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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS QUANTIFICATION OF REMOGLIFLOZIN AND TENELIGLIPTIN IN PURE AND TABLET DOSAGE FORM

T. Prasanthi*, Lakshmana Rao Atmakuri and G. Uha

Department of Pharmaceutical Analysis, V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, Andhra Pradesh, India.

ABSTRACT

A simple, sensitive, accurate and precise RP-HPLC method was developed for the simultaneous determination of Remogliflozin and Teneligliptin in pure and pharmaceutical dosage form. The components were separated on Agilent Eclipse XDB C18 column (150×4.6 mm,3.5 µ) using the mobile phase composed of acetonitrile and 0.1% TFA (65:35, v/v). The response was detected at 225nm. The peaks of Remogliflozin and Teneligliptin were retained at 3.25 min and 3.87 min respectively. The optimized method was validated as per ICH recommendations. The developed method produced linear response in the range of 25-150 µg/mL for Remogliflozin and 2.5-15 µg/mL for Teneligliptin with a good correlation coefficient. Low % RSD values and excellent recoveries demonstrated the precision and accuracy of the method. The limits of detection for Remogliflozin and Teneligliptin were 3 and 0.3 µg/ml, while the limits of quantification were 10 and 1 µg/ml respectively. Stability studies were carried out under acidic, alkaline, oxidative, photolytic and thermal conditions and no significant degradation was observed. The proposed method was so rapid, precise and economical that can be applied successfully for simultaneous estimation of both Remogliflozin and Teneligliptin in bulk and combined tablet formulation.

Keywords: Remogliflozin, Teneligliptin, RP-HPLC, Linearity and Precision.

INTRODUCTION

Remogliflozin(Figure 1) is chemically known as 5-Methyl4-[4-(1-methyl ethoxy) benzyl]-1-(1-methylethyl)- 1H-pyrazol-3-yl-6-O-(ethoxycarbonyl)-β-Dglucopyranoside. It inhibits the sodium-glucose transport proteins, which are responsible for glucose reabsorption in the kidney[1]. Blocking this transporter causes blood glucose to be eliminated through the urine.

Teneligliptin (Figure 2) is chemically 4-[4-(5-methyl-2-phenylpyrazol-3-yl) piperazin-1-yl]pyrrolidin-2-yl]-(1,3-thiazolidin-3-yl) methan-one. Teneligliptin inhibits the action of DPP-4 enzymes and slows down the rapid degradation of incretins[2]. It also increases insulin synthesis by the pancreas and decreases glucagon levels which are a counter-hormone of insulin, thereby further decreasing blood sugar levels.

A survey of literature found that few HPLC methods[3-7] were reported for estimation of Remogliflozin and Teneligliptin alone but no method was described for estimation of these drugs in combined dosage form. Hence an attempt was made to develop a simple, rapid and accurate RP-HPLC method for estimation of Remogliflozin and Teneligliptin in pure and tablet dosage form.

MATERIALS AND METHODS

Instrument

Waters e 2695- Alliance HPLC with Empower 2.0software was used for chromatographic studies.

CHEMICALS

Remogliflozin and Teneligliptin pure samples were procured from Spectrum Pharma Research Solutions, Hyderabad. HPLC grade acetonitrile, AR

grade ortho phosphoric acid and triflouro acetic acid were purchased from E. Merck (India) Ltd. Commercial tablets were purchased from local market. Triple distilled water was used throughout experiment.

Preparation of Mobile Phase
Mobile phase was prepared by mixing 0.1%TFA and ACN taken in the ratio 30:70 v/v. It was filtered through 0.45µ membrane filter to remove the impurities which may interfere in the final chromatogram.

Preparation of standard stock solution
Weigh and transfer accurately about 100 mg of Remogliflozin and 10 mg of Teneligliptin working standard into a 100 ml clean dry volumetric flask, add about 70 ml of mobile phase, sonicate for 5 minutes, and dilute to volume with mobile phase.

Diluted standard
Pipette out 5ml of the standard stock solution and dilute to 50 ml with diluent.

Preparation of sample stock solution
Twenty tablets were accurately weighed and ground to fine powder. An accurately weighed portion of powder sample equivalent to label claim of one tablet was transferred to a 100 ml volumetric flask. The contents of the flask were sonicated for about 10 min for complete solubility of the drug and volume made up with further quantity of mobile phase.
Further pipette 1ml of the above stock solution into a 10 ml volumetric flask and the volume was made up to the mark with the mobile phase.

RESULTS

Method development
Trials were conducted by using different mobile phases in varying composition. By considering peak parameters the following conditions were optimized. The suitable mobile phase identified was acetonitrile and 0.1% TFA (70:30 v/v). Flow rate of mobile phasewas 1ml/min; column temperature was maintained at 30°C. The eluted compounds were monitored at a wavelength of 225nm. The sample injection volume was 10µL. Mobile phase was degassed by sonicator prior to use. All determinations were performed at ambient temperature. The peaks of Remogliflozin and Teneligliptin were found to be 3.25 and 3.87 min respectively. The optimized chromatogram was shown in Figure 3.

VALIDATION

System Suitability
Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated and the values are depicted in Table 1.

Linearity
Linearity was assessed by preparing serial dilutions of 25-150µg/mL for Remogliflozin and 2.5-15µg/mL for Teneligliptin. The absorbance was detected at225 nm and each measurement was carried out in triplicate. Linearity graph was constructed by taking concentration on X-axis and peak area on Y-axis. The correlation coefficient, linearity results were presented in Table 2 and the calibration curves were represented in Figure 4 and 5.

Precision
Precision provides the indication of random errors and can be expressed in terms of intra-day and inter-day precision. To study the intra-day precision, the analysis of drugs was repeated for six times in the same day and for inter-day precision the analysis of drugs was carried out for six days. %RSD was calculated and the results were given in Table 3.

Accuracy
The accuracy test serves to highlight how closely the results from the method under validation agree with a recognized standard value. Accuracy was performed by spiking 50%, 100%, and 150% levels of standard solution to pre-analysed sample solution of the labeled amount as per the test method. The average % recovery of was calculated. The accuracy results were tabulated in Table4.

Robustness
To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision. The robustness results were furnished in Table 5.

Limit of detection and Limit of quantification
For this study six replicates of the analyte at lowest concentration were measured and quantified. The LOD and LOQ values were given in Table 6.

Assay

The standard solutions and sample solutions were determined at 225 nm and the amount of the drugs present in the tablet dosage form was calculated. The assay results were shown inTable 7.

Degradation Studies

Forced degradation is carried out to produce representative samples for developing stability-indicating methods for drug substances and drug products. The samples were stressed under acid, alkali, peroxide, thermal, photolytic and hydrolytic conditions and percentage degradation was calculated. The degradation results were shown in Table 8.

CONCLUSION

A simple, precise, accurate stability indicating reversed-phase HPLC method has been developed and validated for analysis of Remogliflozin and Teneligliptin in pure and commercial dosage form.. The run time was relatively short, which enables rapid quantitation of many samples in routine and quality control analysis of formulations. The optimized solvent system was used throughout the experimental work and no interference from any excipients was observed. The developed RP-HPLC method was validated as per the ICH guidelines. These results have proven that method could find practical application as a quality-control tool for simultaneous determination of Remogliflozin and Teneligliptin in pharmaceutical dosage forms in quality-control laboratories.

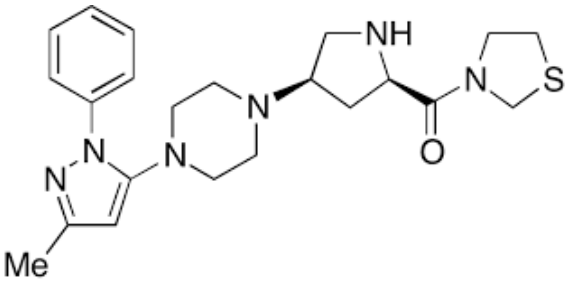

Fig. 1: Structure of Remogliflozin

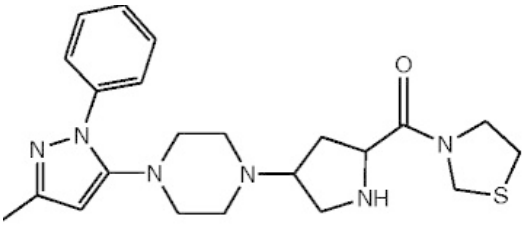

Fig. 2: Structure of Teneligliptin

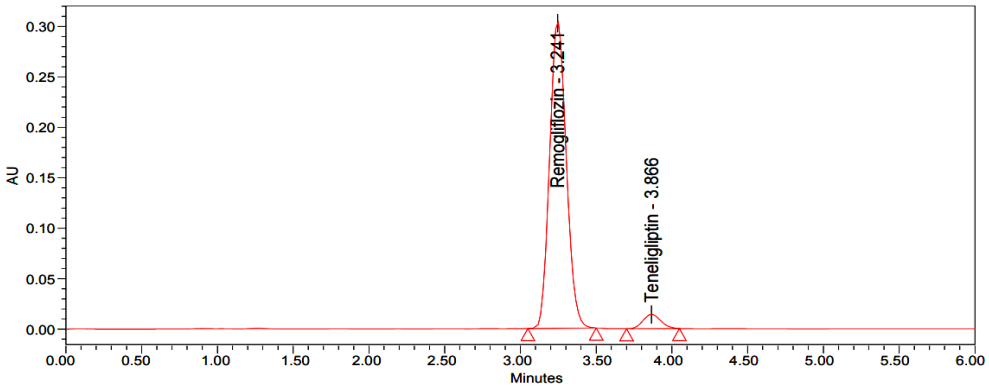

Fig. 3: Optimized Standard Chromatogram

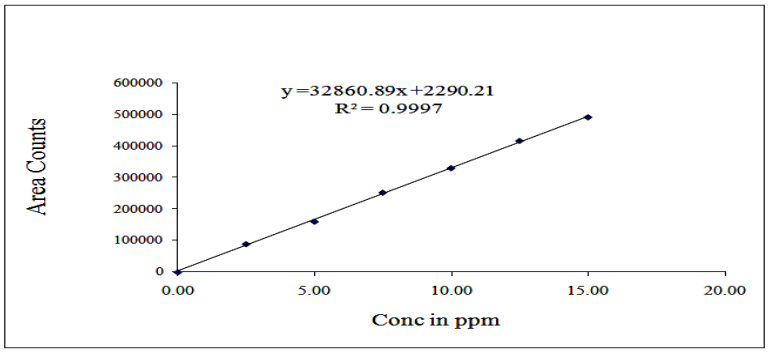

Fig. 4: Linearity curve of Remogliflozin

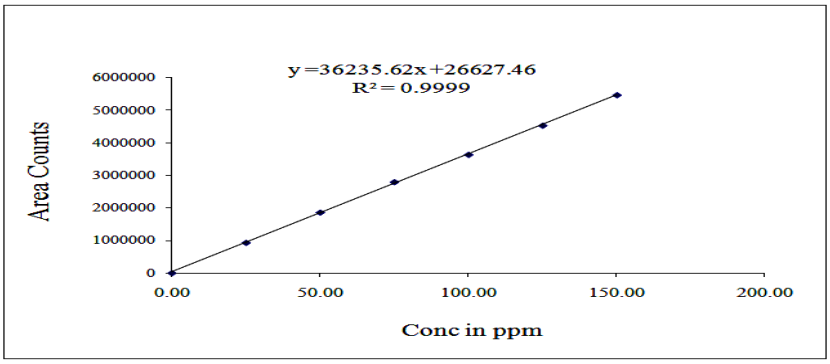

Fig. 5: Linearity curve of Teneligliptin

Table 1: System suitability results

S. No.	Parameter	Remogliflozin	Teneligliptin
1	Retention time	3.241	3.866
2	Plate count	4160	5077
3	Tailing factor	1.14	1.05
4	Resolution	----	2.89
5	%RSD	0.32	0.37

Table 2: Linearity results

S. No.	Remogliflozin		Teneligliptin	
	Conc. (µg/mL)	Peak area	Conc. (µg/mL)	Peak area
1	25	929639	2.5	89046
2	50	1859735	5	160773
3	75	2794721	7.5	252648
4	100	3632381	10	330091
5	125	4529283	12.5	416974
6	150	5464333	15	491696
Slope	36235.62		32860.89	
Intercept	26627.46		2290.21	
R^2	0.9999		0.9997	

Table 3: Results of Precision

S. No	Intra-day		Inter-day	
	Area of Remogliflozin	Area of Teneligliptin	Area of Remogliflozin	Area of Teneligliptin
1	3702261	334747	3652261	334388
2	3654589	331866	3699950	332178
3	3625473	330541	3591334	334878
4	3593675	332729	3606891	331064
5	3670969	331376	3618068	335394
6	3685239	332263	3706522	334768
Mean	3655368	332254	3645838	333778
S.D	40087.318	1435.03	48807.047	1737.783
%RSD	1.1	0.43	1.34	0.52

Table 4: Accuracy results

% Level	Remogliflozin			Teneligliptin		
	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	%Recovery	Amount Spiked	Amount recovered	% Recovery
50	50	49.7	99.4	5	5.03	100.6
100	100	100.82	100.8	10	10.12	101.2
150	150	148.58	99.1	15	14.76	98.4

Table 5: Robustness results

S.No.	Condition	%RSD of Remogliflozin	%RSD of Teneligliptin
1	Flow rate (-) 0.9mL/min	0.81	0.73
2	Flow rate (+) 1.1mL/min	0.43	0.65
3	Mobile phase (-) 60B:40A	0.59	0.73
4	Mobile phase (+) 50B:50A	0.74	0.52
5	Temperature (-) 25°C	0.23	0.92
6	Temperature (+) 35°C	0.67	0.39

Table 6: LOD and LOQ results

Name of drug	LOD(µg/mL)	LOQ(µg/mL)
Remogliflozin	3	10
Teneligliptin	0.3	1

Table 7: Results for Assay

Brand	Drug	Avg sample area (n=5)	Label amount (mg)	Amount found (µg/mL)	% assay
Zeta Plus R	Remogliflozin	3641257	100	99.99	99.99
	Teneligliptin	331264	10	10.03	100.3

Table 8: Degradation results

Type of Degradation	Remogliflozin		Teneligliptin	
	Area	% Degradation	Area	% Degradation
Acid	3055265	16.1	287097	13.5
Alkali	3099838	14.9	293199	11.6
Peroxide	3014515	17.2	285207	14.0
Thermal	3597236	1.2	329271	0.8
Photolytic	3639135	1.6	331891	0.7

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