RP-HPLC method validation for the simultaneous estimation of remogliflozin and teneligliptin in bulk and pharmaceutical dosage form

Anchal Arora[1], Komal[2], Sanjeeb Kumar Kar[3*], Abhishek[4], Ishwari Rajesh Chaudhari[5], Narendra Kumar Dnyandev Phupate[6],Preeti Avasthi[7]

[1]Department of Pharmacology, All India Institute of Medical Sciences, Bathinda, Punjab, India, Pin Code -151001

[2]Department of Pharmacology, DBM College of Pharmacy, Madina, Gohana,Haryana, India, Pin Code - 131301

[3]Department of Pharmacy, Sarala Birla University, Ranchi, Jharkhand, India, Pin Code - 835103

[4] Department of Pharmacology, Guru Gobind Singh College of Pharmacy, Yamunanagar, Haryana, India, Pin Code - 135001

[5]Department of Pharmaceutical Chemistry, Pataldhamal wadhwani College of Pharmacy, Yavatmal, Maharashtra, India, Pin code - 445001

[6]Department of Pharmaceutical Chemistry, Sudhakarrao Naik Institute of Pharmacy, Pusad, Maharashtra, India, Pin Code - 445204

[7]Deaprtment of Pharmacy, Maharaja Agrasen School of Pharmacy, Maharaja Agrasen University, Atal Shiksha Kunj,vill. Kalujhanda Tehsil Baddi, Distt. Solan (H.P.), India, Pin Code - 174103

*** Corresponding Author: Dr. Sanjeeb Kumar Kar**

ABSTRACT

A straightforward, accurate, and long-lasting reverse phase RP-HPLC has been verified for the simultaneous quantification of remogliflozin and teneligliptin in API and pharmaceutical dosage form. This method makes use of an Aglient column, Aglient HPLC chromatographic separation, and PDA detection along with a straightforward isocratic mobile phase made up of Acetonitrile: KH2 taken in the ratio (35:65). Remogliflozin and teneligliptin's average retention times were discovered to be 2.263 min. and 2.994 min. respectively, indicating the approach can be employed for the regular testing. Remogliflozin and teneligliptin's linearity was found to be linear with a r2 of 0.999 for all drugs, suggesting that the method can produce acceptable responsiveness. The RSD should not be greater than 2.0% according to the precision acceptance. Remogliflozin & teneligliptin were found to have % RSDs of 0.5 and 0.6, respectively, demonstrating the accuracy of the approach.

Key words: *Validation, remogliflozin, teneligliptin, RP-HPLC.*

INTRODUCTION

The effectiveness and safety of a medication are significantly influenced by the quality of the medicine. For customers to have access to safe and effective medicinal formulations, quality assurance & management of pharmaceutical and chemical formulations are crucial.

Hence Analyzing pure medicinal ingredients and their pharmaceutical dose forms is essential to determining whether a chemical is appropriate for usage in patients [1]. The caliber of the data generating techniques used determines the caliber of the analytical data. To ensure that medications and their formulations are legally certified by regulatory bodies, it is crucial to create tough and reliable analytical methods [2–3].

Trials examining the therapy and underlying science of Type 2 Diabetes Mellitus and Diabetes Mellitus, Type 2 have used remogliflozin etabonate. The sodium-glucose transport proteins (SGLT), which are in charge of glucose reabsorption in the kidney, are inhibited by remogliflozin. Blood glucose is removed through the urine when this transporter is blocked (Dosage forms: Tablet; Brand names: Remozen V) [4,5].

Management for Type 2 Diabetes Mellitus has been studied with teneligliptin. A sodium glucose co-transporter-2 (SGLT-2) inhibitor is teneligliptin. The SGLT2 co-transporters in the kidney are in charge of reabsorbing glucose from the filtrate of the glomerular system. The glucuretic action brought on by SGLT2 inhibition lowers renal glucose tolerance and renal absorption, increasing renal excretion of glucose. Additionally, it helps with weight loss, lowers blood pressure, and results in lessened hyperglycemia [6–9].

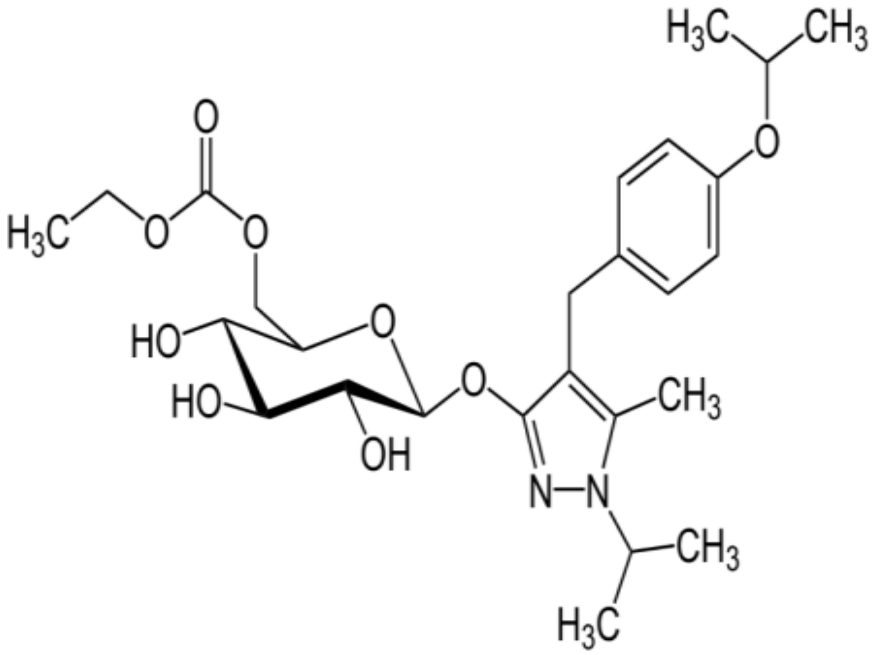

Fig. 1: Structure of Remogliflozin

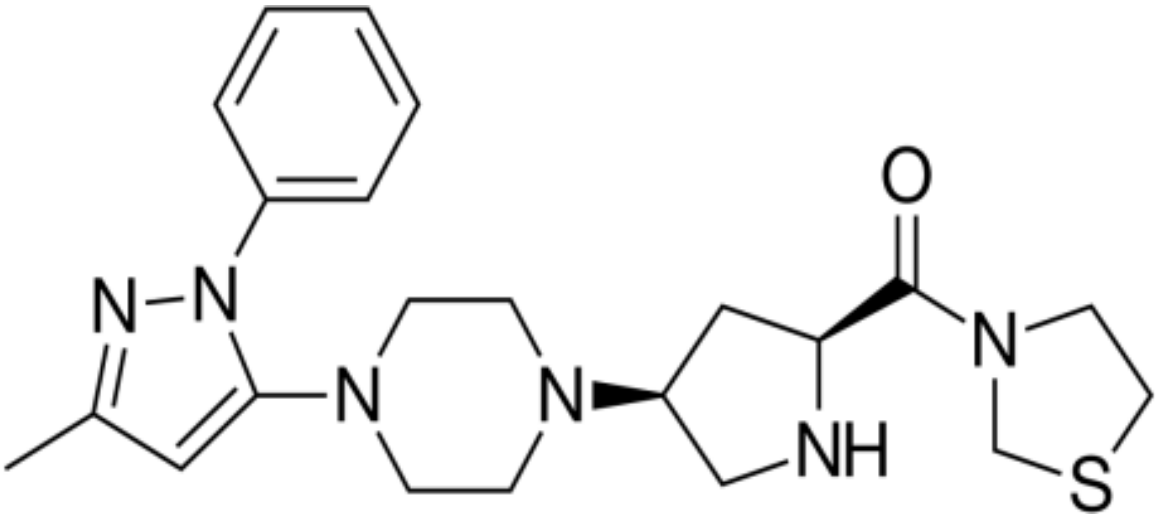

Fig. 2: Structure of Teneligliptin

In accordance to a literature review, certain approaches for estimating these medicines simultaneously, separately, or in combination with other drugs have been published. UV-Spectroscopy technique (tablet, extended-release, Jardiance brand) [10–11].

It is combined with a healthy diet and exercise. A sodium-glucose cotransporter 2 (SGLT2) antagonist is teneligliptin. It functions by raising the quantity of sugar excreted in the urine and reducing the quantity of glucose that the body absorbs [12–14]. RP-HPLC has not been used to simultaneously estimate remogliflozin and teneligliptin in pharmaceutical dose form, according to a basic evaluation of the literature. This study's primary goal is to create a quick, accurate, and easy RP-HPLC approach for estimating remogliflozin and teneligliptin in pharmaceutical dose form and bulk. According to ICH standards, a validated method was used to estimate the concentrations of remogliflozin and teneligliptin.

MATERIALS AND METHODS

Glenmark Pharma, Himanchal Pradesh has provided the remogliflozin and teneligliptin pure API drugs. Rankem, India provided all of the chemicals and buffers utilized in this work. Combination of remogliflozin and teneligliptin tablets (Zita PLUS-R) received from local market,Bhopal, Madhya Pradesh, India.

Instrumentation and Chromatographic Conditions

AGILENT HPLC, model G4-286b-HPLC system with photo diode array detector was used for the development and method validation with an automated sample injector. Agilent (150mm 4.5mm 3.5mm) column was used for the separation. The mobile phase used was KH2: Acetonitrile (65:35). having flow rate of 1ml/min, detected wavelength of 240nm, column temperature of 30°C, injection volume was set at 10µL and run time was 6min. The data acquired was at 240nm and the output signal was monitored and integrated using Empower2 Software.

Preparations of SolutionsDiluent

The diluent used was Acetonitrile and Water in the ratio of (50:50) as per solubility of drugs.

Preparation of Stock Solutions [15-16]

Accurately weighed 25mg of remogliflozin, 2.5mg of teneligliptin and transferred to 50ml and 50ml volumetric flasks separately. 3/4 Th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and from these stock solutions take 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (50 µg/ml of remogliflozin and 5µg/ml of teneligliptin).

Preparation of Sample Solutions

10 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, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters and 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent (50µg/ml of remogliflozin and 5µg/ml of teneligliptin).

Preparation of Buffer [17]

0.01N KH_2PO_4 Buffer:

In a 1000 ml volumetric flask , accurately weigh 1.36 g of potassium dihydrogen ortho phosphate. Add 900 ml of milli-Q water, degas, sonicate, and then fill the remaining volume with water. Finally, adjust pH to 5.4 using dilute formic acid.

0.1% Formic acid buffer: 1ml of conc. formic acid was diluted to 1000ml with water.

Method Development [18, 19]

According to ICH criteria, the technique validation of HPLC was completed for the combined estimation of the drug substances remogliflozin and teneligliptin in order to show that the method is suggested for routine testing.

RESUTS AND DISCUSSION

Remogliflozin and teneligliptin were eluted at 2.263min and 2.994 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

System Suitability Data

System suitability: The system suitability was performed for each validation parameters by injecting standard solutions containing (50µg/ml of remogliflozin and 5µg/ml of teneligliptin). The % RSD for the area of six standard injections results should not be more than 2%. System suitability parameters are shown in figure 3 and values and mentioned in Table 1.

Specificity Data

Specificity (Selectivity): Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific. Representative chromatogram is shown in Figure 4 and the experimental data is shown in Table 2.

Table 1: System Suitability Parameters for Remogliflozin and Teneligliptin

S. No.	Teneligliptin			Remogliflozin			
Injection	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.237	7555	1.20	2.950	11892	1.23	6.5
2	2.244	7803	1.17	2.954	12415	1.26	6.7
3	2.257	7636	1.16	2.989	11468	1.33	6.7
4	2.257	8116	1.16	2.990	12118	1.28	6.6
5	2.258	6584	1.25	2.994	12455	1.29	6.6
6	2.260	7925	1.17	2.997	11839	1.24	6.6

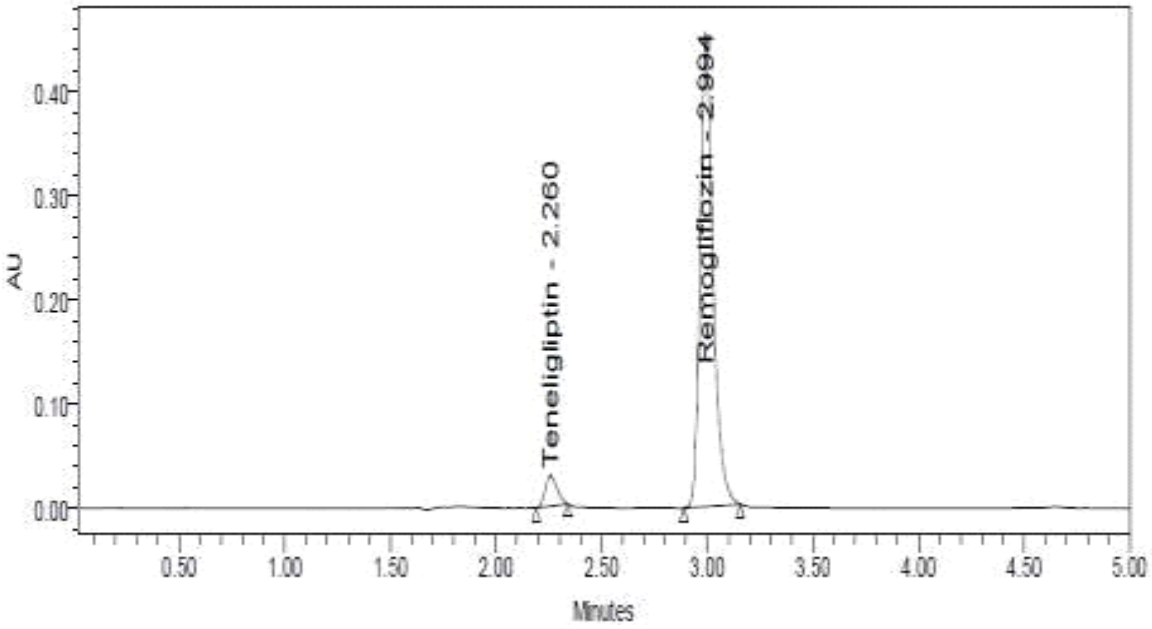

Fig. 3: System suitability chromatogram of remogliflozin and teneligliptin

Table 2: Specificity data of remogliflozin and teneligliptin

Sample name	Retention time (mins)
Remogliflozin	2.263
Teneligliptin	2.994

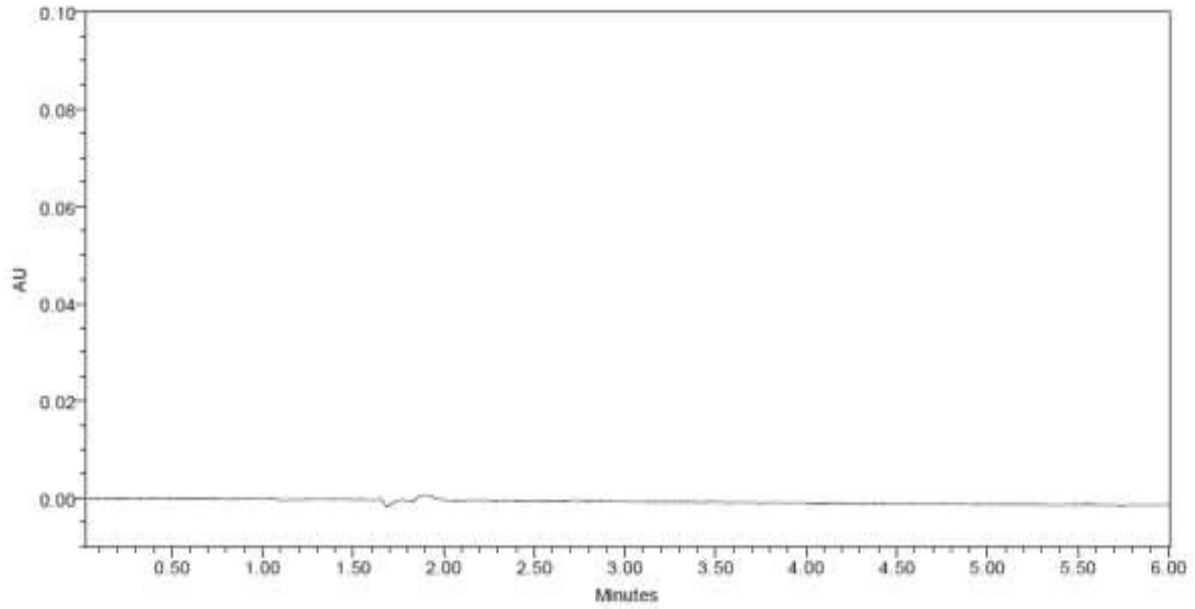

Fig. 4: Blank chromatogram of remogliflozin and teneligliptin

Table 3: Linearity of Remogliflozin and Teneligliptin

Remogliflozin		**Teneligliptin**	
Conc. (µg/mL)	**Peak area**	**Conc. (µg/mL)**	**Peak area**
0	0	0	0
12.5	497501	1.25	35433
25	995001	2.5	67916
37.5	1508074	3.75	104299
50	1995210	5	135732
62.5	2482220	6.25	172134
75	2885003	7.5	206097

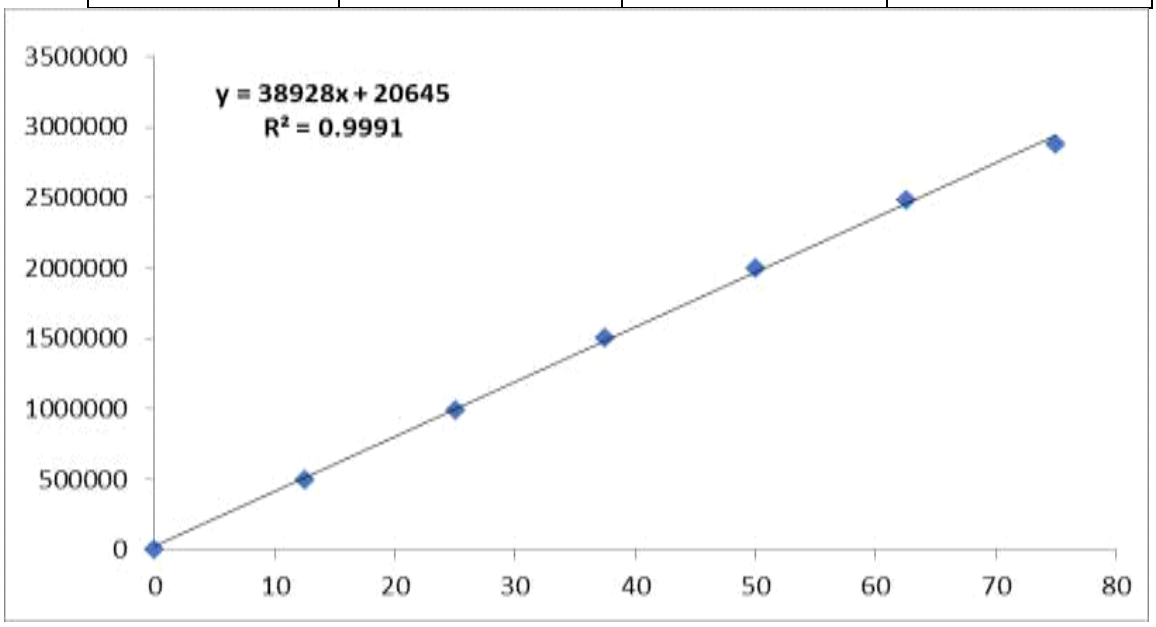

Fig. 5: Calibration of remogliflozin

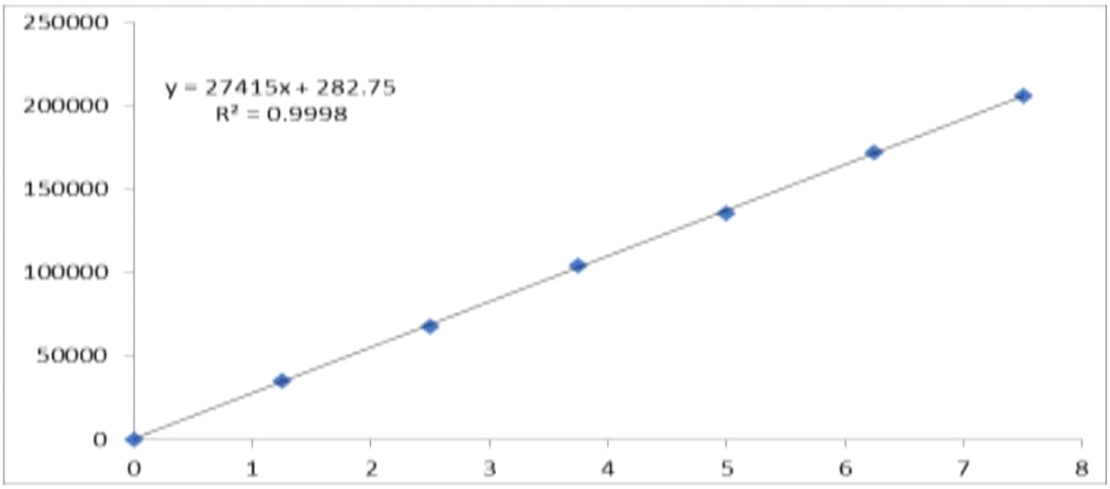

Fig. 6: Calibration curve of teneligliptin

Six linear concentrations of remogliflozin (12.5-75µg/ml) and teneligliptin (1.25-7.5µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for remogliflozin was y = 38928x + 20645. and of teneligliptin was y = 27415x + 282.75. Correlation coefficient obtained was 0.999 for the two drugs.

Table 4: Accuracy table of Remogliflozin

% Level	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % recovery
50	25	24.94	99.76	99.63
	25	24.87	99.49	
	25	24.75	99.01	
100	50	50.17	100.35	
	50	49.98	99.95	
	50	49.87	99.74	
150	75	74.89	99.85	
	75	74.46	99.28	
	75	74.44	99.25	

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean % Recovery was obtained as 99.63% and 100.23% for remogliflozin and teneligliptin respectively.

Table 5: Accuracy Table of Teneligliptin

% Level	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % recovery
50	2.5	2.51	100.53	
	2.5	2.51	100.59	

	2.5	2.52	100.99	100.23
100	5	4.96	99.17	
	5	5.04	100.84	
	5	5.00	100.08	
150	7.5	7.57	100.97	
	7.5	7.46	99.46	
	7.5	7.46	99.47	

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.5% and 0.6% respectively for remogliflozin and Teneligliptin. As the limit of Precision was less than "2" the system precision was passedin this method.

Table 6: System Precision Table of Remogliflozin and Teneligliptin

S. No.	Area of remogliflozin	Area of teneligliptin
1.	1907093	111848
2.	1912489	110435
3.	1907366	111567
4.	1903190	110470
5.	1900303	110284
6.	1884684	111647
Mean	**1902521**	**111042**
S.D	**9667.1**	**715.7**
%RSD	**0.5**	**0.6**

The % RSD for the peak areas of drospirenone and estetrol obtained from six replicate injections of standard solutions which was within range of limit (<2%).

Table 7: Sensitivity table of remogliflozin and teneligliptin

Molecule	LOD	LOQ
Remogliflozin	0.42	1.26
Teneligliptin	0.02	0.05

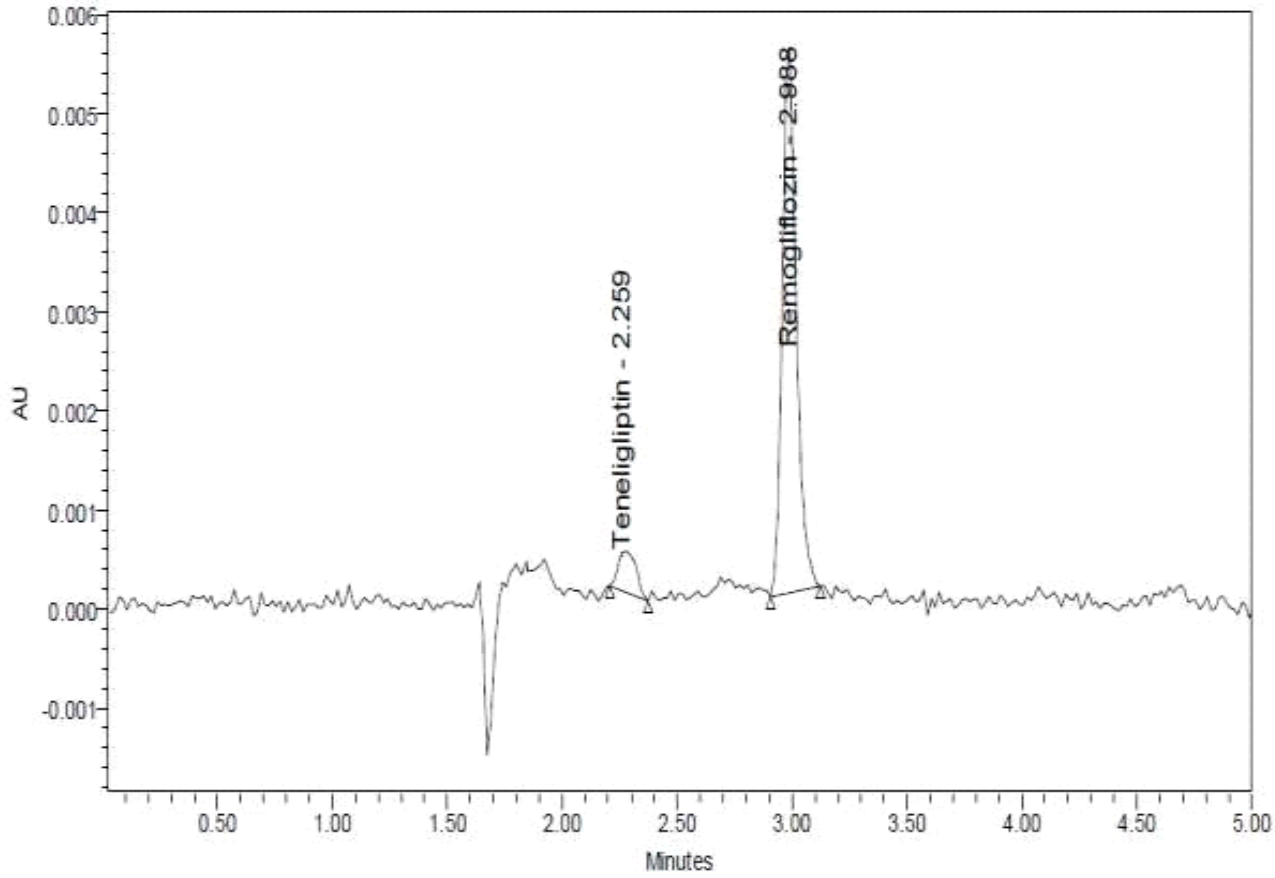

Fig. 7: LOD chromatogram of standard

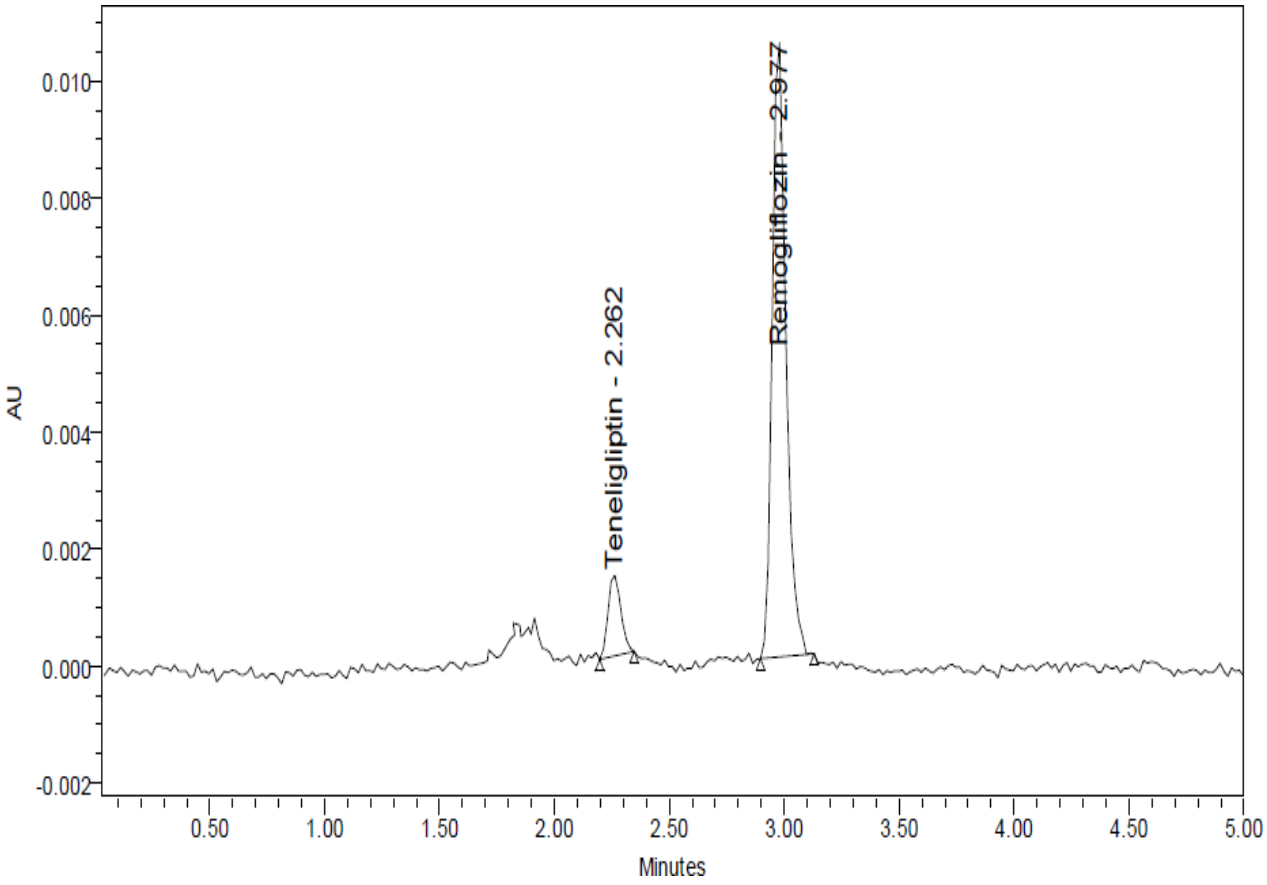

Fig. 8: LOQ chromatogram of standard

Table 8: Robustness data for Remogliflozin and Teneligliptin

S. No.	Condition	% RSD of Remogliflozin	% RSD of Teneligliptin
1	Flow rate (-) 0.9ml/min	0.4	0.1
2	Flow rate (+) 1.1ml/min	0.9	0.6
3	Mobile phase (-) 55B:45A	0.9	0.9
4	Mobile phase (+) 70B:30A	1	0.4
5	Temperature (-) 27°C	0.3	0.2
6	Temperature (+) 33°C	0.4	0.2

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55B:45A), mobile phase plus (70B:30A), temperature minus (27°C) and temperature plus(33°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within thelimit.

Table 9: Assay table of Remogliflozin and Teneligliptin

Drug name	Label claim	%Assay	Brand name
Remogliflozin	100mg	100.54%	Remozen V
Teneligliptin	10mg	100.27%	Jardiance

Zita Plus-R, bearing the label claim remogliflozin 100mg, teneligliptin 10mg. Assay was performed with the above formulation. Average % assay for remogliflozin and teneligliptin obtained was 100.54% and 100.27% respectively.

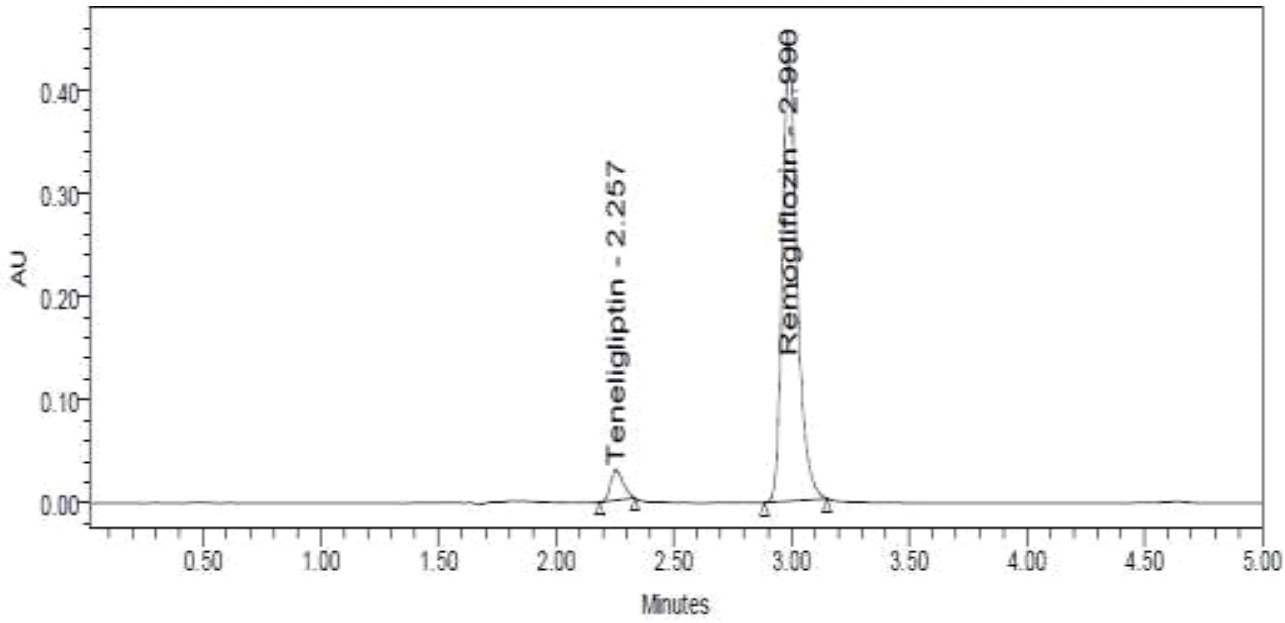

Fig. 9: Chromatogram of working standard solution

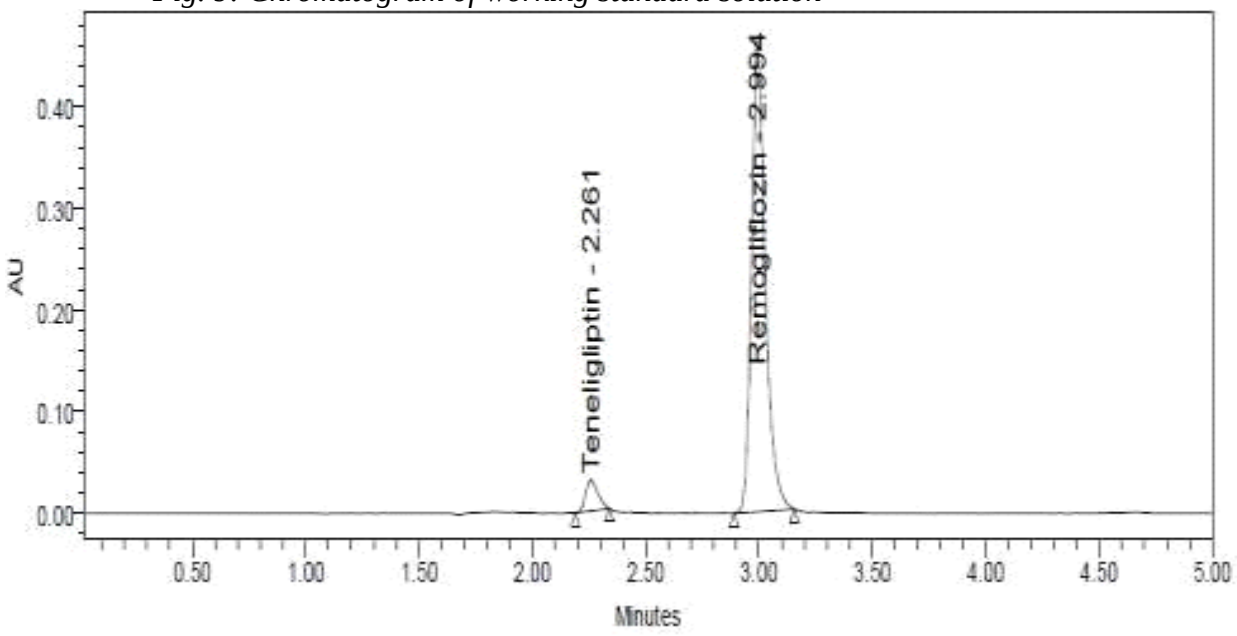

Fig. 10: Chromatogram of working sample solution

A simple, accurate, precise method was developed for the simultaneous estimation of the remogliflozin and teneligliptin in tablet dosage form. Retention time of remogliflozin and teneligliptin were found to be 2.263 min and 2.994 min. % RSD of the remogliflozin and Teneligliptin were and found to be 0.5 and 0.6 respectively. %Recovery was obtained as 99.63% and 100.23% for remogliflozin and teneligliptin respectively. LOD, LOQ values obtained from regression equations of remogliflozin and teneligliptin were 0.26, 0.78 and

0.03, 0.09 respectively. Regression equation of remogliflozin is y = 38928x + 20645. And y = 27415x + 282.75 of teneligliptin. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular quality control test in Industries.

CONCLUSION

For the concurrent stimulation of remogliflozin and teneligliptin in the pharmaceutical dose form, a novel stability indicating RP-HPLC approach was created and validated. The develop approach was accurate, had greater resolutions, required fewer retentions with different degradants, and was less expensive. This approach may thus be used to evaluate processes in pharmaceutical companies and to ensure that drug testing procedures are of a high standard.

ACKNOWLEDGEMENT

We would like to express our special thanks of gratitude to our Institutions for facilitating this research work.

FUNDING

The authors declare that they have not received any financial aid from any source in this study.

DECLARATIONS

The authors declare that they have no competing interests.

REFERENCES

1. C.R. Harish, P. Ramalingam, P.V. Vamshi, N. Ramesh, B. Sreeram, Simultaneous determination of Remogliflozin hydrochloride, Atorvastatin and Glimepiride in tablet dosage forms by RP-HPLC, Am. J. Pharm. Tech. Res. 2012; 2:991-998.
2. Kaushal. C, Srivatsava. B. A Process of Method Development: A ChromatographicApproach. J Chem Pharm Res, Vol.2, Issue 2, 519-545, (2010).
3. Green JM. A Practical guide to analytical method validation, Anal Chem (1996) 305A- 309A.
4. Ananda Kumar Chettupalli, Vivek Kunduru, Narender Boggula, Vasudha Bakshi. Development and Validation of Capecitabine Tablet (Pharmaceutical Dosage Form) By Using RP-HPLC Method. Indo Am. J. P. Sci. 2017; 4(03):550-557.
5. Indian Pharmacopoeia, Indian Pharmacopoeial Commission, Controller of Publication, Government of India, Ministry of health and Family Welfare, Ghaziabad, India, 2 (2010) 1657-1658.
6. Amit MS, Kiran KD, Varsha AR. A simple UV spectrophotometric method development and validation of teneligliptin in tablet dosage form. *Indo Am J Pharm Res.* 2016; 6:14– 21.
7. Sirigiri N, Subramanian NS, Reddy NK. Stability Indicating Method Development and Validation for Simultaneous Estimation of Sitagliptin Phosphate and Metformin HCL in Tablets by HPLC. Int J Pharm Sci Reserch. 2018 Feb ;9(10):4294–302.

8. Narender Boggula et. al. Validation of RP-HPLC method for the estimation of dolasetron in injection, International Journal of Pharmaceutical, Chemical and Biological Sciences 2018; 8(2):210-217.
9. Kapil R, Pushpendra S. Analytical Method Development and Validation for the Simultaneous Estimation of Metformin Hydrochloride and Alogliptin by RP-HPLC in Bulk and Tablet Dosage Forms. Research Journal of Science and Technology. 2021; 13(2):111-8.
10. Kanna KL, Panigrahy UP. Stability indicating method development and validation of remogliflozin etabonate in bulk and pharmaceutical dosage form by RP- HPLC.International Journal of Pharmaceutical Sciences and Research, 2021, 12(8); 4197-4207.
11. Narender Boggula, Dr. P. Shanmuga Pandiyan. Development and Validation of RP- HPLC Method for the Simultaneous Estimation of Dapagliflozin and Saxagliptin in Bulk and Pharmaceutical Dosage Forms. Int J Pharm Sci & Res. 2021; 12(1):314-20.
12. Dave V, Paresh 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. 2021; 14(4):2042-4.
13. Swamy GK, Lalitha R, Mounika C, Soumya B, Kumar DS. A Validated RP-HPLC Method for Simultanious Determination of Metformin and Canagliflozin in Pharmaceutical Formulation. Asian Journal of Pharmaceutical Analysis. 2018 Jun 11;8(2):73-7.
14. Bhavana Goud Ranga, Rithika Sankepally, Sneha Sollu, Venkateswara Rao Pragada, M Akiful Haque, Vasudha Bakshi, Narender Boggula. Analytical Method Development and Validation of Tolvaptan in Bulk and Its Tablet Dosage Form by UV- Spectrophotometry. Indo Am. J. P. Sci. 2022; 09(2):186-193.
15. Saudagar PGT, RB. A Review - Importance of RP-HPLC in Analytical Method Development. Indo Am J Pharm Sci. 2018;05(05):4897–907.
16. Khalil GA, Salama I, Gomma MS, Helal MA. Validated RP-HPLC method for simultaneous detrmination of canagliflozin, dapagliflozin,empagliflozin and metformin. Int J Pharm Chem Biol Sci. 2018;8(1):1–13.
17. Shrikrishna B. Baokar, Sugandha V. Mulgund, Nisharani S. Ranpise. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Vildagliptin and Metformin. Research J. Pharma. Dosage Forms and Tech. 2013; 5(2): 95-98.
18. Bolla, P.K.; Kalhapure, R.S.; Rodriguez, V.A.; Ramos, D.V.; Dahl, A.; Renukuntla, J. Preparation of solid lipid nanoparticles of furosemide-silver complex and evaluation of antibacterial activity. J. Drug Deliv. Sci. Technol. 2019; 49:6–13.
19. P.K. Bolla, V.A. Rodriguez, R.S. Kalhapure, C.S. Kolli, S. Andrews, J. Renukuntla. A review on pH and temperature responsive gels and other less explored drug delivery systems. J. Drug Deliv. Sci. Technol. 2018; 46:416-435.