Advances in Bioresearch
Adv. Biores., Vol 15 (5) September 2024: 150-156

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.5.150156

Advances in Bioresearch

ORIGINAL ARTICLE

Stability Indicating RP-HPLC Method for Remogliflozin and Teneligliptin

Dasari Vasavi Devi*[1], Anil Kumar Dindigala[2], Anantha Makineni[3], P. Anitha[4]
[1]Department of Pharmaceutical Analysis, Annamacharya College of Pharmacy, New Boyanapalli, Rajampet, Annamayya Dt., A.P 516 126, India.
[2] R&D Scientist, Thermo Fisher Scientific, Greenville, NC, USA.
[3]Project Manager - Project and Alliance Management, Adaptive phage Therapeutics Gaithersburg MD, USA.
[4]Department of Pharmaceutics, Annamacharya College of Pharmacy, New Boyanapalli, Rajampet, Annamayya Dt., A.P 516 126, India
*Corresponding author email: drdvas.reddy@gmail.com

ABSTRACT
The stability-indicating chromatographic method was developed and validated for simultaneous estimation of Remogliflozin and Teneligliptin in bulk and tablet dosage form. The RP-HPLC elution was carried out at 210 nm, and the chromatogram was run through Inertsil C18 (4.6 x 150mm, 4.8µm). Mobile phase containing Acetonitrile: OPA buffer with pH 4.4 taken in the ratio 70:30 was pumped through the column at a flow rate of 1.0 ml/min with a temperature maintained at 30°C. The proposed method was validated according to ICH Q2 (R1) guidelines. Remogliflozin and Teneligliptin were eluted at 2.222 min and 2.748 min, respectively. The method is linear from 12.5-75µg/ml for Remogliflozin (R^2 = 0.999) and 1.25- 7.5 µg/mL for Teneligliptin (R^2 = 0.999). The average recovery percentage was 100.07% for Remogliflozin and 100.13% for Teneligliptin at three different levels. The results of method repeatability and intermediate precision were found within acceptable limits. LOD and LOQ values obtained from regression equations of Remogliflozin and Teneligliptin were 0.22, 0.68, and 0.05, 0.15, respectively. Also, the results of the forced degradation study show that the method is stable, indicating that it can distinguish the active analytes peak from the degraded product. The developed stability-indicating method is linear in the studied concentration range and precise, accurate, specific, and robust. Hence, it can successfully be used for routine analysis and stability studies.
Keywords: Remogliflozin, Teneligliptin, RP-HPLC, stability indicating.

Received 04.06.2024 Revised 11.07.2024 Accepted 17.09.2024

How to cite this article:
Dasari Vasavi D, Anil K D, Anantha M, P. Anitha. Stability Indicating RP-HPLC Method for Remogliflozin and Teneligliptin.. Adv. Biores. Vol 15 [5] September 2024. 150-156

INTRODUCTION

Remogliflozin is a sodium-glucose cotransporter 2 (SGLT2) inhibitor. SGLT2 inhibitors are a class of drugs that work by inhibiting the reabsorption of glucose in the kidneys, leading to increased glucose excretion in the urine. This mechanism helps lower blood glucose levels and is particularly useful in people with type 2 diabetes. By reducing glucose reabsorption, SGLT2 inhibitors also lead to modest weight loss and potential blood pressure reduction[1-3]. Teneligliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor. DPP-4 inhibitors are another class of drugs used to manage type 2 diabetes. They work by inhibiting the enzyme DPP-4, which breaks down incretin hormones (GLP-1 and GIP). These hormones play a role in regulating insulin release and glucose levels. By inhibiting DPP-4, teneligliptin increases the levels of these incretin hormones, which in turn promotes insulin release and helps lower blood glucose levels[4-6]. Both Remogliflozin and Teneligliptin can be used as part of a comprehensive treatment plan for individuals with type 2 diabetes, typically in combination with other antidiabetic medications, dietary modifications, and exercise [7].

The review of the literature indicates that only a few analytical techniques, including HPLC[8, 9] UV[10], HPTLC[11], and UHPLC[12], are available for the quantification of remogliflozin both alone and in

combination with other medications in APIs and formulations. The measurement of remogliflzin concentrations in human plasma has been reported using LC-MS-MS techniques[13]. Teneligliptin can be quantified using ultraviolet (UV) spectrophotometry[14, 15], HPLC-UV[16], UVHPTLC[17], HPLC-UPLC[18], high-performance liquid chromatography (HPLC)[19-21], HILIC[22], and LC-MS/MS[23] analytical techniques, either alone or in combination with other drugs.

This research aims to create a simple and accurate HPLC method for identifying Remogliflzin and Teneligliptin in bulk preparation and pharmaceutical formulation. This approach has also been validated in accordance with ICH recommendations. Its degradation experiments for Remogliflzin and Teneligliptin (Figure 1) were conducted in acidic, basic, peroxide, reduction, UV, thermal, and hydrolysis conditions[24-27].

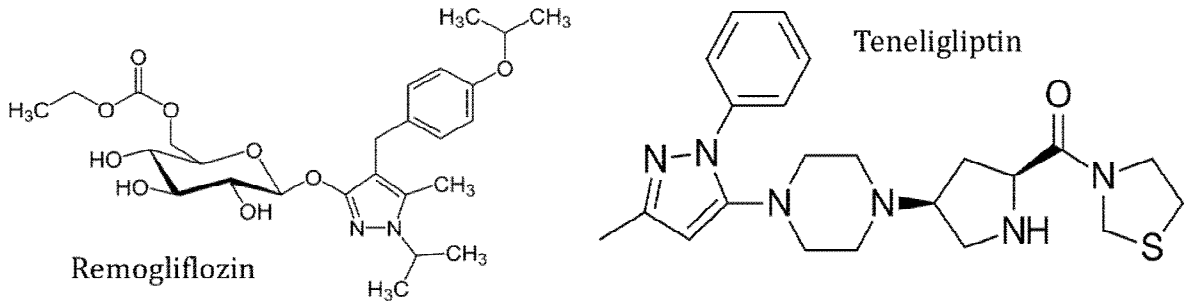

Figure 1: Structure of Remogliflozin and Teneligliptin

MATERIAL AND METHODS

Chemicals and Reagents

Remogliflozin and Teneligliptin pure drugs (API) were received from Spectrum Pharma labs. Combination Remogliflozin and Teneligliptin tablets (Zeta PLUS_R) were received from the local market. Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dehydrogenate ortho phosphate buffer, and Ortho-phosphoric acid were also received. All the above chemicals and solvents are from Rankem.

Preparation of Standard stock solutions

Accurately weighed 50mg of Remogliflozin and 25mg of Teneligliptin and transferred to a 50ml volumetric flask. 3/4th of the diluents were added to the flasks and sonicated for 10 minutes. Flask were made up of diluents and labeled as Standard stock solution 1. (500µg/ml of Remogliflozin and 50µg/ml of Teneligliptin)

Preparation of Sample stock solutions

Twenty tablets were weighed, and the average weight of each tablet was calculated, then, the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, and 50ml of diluents were added and sonicated for 25 min. Further, the volume was made up of diluent and filtered by HPLC filters. (1000µg/ml of Remogliflozin and 100µg/ml of Teneligliptin)

Method Validation

Specificity/Selectivity

The method's ability to distinguish and quantify the analyte in the presence of other components like impurities, degradation products, or matrix components. To ensure no interference at the analyte retention time, analyze blank samples, spiked samples with known analyte concentrations, and samples containing potential interferences.

Linearity

The method's ability to elicit results directly proportional to the concentration of the analyte within a given range. Prepare calibration curves by analyzing samples of known concentrations (typically 5–7 levels) and plot the response vs. concentration. Calculate the correlation coefficient (R^2), which should be ≥ 0.99 for most applications.

Accuracy

The closeness of the test results to the actual value. Analyze known concentrations of the analyte and compare the measured value with the actual value. Express results as a percentage recovery, typically within 98-102% for pharmaceutical assays.

Precision

Perform replicate analyses (typically six) on homogeneous samples and calculate %RSD (Relative Standard Deviation), with the acceptable limit generally being ≤ 2%.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The lowest amount of analytes can be detected but not necessarily quantified. The lowest amount of analyte can be quantitatively determined with acceptable precision and accuracy. Based on the signal-to-noise ratio, a signal-to-noise ratio of 3:1 for LOD and 10:1 for LOQ is typically acceptable. LOD and LOQ can also be estimated from the response's calibration curve slope and standard deviation.

Robustness

The method's capacity to remain unaffected by minor but deliberate variations in method parameters (e.g., changes in pH, temperature, flow rate). Intentionally vary method parameters and assess if the changes affect the results significantly. The method should maintain acceptable performance under varying conditions**.**

Degradation studies

Degradation studies for Remogliflozin and Teneligliptin were conducted under various stress conditions to assess their stability. Oxidative degradation involved adding 1 ml of 20% hydrogen peroxide to 1 ml of stock solution, heating at 60°C for 30 minutes, and analyzing by HPLC after dilution to 50 µg/ml and 5 µg/ml. Acid degradation was performed by refluxing 1 ml of stock solution with 1 ml of 2N hydrochloric acid at 60°C for 30 minutes, followed by HPLC analysis at the same concentrations. Similarly, alkali degradation was conducted by refluxing the stock solution with 2N sodium hydroxide at 60°C for 30 minutes. Dry heat degradation was tested by placing the drug at 105°C for 6 hours, and Photostability was assessed by exposing the drug solution to UV light for seven days or 200 Watt hours/m^2. In neutral degradation studies, the drug was refluxed in water for 6 hours at 60°C. For each condition, the resulting solution was diluted to 50 µg/ml and 5 µg/ml, and 10 µl was injected into the HPLC system to record chromatograms and evaluate sample stability.

RESULTS AND DISCUSSION

The chromatographic analysis was performed using an Inertsil C18 column (4.6 x 150 mm, 4.8 µm) with a mobile phase consisting of acetonitrile (ACN) and 0.1% orthophosphoric acid (OPA) in a ratio of 70:30. The flow rate was set at 1 ml/min, and the detector wavelength was 230 nm. The column temperature was maintained at 30°C, and an injection volume of 10 µL was used. The total run time for each analysis was 6.0 minutes. Both peaks have good resolution, tailing factor, Theoretical plate count, and resolution. The total runtime for each validation parameter was set to 6 minutes.

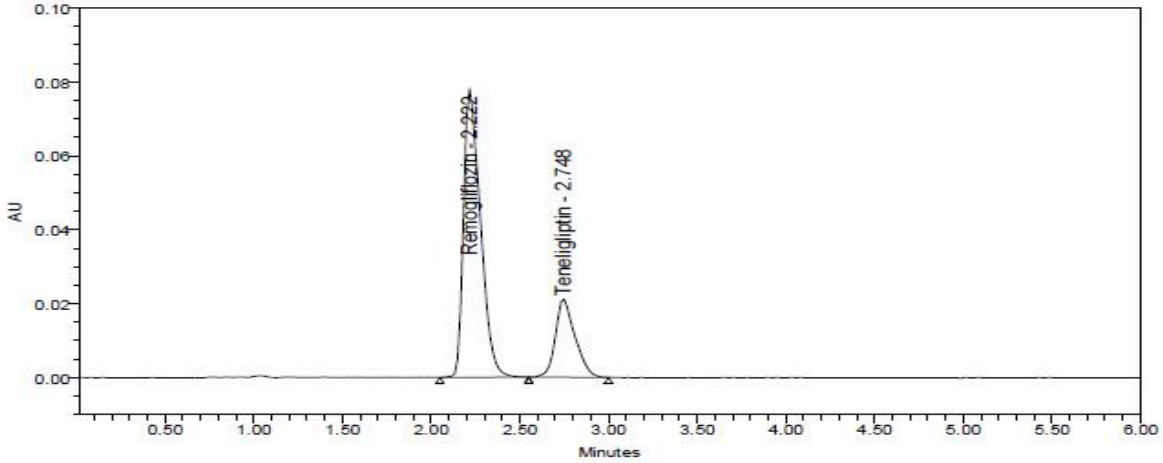

Figure 2: Optimized chromatogram

Method Validation

The validation of HPLC method for the determination of Remogliflozin and Teneligliptin as per the protocol and to demonstrate that the method is appropriate for its intended use was studied for the following parameters. All the validation parameters were carried out according to ICH.

Specificity

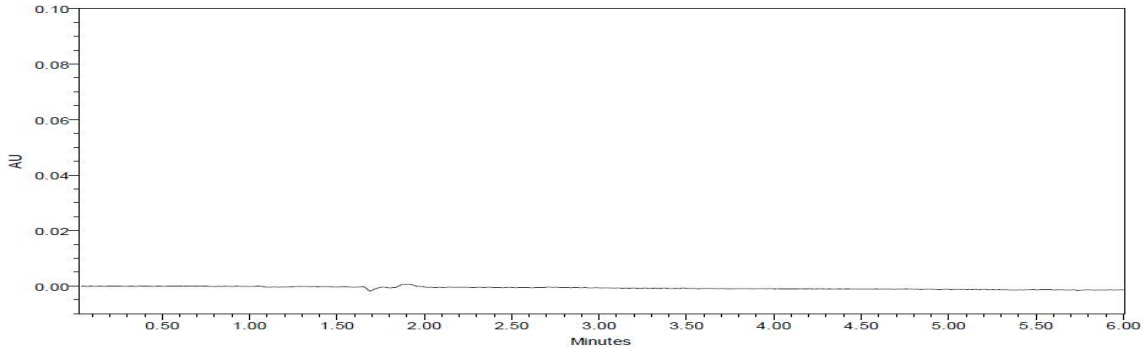

Figure 3: Chromatogram of blank

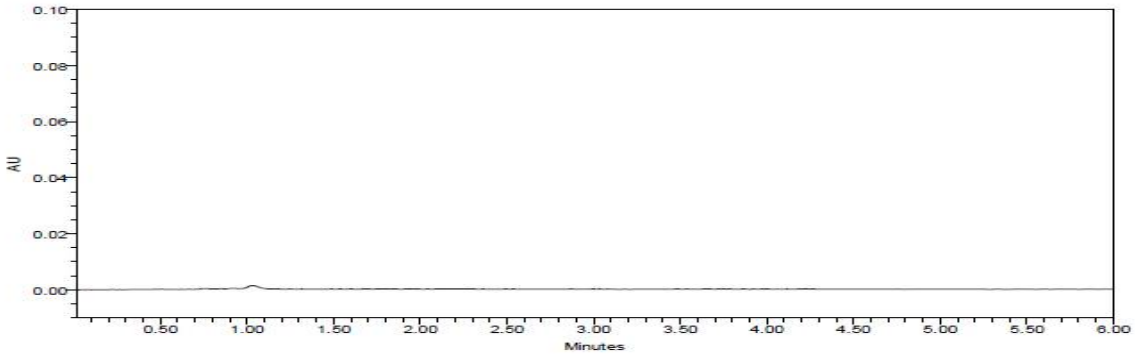

Figure 4: Chromatogram of placebo

Linearity

Six linear concentrations of Remogliflozin (12.5-75µg/ml) and Teneligliptin (1.25-7.5µg/ml) were injected and duplicated. Average areas were mentioned in table No. 2, and the linearity equations obtained for Remogliflozin was y = 11186x + 2361.7, and for Teneligliptin, was y = 22264x + 675.91. The correlation coefficient obtained was 0.999 for the two drugs.

Table No: 1 Linearity table for Remogliflozin and Teneligliptin.

Remogliflozin		Teneligliptin	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
12.5	143069	1.25	28598
25	280259	2.5	56426
37.5	422821	3.75	84300
50	566394	5	112716
62.5	706005	6.25	141217
75	834407	7.5	165903

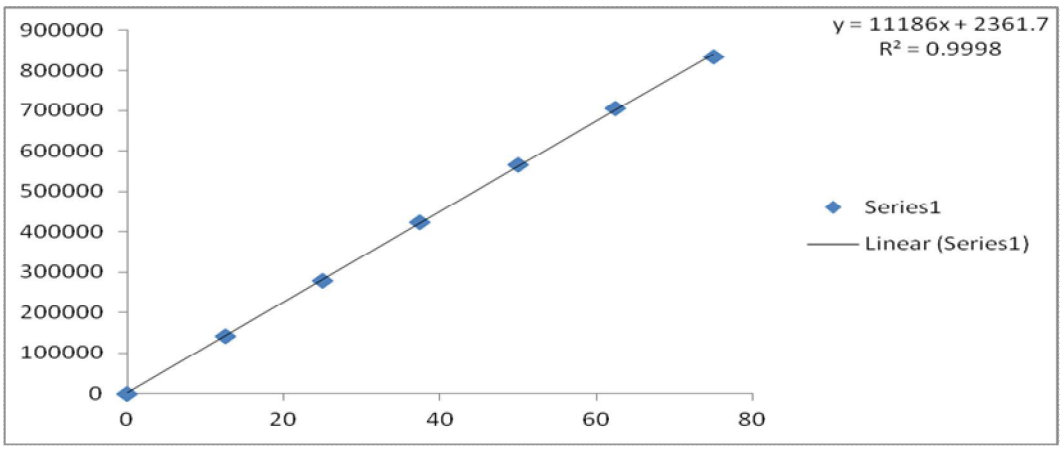

Figure 5: Calibration curve of Remogliflozin

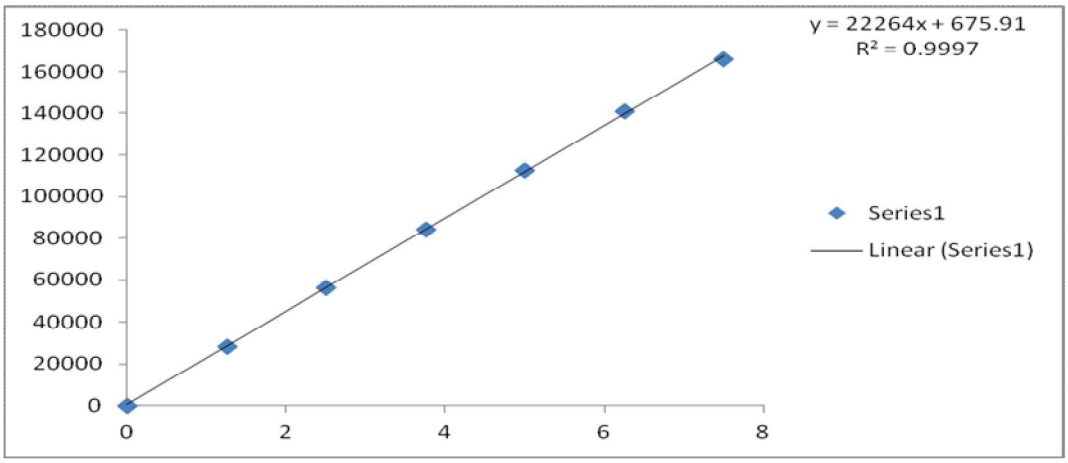

Figure 6: Calibration curve of Teneligliptin

Precision

Method precision

The %RSD (Relative Standard Deviation) for Remogliflozin and Teneligliptin was calculated using six replicate injections. The mean area for Remogliflozin was 564788 with a standard deviation (S.D) of 3147.7, resulting in a %RSD of 0.6%. Similarly, the mean area for Teneligliptin was 113296, with a standard deviation of 719.7, giving a %RSD of 0.6%. These low %RSD values indicate high precision and reproducibility of the method for both compounds.

Intermediate precision (Day-Day Precision)

Intermediate precision of Remogliflozin and Teneligliptin based on six replicate injections. The mean area for Remogliflozin was 565271, with a standard deviation (S.D) of 2437.7, resulting in a %RSD of 0.4%. Teneligliptin's mean area was 118174, with a standard deviation of 535.6, yielding a %RSD of 0.5%. These results indicate good intermediate precision for both analytes, demonstrating the method's consistency when tested under different conditions or over different days.

Accuracy

Samples for three levels of accuracy were prepared using the standard addition method. Triplicate injections were given for each level of accuracy, and the mean Recovery was 100.07% and 100.13% for Remogliflozin and Teneligliptin, respectively.

Sensitivity

Remogliflozin's Limit of Detection (LOD) was 0.22 µg/ml, and the Limit of Quantitation (LOQ) was 0.68 µg/ml. Teneligliptin's LOD was 0.05 µg/ml, and the LOQ was 0.15 µg/ml. These values indicate the method's ability to detect and quantify deficient concentrations of both analytes with high sensitivity.

Robustness

The method's robustness for Remogliflozin and Teneligliptin was evaluated under various conditions by altering the flow rate, mobile phase composition, and temperature. When the flow rate was decreased to 0.9 ml/min, the %RSD for Remogliflozin was 0.6%, and for Teneligliptin, was 0.3%. When the flow rate was increased to 1.1 ml/min, the %RSD values were 0.7% and 1.1%, respectively. For mobile phase composition changes, a ratio of 65B:35A resulted in %RSD values of 0.9% for Remogliflozin and 1.3% for Teneligliptin, while a 75B:25A ratio yielded %RSDs of 0.6% and 0.8%. Lastly, temperature variations at 27°C showed %RSDs of 0.6% and 1.0%, while at 33°C, the %RSDs were 0.5% and 0.6%, indicating that the method is robust under these conditions.

Table No 2: Robustness data for Remogliflozin and Teneligliptin.

S.No.	Condition	%RSD of Remogliflozin	%RSD of Teneligliptin
1	Flow rate (-) 0.9ml/min	0.6	0.3
2	Flow rate (+) 1.1ml/min	0.7	1.1
3	Mobile phase (-) 65B:35A	0.9	1.3
4	Mobile phase (+) 75B:25A	0.6	0.8
5	Temperature (-) 27°C	0.6	1
6	Temperature (+) 33°C	0.5	0.6

Assay

Zeta-Plus-R, bearing the label claim Remogliflozin 100mg, Teneligliptin 10mg. An assay was performed with the above formulation. The average % Assay for Remogliflozin and Teneligliptin obtained was 100.17% and 99.90%, respectively.

Forced Degradation Studies:

The specificity of the method was demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative, reductive, and thermal degradation. The sample was exposed to these conditions, and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient. Regulatory guidance in ICH Q2A, Q2B, Q3B, and FDA 21 CFR section 211 requires developing and validating stability-indicating potency assays.

Table No: 3 Degradation data of Remogliflozin and Teneligliptin

Type of degradation	Remogliflozin			Teneligliptin		
	Area	%Recovered	% Degraded	Area	%Recovered	% Degraded
Acid	530635	94.12	5.88	103170	90.97	9.03
Base	521578	92.51	7.49	105287	92.83	7.17
Peroxide	525492	93.20	6.80	107309	94.62	5.38
Thermal	545475	96.75	3.25	109995	96.99	3.01
UV	538129	95.45	4.55	106301	93.73	6.27
Water	555576	98.54	1.46	112651	99.33	0.67

Conclusion

A simple, Accurate, precise method was developed to estimate the Remogliflozin and Teneligliptin in tablet dosage form simultaneously. The retention time of Remogliflozin and Teneligliptin was found to be 2.222 min and 2.748 min. %RSD of the Remogliflozin and Teneligliptin were found to be 0.4 and 0.9, respectively. %Recovery was obtained as 100.07% and 100.13% for Remogliflozin and Teneligliptin, respectively. LOD and LOQ values obtained from regression equations of Remogliflozin and Teneligliptin were 0.22, 0.68, and 0.05, 0.15, respectively. The regression equation of Remogliflozin is y = 11186x + 2361.7. And Y = 113497x + 1822.1of Teneligliptin. Retention times were decreased, and that run time was decreased, so the method developed was simple and economical and can be adopted in regular Quality control tests in Industries.

References

1. Scheen AJ. (2015). Pharmacokinetics, Pharmacodynamics and Clinical Use of SGLT2 Inhibitors in Patients with Type 2 Diabetes Mellitus and Chronic Kidney Disease. Clin Pharmacokinet. 54(7):691-708.
2. Viswanathan Mohan, Ambrish Mithal, Shashank R Joshi, S R Aravind, and Subhankar Chowdhury. (2020). Remogliflozin Etabonate in the Treatment of Type 2 Diabetes: Design, Development, and Place in Therapy. Drug Des Devel Ther. 14(1): 2487–2501.
3. Choi CI. (2016). Sodium-glucose cotransporter 2 (SGLT2) inhibitors from natural products: discovery of next-generation antihyperglycemic agents. Molecules. 21(9):1136.
4. FDA Thailand Product Information: Tenelia (teneligliptin) oral tablets.
5. Surendra Kumar Sharma, A Panneerselvam, KP Singh, Girish Parmar, Pradeep Gadge, and Onkar C Swami (2016). Teneligliptin in management of type 2 diabetes mellitus. Diabetes Metab Syndr Obes., 9(1): 251–260.
6. Kishimoto M. (2013). Teneligliptin: a DPP-4 inhibitor for the treatment of type 2 diabetes. Diabetes Metab Syndr Obes. 12(2); 6:187–195.
7. Rahul Kodgule, Monika Tandon, Rajesh Gaikwad, Amol Pendse, Kiran Khaladkar, Manoj Kumar, Sumit Bhushan, Sachin Suryawanshi, Hanmant Barkate (2022). A Randomized, Double-blind, Active-controlled Study of Remogliflozin Etabonate 100 mg plus Teneligliptin 10 mg Twice-daily versus Teneligliptin 20 mg Once daily as add-on to Metformin Monotherapy in Indian Diabetic Patients. J Diabetes Treat., 7(2): 1-16.
8. Bhatkar TV, Badkhal AV and Bhajipale NS (2020). Stability indicating RP-HPLC method development and validation for the estimation of remogliflozin etabonate in bulk and pharmaceutical dosage form. International Journal of Pharmaceutical Research .12(2): 160-9.
9. Dimal A. Shah, Ishita I. Gondalia, Vandana B. Patel, Ashok Mahajan and Usmangani K. Chhalotiya (2020). Stability indicating liquid chromatographic method for the estimation of remogliflozin etabonate. J Chem Metrol . 14(6): 125-32.
10. Vidhi Dave and Patel Paresh (2021). Method development and Validation of UV Spectrophotometric estimation of Remogliflozin Etabonate in bulk and its tablet dosage form. Res J of Pharmacy and Technolo. 14(4): 2042-4.
11. Dimal AS, Ishita IG, Vandana BP, Ashok M, Usmangani C and Dhruti CN (2021). Stability indicating thin-layer chromatographic method for estimation of antidiabetic drug Remogliflozin etabonate. Future Journal of Pharmaceutical Sciences.,7(2): 1-12.

12. Patel VA, Pandya CV, Patel ZJ, Patel DR and Pandya AC (2021). Development and validation of novel RP-UHPLC/ DAD methods for simultaneous quantification of Remogliflozin and Metformin in bulk and formulation. Rasaya J Chem 14(2): 1384-1393.
13. Polli JW, Humphreys JE, Harmon KA, Webster LO, Reese MJ and MacLauchlin CC (2021). Assessment of Remogliflozin etabonate, a sodium dependent glucose co-transporter-2 inhibitor, as a perpetrator of clinical drug interactions: A study on Drug transporters and metabolic enzyme. J Diabetes Metab 3(5): 1-8.
14. Amit MS, Kiran KD and Varsha AR (2016): A simple UV spectrophotometric method development and validation of teneligliptin in tablet dosage form. Indo Am J Pharm Res 6(1): 14-21.
15. Manjusha DK and Barhate VD (2016) Spectrophotometric determination of an anti-diabetic drug Teneligliptin bulk and pharmaceutical formulations. W J Pharm Res .5(4): 1625-1632.
16. Atul TH, Rathod EA, Gupta KR and Umekar MJ (2016). HPLC and UV spectrophotometric estimation of Teneligliptin from tablet dosage form. J Pharm Biomed Anal 2016; 4(7): 148-156.
17. Shinde VC, Aher KB, Bhavar GB, Kakad SJ and Chaudhari SR (2016). Development and validation of a UV spectrophotometric method and a high-performance thin-layer chromatographic (HPTLC) method for estimating teneligliptin hydrobromide in pharmaceutical preparations. Der Pharm Lett 8(2): 291-301.
18. Ganeshkumar TNV, Vidyadhara S, Niteen AN, Saisilpa Y and Rajyalakshmi M (2016). Method development, validation, and stability studies of teneligliptin by RP-HPLC and identification of degradation products by UPLC tandem mass spectroscopy. J Anal Sci Tech . 7(4): 18-23.
19. Chandana M, Prasad Rao M, Samrajyam B, Sireesha, KSKD and Naga Premi VV (2016) Analytical method development and validation of teneligliptin in pharmaceutical dosage form by RP-HPLC method. J Heal Sci Nur. 1(1): 1-12.
20. Shailesh VL, Kamna RP, Jani GK and Sachin BN (2016). Simultaneous estimation of teneligliptin hydrobromide hydrate and its degradation product by RP-HPLC method. J Pharm Sci Biosci Res 6(5): 254-261.
21. Dasari Vasavi Devi, Dugasani Swarnalatha And Gopireddy Venkatasubba Reddy(2018). Chemometric Assisted Method Development for Teneligliptin and Metformin by Stability Indicating RP-HPLC Technique and its Validation. Asian J. Chem. 30, (12): 2704-2710.
22. Mahesh Attimarad, Venugopal KN and Chohan H Muhammad: An experimental design approach to quantitative expression for quality control of a multicomponent anti diabetic formulation by the HILIC method. Molecules .27(10): 3135-3152.
23. Chunduri RHB and Dannana G(2022)Development and validation of LC-MS/ MS method for quantification of teneligliptin in human plasma and its application to a pharmacokinetic study. W J Pharm Pharm Sci, 5(9):833-850.
24. ICH Harmonised Tripartite Guideline (2003), Validation of analytical procedures: Text and methodology, Q2 (R1), International Conference on Harmonization, Geneva (2005): 1-13.
25. International Conference on the Harmonization, (2003) ICH Harmonized Tripartite Guideline. Stability Testing of New Drug Substances and Products Q1A (R2), 32-35.
26. International Conference on the Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures:Text and Methodology Q2 (R1), November (2005).
27. Bakshi M, Singh S.(2002). Development of validated stability-indicating assay methods--critical review. J Pharm Biomed Anal.;28(6):1011-40.