Research Article **ISSN: 2395 – 5597**

International Journal of Engineering and Robot Technology

Journal home page: www.ijerobot.com

https://doi.org/10.36673/IJEROBOT.2022.v09.i01.A02

VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF REMOGLIFLOZIN ETABONATE IN BULK AND TABLET DOSAGE FORM

D. Lavanya Satya Kumari[*1], H. G. Sowmya[1], G. S. Naveen Kumar[1]

[1*]Department of Pharmaceutical Analysis, Bharathi College of Pharmacy, Bharathi Nagar, Mandya, Karnataka, India.

ABSTRACT

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Remogliflozin Etabonate in pharmaceutical dosage form. Chromatographic separation of Remogliflozin Etabonate was achieved on Waters Alliance-e2695, by using Luna Phenyl Hexyl (150x4.6mm, 3.5µ) column and the mobile phase containing Acetonitrile and Ammonium formate pH-3.0 in the ratio of 70:30% v/v. The flow rate was 1.0ml/min; detection was carried out by absorption at 275nm using a photodiode array detector at ambient temperature. LOD and LOQ were found to be 0.30µg/ml and 1µg/ml respectively and retention time was found to be 2.271 mins. The % Recovery was found to be 99.83%. The number of theoretical plates and tailing factor for Remogliflozin Etabonate were NLT 2000 and not more than 2 respectively. % Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate and robust method for quantitative analysis of Remogliflozin Etabonate.

KEYWORDS

Remogliflozin etabonate, RP- HPLC, Validation and Pharmaceutical formulations.

Author for Correspondence:

Lavanya Satya Kumari D,
Department of Pharmaceutical Analysis,
Bharathi College of Pharmacy, Bharathi Nagar,
Mandya, Karnataka, India.

Email: lavanyadalai87@gmail.com

INTRODUCTION

Remogliflozin etabonate ISA drug of the gliflozin class for the treatment of non-alcoholic steatohepatitis ("NASH") and type 2 diabetes. Remogliflozin was discovered by the Japanese company Kissei Pharmaceutical and is currently being developed by BHV Pharma. Remogliflozin was commercially launched first in India by Glenmark in May 2019. Remogliflozin etabonate is anorally available prodrug of Remogliflozin etabonate, a benzyl pyrazole glucoside-based

inhibitor of renal sodium-glucose co-transporter subtype 2 (SGLT2) with antihyperglycemic activity. Upon administration and absorption, the inactive prodrug is converted to its active for Remogliflozin and acts selectively on the sodium-glucose co-transporter subtype 2 (SGLT2).

Literature survey revealed that there were few analytical methods have been reported for the determination of the Remogliflozin etabonate in pure drug and pharmaceutical dosage form by using UV-Spectrophotometric[1-5], HPLC[6-12] and HPTLC[13-15] so far.

The aim of the present work is to develop and validate a novel, rapid, precise and specific RP-HPLC method for estimation of Remogliflozin etabonate in bulk and tablet dosage form.

MATERIAL AND METHODS

Material and reagents

The Remogliflozin etabonate was obtained as a gift sample from the pharmaceutical industry and REMO tablet was obtained from Pharmacy store. Acetonitrile, Ammonium formate and distilled water were obtained Bharathi College of pharmacy, Bharathinagara, KM Doddi, Maddur Taluk, Mandya District, India. All chemicals used are of HPLC grade. Distilled water was used throughout the experiment.

Instrumentation

Chromatographic separation was performed on aWaters Alliance-e2695, by using Luna Phenyl Hexyl (150x4.6mm, 3.5µ) columnis used.

Chromatographic conditions

Preparation of solutions Mobile phase preparation

Preparation of Ammonium formate buffer solution

6.30g of Ammonium formate is dissolved in 1litre of HPLC grade water. Filter through 0.45µ nylon filter, after that adjust its pH-3.0 with ortho phosphoric acid.

Preparation of Mobile Phase

Mobile phase was prepared by mixing Ammonium formate pH-3.0 and ACN taken in the ratio 30:70. It was filtered through 0.45µ membrane filter to remove the impurities which may interfere in the final chromatogram.

Preparation of sample Solution

Accurately weighed and transfer 217mg of Remogliflozin etabonate, sample into a 100mL clean dry volumetric flask add diluent and sonicate it up to 30 min to dissolve and centrifuge for 30min to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45-micron injection filter (Stock solution). Further pipette 5 ml of the above stock solutions into a 50ml volumetric flask and dilute up to the mark with diluent (100ppm of Remogliflozin Etabonate).

Preparation of Standard solution

Accurately weigh and transfer 100mg of Remogliflozin Etabonate working standard into a 100ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 5ml of the above stock solutions into a 50ml volumetric flask and dilute up to the mark with diluent. (100ppm of Remogliflozin Etabonate).

System suitability requirements from stock and standard solutions

Tailing factor: NMT 2.0

Theoretical Plates: NLT 2000

RESULTS AND DISCUSSION

Validation of the proposed method

The proposed method was validated as per ICH guidelines[16-18]. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

Specificity

Specificity of an analytical method is ability to measure specifically the analyte of interest without interference from blank and known impurities. For this purpose, blank chromatogram, standard chromatogram and sample chromatogram were recorded. The chromatogram of blank shows no response at the retention times of drugs which confirms the response of drug was specific.

Linearity

Preparation of stock solution

Accurately weigh and transfer 100mg of Remogliflozin Etabonate working standard into a 100ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Preparation of Level – I (25ppm of Remogliflozin Etabonate)

1.25ml of above stock solutions has taken in different 50ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – II (50ppm of Remogliflozin Etabonate)

2.5ml of above stock solutions has taken in different 50ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – III (75ppm of Remogliflozin Etabonate)

3.75ml of above stock solutions has taken in different 50ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level –IV (100ppm of Remogliflozin Etabonate)

5ml of above stock solutions has taken in different 50ml of volumetric flasks, dilute up to the mark with diluent

Preparation of Level – V (125ppm of Remogliflozin Etabonate)

6.25ml of above stock solutions has taken in different 50ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – VI (150ppm of Remogliflozin Etabonate)

7.5ml of above stock solutions has taken in different 50ml of volumetric flasks, dilute up to the mark with diluent.

The linearity of the response of the drug was verified at six concentration levels, ranging from 25-150µg/ml of Remogliflozin etabonate in each linearity level were prepared. 10µl of each concentration was injected into the HPLC system. The response was read at 275nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were presented in Table No.3.

Precision

Precision of the method was performed as intraday precision, Inter day precision. To study the intraday precision, six replicate standard solutions (100µg/ml) of Remogliflozin etabonate was injected. % RSD was calculated and it was found to be 0.80 and interday precision done same as intraday, six replicate standard solutions (100µg/ml) of Remogliflozin etabonate was injected. % RSD was calculated and it was found to be 0.43which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table No.4.

Accuracy

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 50, 100 and 150% of the label claim of the tablet (100mg of Remogliflozin etabonate). The recovery values for Remogliflozin etabonate ranged from 98.0 to 102.0%. The average recoveries of three levels of Remogliflozin etabonate were found to be 99.6-100.7%. The results are shown in the Table No.5.

Limit of detection and Limit of quantification

The limit of detection (LOD) limit of quantification (LOQ) of the drug carry was calculated using the following equation as per international conference harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

LOD for Remogliflozin Etabonate was found to be 0.30µg/mL and LOQ for Remogliflozin Etabonate was found to be 1µg/ml. Results were shown in Table No.6.

Robustness

Robustness is the measure of the capacity of the analytical method to remain unaffected by small but deliberate variation in the procedure. The robustness of the method was evaluated by analysing the system suitability standard and evaluating system suitability parameter data after varying, individually, the HPLC

pump flow rate (0.9ml/min to 1.1ml/min.) and organic phase change shown in Table No.7.

Acceptance criteria

System suitability should pass as per test method at variable conditions.

Table No.1: HPLC method development parameters

S.No	HPLC method development parameters	
1	Column	Luna Phenyl Hexyl (150x4.6mm, 3.5µ)
2	Flow rate	1.0ml /min
3	Wavelength	275nm
4	Column temperature	25°C
5	Injection volume	10µL
6	Run time	5 minutes
7	Diluents	Mobile phase
8	Elution	Isocratic mode

Table No.2: Specificity of Remogliflozin etabonate

S.No	Name of the solution	Retention time in min
1	Blank	0
2	Remogliflozin etabonate (Standard)	2.271

Table No.3: Linearity of Remogliflozin etabonate

S.No	Concentration (µg/ml)	Peak area* (mv)
1	25	581124
2	50	1175605
3	75	1814569
4	100	2360358
5	125	2999546
6	150	3536489

*Average of six determinations

Table No.4: Results of Precision of Rosuvastatin calcium

S.No	Intraday Names	Studies Peak area	Interday Names	Studies Peak area
1	Injection-1	2321541	Injection-1	2341065
2	Injection-2	2350185	Injection-2	2367198
3	Injection-3	2373161	Injection-3	2352315
4	Injection-4	2340535	Injection-4	2335422
5	Injection-5	2319774	Injection-5	2316944
6	Injection-6	2351628	Injection-6	2322887
7	AVG	2342804	AVG	2339305
8	STDEV	20206.60	STDEV	18628.04
9	%RSD	0.86	%RSD	0.80

Table No.5: Results of recovery of Remogliflozin etabonate

S.No	%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean %Recovery
1	50%	1172458	50	50.29	100.7	99.83
2	100%	2312887	100	99.2	99.2	
3	150%	3484511	150	149.47	99.6	

*Average of three determinations

Table No.6: System suitability parameters

S.No	Parameters	Remogliflozin etabonate
1	Linearity	25-150µg/ml
2	Regression equation	y = 23759x-801
3	Correlation coefficient	R^2 = 0.9997
4	Retention time	2.271min
5	Run time	5min
6	Limit of detection(LOD)	0.30µg/ml
7	Limit of quantification(LOQ)	1µg/ml
8	Tailing factor	0.95
9	Theoretical Plate	12758

Table No.7: Robustness results for Remogliflozin etabonate

S.No	Parameter	Remogliflozin Etabonate				
		Condition	Retention time (min)	Peak area	Tailing	Plate count
1	Flow rate Change (mL/min)	Less flow (0.9ml)	3.352	2556140	1.05	12821
		Actual (1ml)	2.271	2329162	0.99	12754
		More flow (1.1ml)	1.998	2204556	0.96	12713
2	Organic Phase change	Less Org (63:37)	3.299	2620487	1.01	12880
		Actual (70:30)	2.278	2333584	0.95	12758
		More Org (77:23)	2.002	2047602	0.92	12687

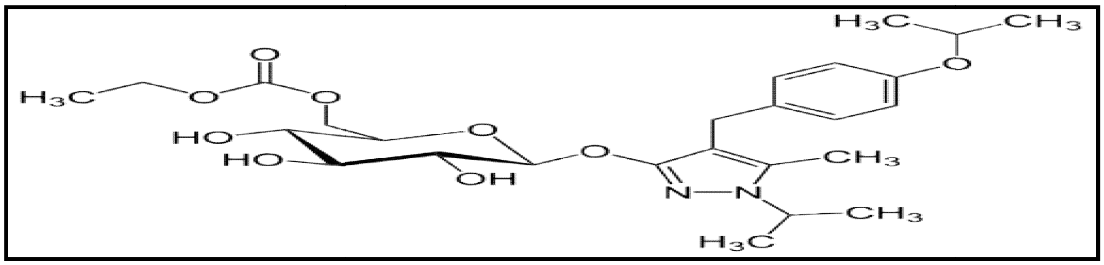

Figure No.1: Chemical structure of Remogliflozin etabonate

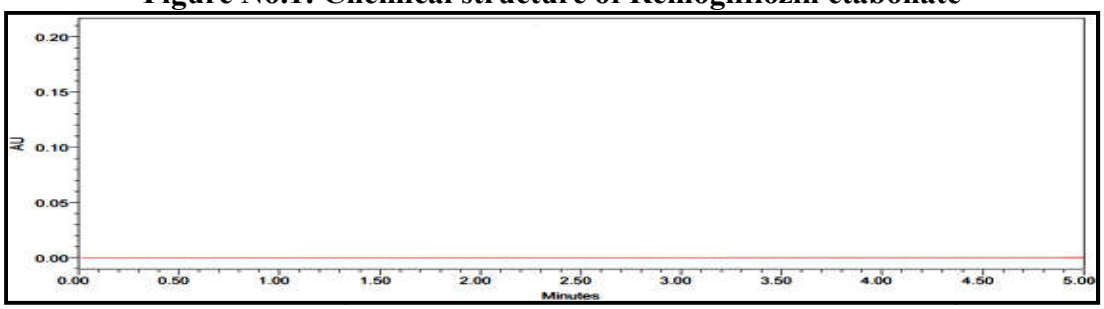

Figure No.2: Chromatogram of blank solution of Remogliflozin etabonate

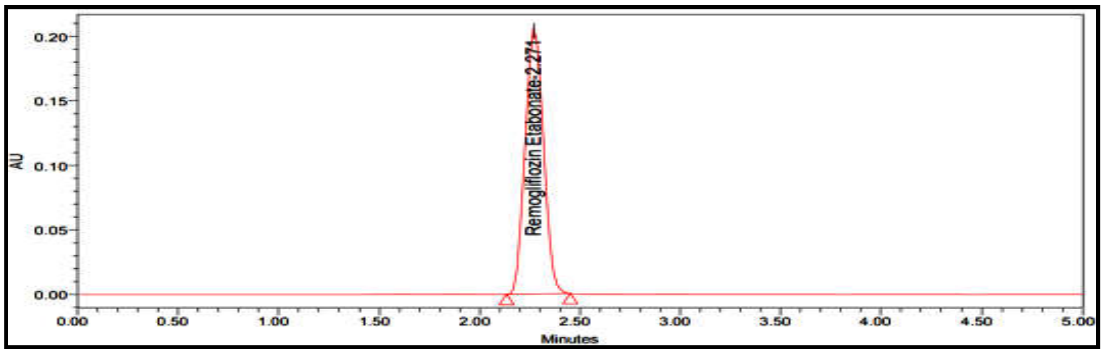

Figure No.3: Chromatogram of standard solution of Remogliflozin etabonate

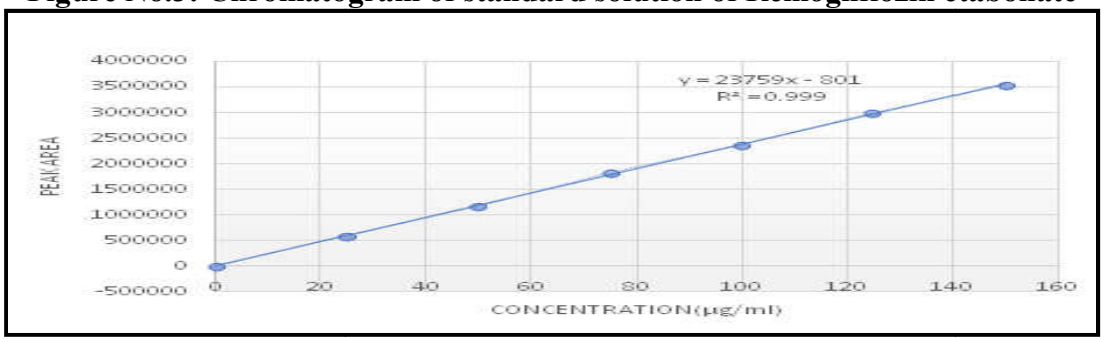

Figure No.4: linearity of Remogliflozin etabonate

CONCLUSION

The present analytical method was validated as per ICH guidelines and met the acceptance criteria. It was concluded that the developed analytical method was simple, accurate, economical and sensitive, and can be used for routine analysis of Remogliflozin etabonatein bulk drug and pharmaceutical dosage forms.

ACKNOWLEDGEMENT

We authors wish to thank our management, principal of Pharmacy College for providing all facilities in the college.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Vidhi D, Patel P. Method development and validation of UV spectrophotometric estimation of remogliflozin etabonate in bulk and its tablet dosage form, Research *Journal of Pharmacy and Technology,* 14(4), 2021, 2042-2044.
2. Fathima H, Havannavar N T, Jahan A. Estimation and validation of remogliflozin etabonate in dosage form and in bulk drug by spectrophotometric method, *Asian Journal of Research in Chemistry,* 15(2), 2022, 166-170.
3. Attimarad M, Nair A B, Nagaraja S, Aldhubiab B E, Venugopala K N, Pottathil S. Smart UV derivative spectrophotometric methods for lsimultaneous determination of metformin and remogliflozin: Development, validation and application to the formulation, *Indian J Pharm Educ Res,* 55(1), 2021, 293-302.
4. Itigimatha N, Chadchan K S, Hadagali M D. Simple and sensitive RP-HPLC and UV spectroscopic methods for the determination of Remogliflozin etabonate in pure and pharmaceutical formulations, *Turkish Journal of Pharmaceutical Sciences,* 19(2), 2022, 213.
5. Srinivasan R, Ahamed C S. Validation of developed analytical methods for the determination of remoglifozin and ertuglifozin in pharmaceutical dosage forms-An updated review, *Int. J. Pharm. Sci. Rev. Res,* 72(1), 2022, 29-34.

6. Trivedi S V. Stability indicating RP-HPLC method development and validation for simultaneous estimation of remogliflozin etabonate and metformin HCL in synthetic mixture and tablet dosage form, *World Journal of Pharmaceutical Research,* 10(10), 2021, 981-993.
7. Attimarad M, Elgorashe R E, Subramaniam R, Islam M M, Venugopala K N, Nagaraja S, Balgoname A A. Development and validation of rapid RP-HPLC and green second-derivative UV spectroscopic methods for simultaneous quantification of metformin and remogliflozin in formulation using experimental design, *Separations,* 7(4), 2020, 59.
8. Attimarad M, Venugopala K N, Islam M M, Shafi S, Altaysan A I. Rapid simultaneous quantitative analysis of hypoglycemic agents by RP HPLC: Development, validation and application to medicine, *Indian J. Pharm. Edu. Res,* 56(2), 2022, 564-572.
9. Suresh Mankar S, Younas M, Kalode V, Jirvankar P, Phutane P, Chandewar S, Armarkar A, Pethe A. Selective SGLT2 inhibitor's estimation and validation from galenical form by RP-HPLC method, *Journal of Pharmaceutical Research International,* 33(57B), 2021, 57-68.
10. Dhara V, Hetvi C. Development and validation of high-performance liquid chromatography method for simultaneous estimation of Remogliflozin etabonate and vildagliptin in pharmaceutical dosage form, *World Journal of Pharmacy and Pharmaceutical Sciences,* 10(4), 2022, 404-411.
11. Suryavanshi V D, Sharma S, Sahu J K. Review on characteristics and analytical methods of remogliflozin etabonate: An update, *Mini Reviews in Medicinal Chemistry,* 22(9), 2022, 1341-1350.
12. Patel V A, Pandya C V, Patel Z J, Patel D R, Pandya A C. Development and validation of novel RP-UHPLC/DAD methods for simultaneous quantification of Remogliflozin and metformin in bulk and formulation, *J. Chem,* 14(2), 2021, 1384-1393.
13. Shah D A, Gondalia II, Patel V B, Mahajan A, Chhalotiya U, Nagda D C. Stability indicating thin-layer chromatographic method for estimation of antidiabetic drug Remogliflozin etabonate, *Future Journal of Pharmaceutical Sciences,* 7(1), 2021, 1-2.
14. Badke S V, Kakad K S, Malode S S. High-performance thin-layer chromatography (HPTLC) method development and validation for determination of Remogliflozin etabonate and vildagliptin in bulk and its tablet formulation, *Int J App Pharm,* 14, 2022, 147-153.
15. Jadhav S R, Deshpande P B, Swami S, Supe D. A validated stability indicating high performance thin layer chromatographic method for determination of Remogliflozin etabonate in tablet dosage form, *Int. J. Chem Tech. Res,* 14(3), 2021, 382-390.
16. ICH, Q2A Text on Validation of Analytical Procedures, 1994.
17. ICH, Q2B Validation of Analytical Methodology, 1996.
18. ICH, Q2 (R1) Validation of Analytical Procedures: Text and methodology, 2005.

Please cite this article in press as: Lavanya Satya Kumari D *et al*. Validated RP-HPLC method for the estimation of Remogliflozin etabonate in bulk and tablet dosage form, *International Journal of Engineering and Robot Technology,* 9(1), 2022, 9-15.