties of Remogliflozin extraction was carried out using Acetonitrile solvent.
Experiments with spiked compounds resulted in recoveries of analyte 92.61 % - 114.85%.

Ruggedness
The percent recoveries and percent CV of Remogliflozin determined by analyst usingh a specific column were within acceptable criteria in HQC, LQC, MQC and LLQC samples. The results proved method is ruggedness.

Auto sampler carryover
Peak area response of Remogliflozin, wasn't observed within the blank rat plasma samples after successive injections of LLQC and ULQC at the retention times of Remogliflozin. In auto sampler carryover this method doesn't exhibit auto sampler carryover.

Stability
Remogliflozin solution was prepared with diluents for solution stability analysis and placed in a refrigerator at 2-8^{o}C. Fresh stock solutions were associated with stock solutions that were prepared 24 hours earlier. The plasma stability of the bench top and auto sampler was stable for twenty four hours, and 24 hours at 20^{o}C in the auto sampler. It became apparent from future stability that Remogliflozin was stable at a storage temperature of -30^{o}C for up to 24 hours. The overall stability results of Remogliflozin has been stated in the below table 5 [33].

Table 3. Stability results of Remogliflozin

Stability experiment spiked plasma		**Spiked plasma conc.(n=6,ng/ml)**	**Conc.measured (n=6,ng/ml)**	**%CV**
Bench top Stability	HQC	525	525.4124	0.30
	LQC	175	175.332	1.22
Auto sampler stability	HQC	525	525.53454	0.12
	LQC	175	175.50167	0.67
Long term (Day28) Stability	HQC	525	525.3348	0.06
	LQC	175	175.4724	0.17
Wet extract Stability	HQC	525	525.503	0.565
	LQC	175	175.510	1.58
Dry extract Stability	HQC	525	525.4348	0.56
	LQC	175	175.255	1.43

Freeze thaw Stability	HQC	525	525.501	0.515
	LQC	175	175.449	1.32
Short term Stability	HQC	525	525.3264	0.04
	LQC	175	175.474	0.20

4. Conclusion

For the primary time higher sensitive HPLC-ESI-LCMS/MS method was developed and validated for the determination of Remogliflozin in rat plasma. Here the described method is rugged, fast, reproducible bio analytical method. This method was validated according to USFDA guidelines. Simple and efficient method was developed and may be utilized in pharmacokinetic studies and to see the investigated analyte in body fluids.

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Conflicts Of Interest

Authors declare that there were no conflicts of interest.

Funding Source

None

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