Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 8 (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

LC-MS/MS Based Quantitative Analysis of Remogliflozin in Rat Plasma: A Kinetic Study Approach

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Cite this paper as: Smit J. Patel, Hiralben Mehta, Basheer Shaikh, Nadeem Khan, (2025) LC-MS/MS Based Quantitative Analysis of Remogliflozin in Rat Plasma: A Kinetic Study Approach. *Journal of Neonatal Surgery*, 14 (8), 503-508.

ABSTRACT

This research utilized high-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method to quantitatively measure concentration of remogliflozin in rat plasma using bixafen as the internal standard. Samples were prepared by aqueous acetonitrile-based protein precipitation technique. Remogliflozin was then analysed by isocratic elution chromatography with a mobile phase consisting of 0.1% formic acid in milli-Q water (20): acetonitrile (80). Remogliflozin was monitored by m/z 451.4→289.4 transition for quantification and m/z 451.4→111.2 transition for qualification, and bixafen was determined by m/z 414.0→ 394 by multiple reaction monitoring (MRM) in positive ion electrospray ionization (ESI) source. Method exhibited good linearity in the range of 15 ng/mL to 2009 ng/mL. During pharmacokinetic experiments involving oral administration of remogliflozin in rat, the time to reach maximum concentration (T_{max}) $_{was}$ found to 0.5 h in both males and females. The maximum concentration (C_{max}) was found to be 201 ng/mL in males and 293 ng/mL in females while the last area under the concentration-time curve (AUC_{last}) was determined to be 448 ng*h/mL for males and 297 ng*h/mL for females.

Keywords: *Remogliflozin, Rat Plasma, UPLC-MS/MS, Isocratic Elution, Bixafen.*

1. INTRODUCTION

Remogliflozin, a SGLT-2 inhibitor, chemically 5-methyl-1-(1-methyl ethyl)-4- ({4-[(1-methyl ethyl)oxy]phenyl}methyl) -1H-pyrazole -3-yl- β-D-glucopyranoside) **(Figure 1)** is used to treat progressive diabetes[1]. Although traditional oral hypoglycemic agents alone or in combination are preferred for T2DM unless adequate glycemic control is not achieved and at this stage generally insulin is added in the treatment. Therapeutic strategies to inhibit the renal sodium glucose co-transporter 2 (SGLT2) have been developed and advanced very quickly in recent years for T2DM. SGLT2 is a glucose transporter receptor characterized by low affinity and high capacity specifically found in the S1 and S2 segments of the renal proximal tubule which mediate sodium and glucose co-transport[2,3]. The most reliable category of SGLT-2 inhibitors has been recognized as those containing a C-aryl glycosidase moiety, leading to the discovery of remogliflozin, dapagliflozin, canagliflozin, bexagliflozin, and sotagliflozin[4,5]. This SGLT 2 inhibitors function by reducing the kidneys ability to reabsorb glucose thereby enhancing excretion of glucose form the body[6].

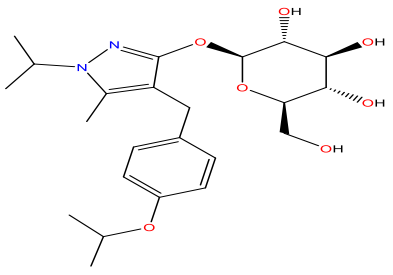

Figure 1: Molecular structure of remogliflozin

Accurate and precise measurement of plasma remogliflozin represents an effective strategy that enables the researcher to define pharmacodynamics and pharmacokinetic relationships in animal models[7-10]. Therefore, there is a necessity for straightforward and reliable method to identify and quantify remogliflozin in biological matrices like plasma. In this study, developed LC-MS/MS method was used for determining remogliflozin level in rat plasma[11].

2. EXPERIMENITAL

Materials:

Remogliflozin (99.97% purity) was provided by Glenmark Pharmaceuticals, Nasik, India. Bixafen was purchased from Sigma Aldrich (99.3% purity) and utilized as an internal standard. All the reagents and chemical employed during experiment were of HPLC grade quality and sourced from Biosolve, India.

Instrumentation:

The LC-MS/MS analysis was performed on API 4000 Triple Quad Mass Spectrometer coupled with Nexera X2 UPLC

system. X select CSH fluorophenyl (150 mm x 4.6 mm, 3.5 µm) column was utilized and the column temperature was set at 40 °C. 0.1% formic acid in milli-Q water (20%) and acetonitrile (80%), v/v was used as a mobile phase for isocratic elution method. The autosampler was kept at 15 °C and the injection volume was 10 µL. Flow rate was maintained at 0.7 mL/min. Each injection has run time of 4 minutes. The separated compounds were detected by CEM (channel electron multiplier) detector. Quantification was achieved by multiple reaction monitoring (MRM) mode with transitions of m/z 451.4→289.4 and m/z 451.4→111.2 for remogliflozin and m/z 414.0→394.0 for bixafen. Data acquisition and control of instruments were done by Analyst software 1.6.3.

Preparation of Standard and Quality Control Samples

Acetonitrile served as a solvent for the preparation of stock solution of approximately 1 mg/mL remogliflozin and bixafen, each separately. A stock solution of remogliflozin was subsequently diluted with mobile phase to prepare a standard working solution. For the preparation of calibration curve, 5 µL of the working solution was spiked into 45 µL of blank rat plasma. The range of the concentrations in the calibration curve was around 15-2009 ng/mL. Internal standard (IS) stock solution was diluted with mobile phase to prepare the working solution and similar to calibration curve standard, the internal standard was spiked 5% in the rat plasma.

Sample Preparation:

Samples were prepared by using protein precipitation technique which is one of the feasible method of extraction[12]. Specifically, 5 µL of the internal standard working solution was added to 45 µL of the plasma samples in the Eppendorf tube. This mixture was then precipitated with 1.5 mL of mobile phase and the sample was vortexed for 10 minutes at 2000 rpm. Subsequently, centrifugation was performed at 14000 rpm at 4±1 °C for 10 minutes to isolate supernatant, from which 10 µL of supernatant was injected in LC-MS/MS for analysis.

Pharmacokinetic Study:

For pharmacokinetics study of remogliflozin, healthy, young adult rats (Rattus norvegicus) of Wistar (RccHan:WIST) strain were used. Female rats were nulliparous and non-pregnant. At the initiation of dosing, rats were 6 to 7 weeks old and the body weight variation among the rats were within ±20% of the mean body weight for each sex. Total 6 male and 6 female rats were used. Remogliflozin was administered as a single dose at 20 mg/kg body weight by oral route to male and female rats. Constant dose volume(s) of 10 mL/kg was used, and individual dose volumes were adjusted according to the most recently recorded body weight of each rat. Blood was collected through retro-orbital plexus as this is the one of the preferred method of blood collection on day 1 at 30 minute and 1, 2, 4, 8, and 24 h from rats[13]. Plasma samples were analyzed using validated bioanalytical method[11]. Pharmacokinetic analyses of plasma concentration vs. time were performed using WinNonlin® software. Rats were humanely sacrificed after the last blood collection time point. The institutional animal ethics committee has approved to perform this work (Approval Number: JRF/IAEC/2024/11).

3. RESULTS AND DISCUSSION

Method and Analytical Parameters

The analytical method for determination of remogliflozin was validated as per US FDA[14].

Linearity

Linearity of the method was carried out by injecting eight concentrations of remogliflozin prepared in the plasma matrix in the range 15 to 2009 ng/mL into the LC-MS/MS system. All samples were analyzed using optimized LC-MS/MS conditions and data of the remogliflozin presented in Table 1. Calibration curve is presented in figure 2 and representative chromatogram is presented in figure 3. At all the selected concentration levels of the calibration curves, back calculated amounts of the calibration standards were always less than ±15% of nominal values. The regression equation was y==0.00133x + 0.00325

with r value of 0.9966.

Table 1: Linearity data of remogliflozin

Nominal Concentration (ng/mL)	Recovered Concentration (ng/mL)	Accuracy (%)	Regression Equation
15	15.61	99.42	Y = 0.00133 x + 0.00325 (r=0.9966)
31	31.76	101.16	
62	64.78	103.16	
125	123.62	98.89	
251	216.66	86.26	
502	497.92	99.12	
1004	1014.94	101.02	
2009	2100.23	104.86	

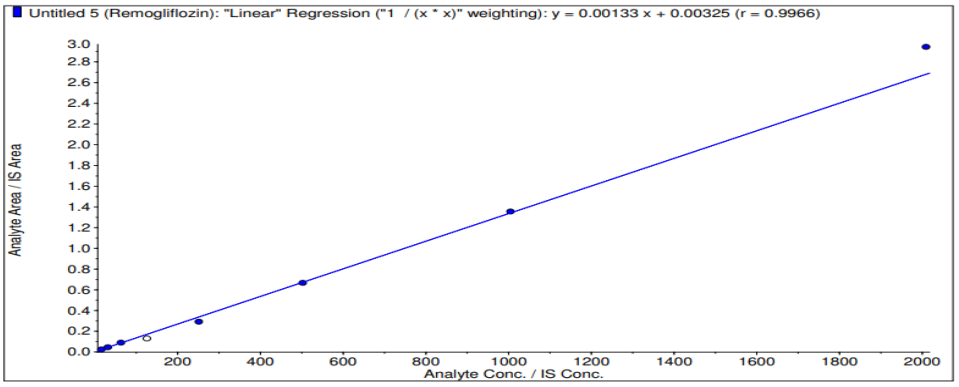

Figure 2: Calibration curve of remogliflozin

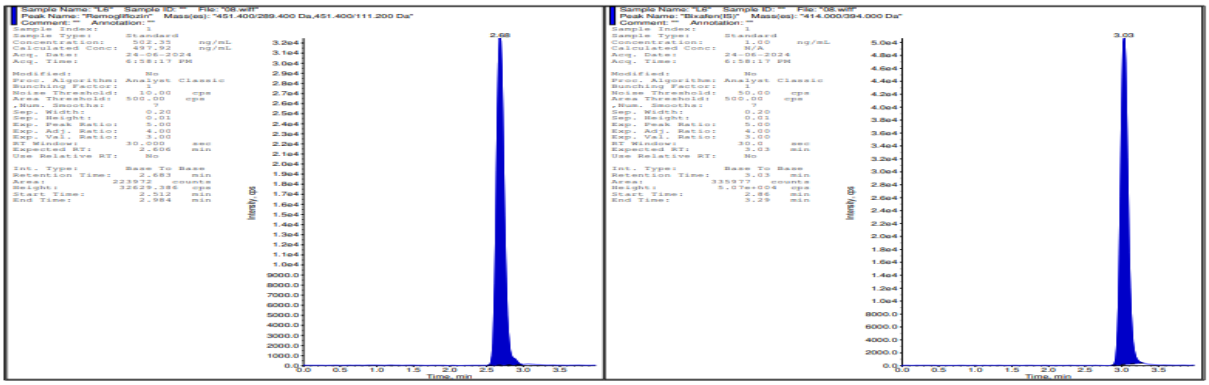

Figure 3: Chromatogram of linearity solution

Pharmacokinetics Study

Collected samples were extracted and analysed by LC-MS/MS. Results of the samples analysis were listed in the Table 2. Representative chromatogram is presented in Figure 4.

Table 2: Results of sample analysis

	0 h (ng/mL)	0.5 h (ng/mL)	1 h (ng/mL)	2 h (ng/mL)	4 h (ng/mL)	8 h (ng/mL)	24 h (ng/mL)
Male	0	460.2	122.99	49.72	14.4	6.88	0
	0	283.49	210.65	54.64	1.29	3.04	0
	0	137.92	341.22	56.32	29.97	0	0
Mean	0	293.87	224.95	53.56	15.22	3.31	0

	0 h (ng/mL)	0.5 h (ng/mL)	1 h (ng/mL)	2 h (ng/mL)	4 h (ng/mL)	8 h (ng/mL)	24 h (ng/mL)
Female	0	191.45	68.89	41.15	11.78	1.02	0
	0	106.56	122.23	46.9	59.04	6.15	0
	0	306.12	18.16	35.96	1.82	7.11	0
Mean	0	201.38	69.76	41.34	24.21	4.76	0

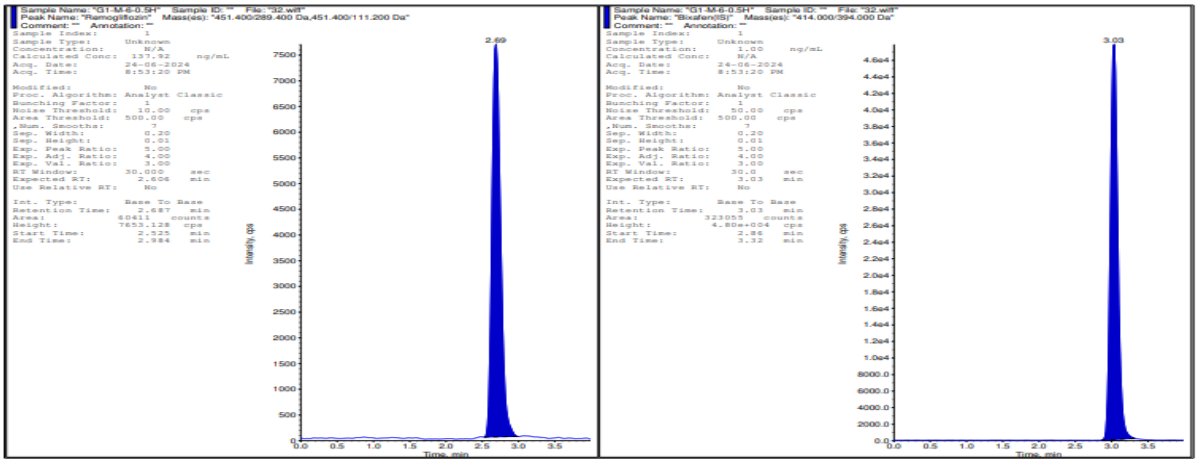

Figure 4: Chromatogram of the plasma sample analysis

Individual sample data were further analysed by WinNonlin Software to determine pharmacokinetic parameter. The various parameters results were listed in Table 3.

Table 3: Pharmacokinetic data

Sex	Dose (mg/Kg)	C_{max} (ng/mL)	T_{max} (h)	AUC_{last} (ng*h/mL)	$T_{1/2}$ (h)	Vd_F (mL/Kg)	Cl_F (mL/h/Kg)
Male	20	293.87	0.5	448.27	1.53	97083.5	43899.35
Female	20	201.38	0.5	297.17	1.85	172454.44	64539.21

The pharmacokinetic data showed that remogliflozin was rapidly absorbed in both male and female rats following oral administration at a dose of 20 mg/kg. The time to reach maximum plasma concentration (T_{max}) was consistent across sexes, occurring at 0.5 hours post-dosing, indicating a rapid onset of absorption. The mean peak plasma concentration (C_{max}) and area under curve (AUC_{last}) observed was higher in males (293.87 ng/mL and 448.27 ng·h/mL) compared to females (201.38

ng/mL and 297.17 ng·h/mL), respectively suggesting a sex-related difference in systemic exposure. The elimination half-life ($T_{1/2}$) was slightly longer in females (1.85 h) than in males (1.53 h), indicating a marginally slower elimination in females. Drug was completely metabolised in 24 h in both male & female rats indicating no significant difference in the duration of exposure. These findings indicated that remogliflozin exhibits rapid absorption and complete elimination in both male and female rats, with minor sex-based differences in pharmacokinetic parameters. Absorption profile is presented in figure 4.

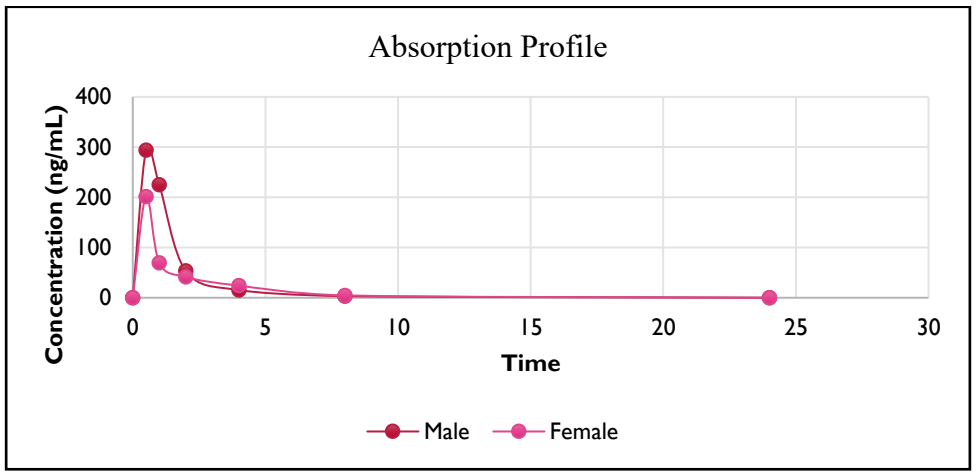

Figure 5: Absorption Profile of Remogliflozin in Rat (male & female)

Conclusion

For estimation of remogliflozin in plasma, simple, rapid and validated bioanalytical method of LC-MS/MS was used. The method used bixafen as internal standard. Small quantity of sample requirement and short run time allow the rapid analysis of large number samples. After single oral dose in rat, T_{max} achieved in 0.5 h in both sexes and drug completely metabolized within 24 h. The assay can be used for both repeat dose pharmacokinetic and biomedical analysis of remogliflozin during preclinical and clinical stages.

Acknowledgement

The authors are thankful to Jai Research Foundation, India for providing the facility and necessary guidance to do work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors made substantial contributions to this manuscript, engaged in the review, editing process and approved the final version for publication.

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