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DEVELOPMENT & VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF REMOGLIFLOZIN & TENELIGLIPTIN IN THE PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A rapid, simple, precise & accurate RP-HPLC method was developed & validated for simultaneous estimation of Remogliflozin & Teneligliptin in the pharmaceutical dosage form. The separation of the components was done by C18 column with optimized mobile phase at flow rate of 1.0 ml/min & detected at 229 nm. The retention times of remogliflozin & teneligliptin were 2.205 min & 3.666 minutes respectively. The method was validated as per ICH guidelines which showed good system suitability with USP plate counts of 6600 & 6765, tailing factors of 1.52 & 1.70. The %RSD values were found to be below 2% for precision studies. The LOD & LOQ values were found to be 0.0508 µg/ml & 0.1693 µg/ml for remogliflozin & 0.0208 µg/mL & 0.0695 µg/mL for teneligliptin. The developed method was proved to be sensitive, robust & suitable for routine quality control analysis of combined dosage form.

INTRODUCTION:

Diabetes mellitus is a long term metabolic condition that affects the glucose level in the bloodstream, this condition arises when the body either fails to produce an adequate amount of insulin or the insulin produced is not utilized effectively. [9,10]. Combination therapy has been popular in the treatment of type 2 diabetes mellitus due to enhanced glucose control & fewer adverse effects [10,11]. Remogliflozin belongs to a new class of drugs called SGLT2 inhibitors which treats type 2 diabetes mellitus. It decreases the blood glucose level by inhibiting renal glucose reabsorption & promoting urinary glucose excretion [9,13]. Teneligliptin belongs to a new class of drugs known as DPP-4 inhibitors, which can improve the action of incretin hormones, which stimulate insulin production and reduce glucagon production [10,11].

Remogliflozin & Teneligliptin is widely used for treating diabetic patients with a view to enhancing glycaemic control [1,6,8]. There is a necessity to establish an analytical technique capable of the simultaneous estimation of these drugs in pharmaceutical dosage forms [1,4,5,7]. Various analytical techniques have been documented for the estimation of Remogliflozin & Teneligliptin both individually & in combination, utilizing the RP-HPLC technique [1, 3, 6, 8]. However, there remains a requirement for a simple, rapid, cost-effective & precise RP-HPLC method for routine quality control analysis. Therefore, the present study aims to

develop & evaluate a method for the simultaneous measurement of Remogliflozin & Teneligliptin using RP-HPLC, in accordance with ICH guidelines.

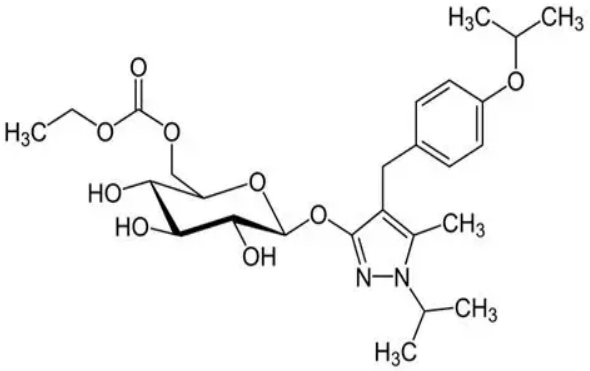

Figure 1 Structure of Remogliflozin

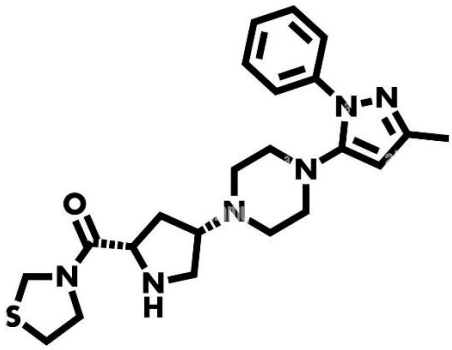

Figure 2 Structure of Teneligliptin

EXPERIMENTAL PROCEDURE

Chemicals & reagents:

Teneligliptin & remogliflozin were acquired as a gift sample from Hetero Laboratory, Hyderabad. HPLC grade Methanol, Acetonitrile, Water & Ammonium Acetate was purchased from Fisher Scientific (India).

Instrumentation:

The development & validation of the method were performed using a WATERS HPLC, specifically the 2695 SYSTEM model, which was equipped with a photodiode array detector. This system also included an automatic sample injector & Empower 2 software.

Chromatographic Conditions:

A C18 column with an optimized mobile phase made of buffer & organic solvent was used to perform chromatographic separation. Analysis was conducted under the following detection wavelength: 229 nm & flow rate: 1.0 ml/min. A 20 µl sample injection volume was employed & the method's overall run time was kept at 6 minutes.

Mobile phase preparation:

A mobile phase was made by mixing HPLC grade acetonitrile & 0.01M ammonium acetate buffer in a ratio of 65:35 v/v. The ammonium acetate buffer was prepared by accurately weighing 0.77 g of ammonium acetate & dissolving it in about 700 ml of HPLC grade water in a 1000 ml volumetric flask. The solution was sonicated until it was completely dissolved and the final volume was made up to 1000 ml with HPLC grade water. A filter (0.45 µm) & sonicator are used to filter & degas the prepared buffer. The buffer & acetonitrile were then completely mixed, re-filtered through 0.45 µm membrane filter & sonicated for 10 minutes before use in the RP-HPLC analysis.

Preparation of the Standard Solution:

Remogliflozin & Teneligliptin primary stock solution (1000 µg/ml & 100 µg/ml respectively) was prepared by dissolving 100 mg of Remogliflozin & 10 mg of Teneligliptin with diluent in 100 ml volumetric flask followed by sonication for 10-15 minutes. The solutions were labelled as standard stock solutions & the volume is then adjusted to the mark with the same diluent.

Preparation of the working standard solutions:

The working standard solutions were prepared by diluting the primary stock solution to obtain concentration ranges of Remogliflozin (50 – 150µg/ml)
& Teneligliptin (5 – 15µg/ml).

Preparation of the sample solution:

Twenty tablets were carefully weighed & ground to a fine powder. This powder was placed in a 100 ml volumetric flask, corresponding to 100mg of Remogliflozin & 10mg of Teneligliptin. About 70ml of diluent was added & the mixture sonicated for 15 minutes, shaking occasionally. The solution was cooled & then diluted to the mark. The final solution was then filtered with 0.45 µm membrane filter.

RESULTS & DISCUSSION:

Method Validation:

The developed RP-HPLC method was validated according to ICH guidelines for : robustness, accuracy, precision, linearity, system suitability, LOD & LOQ.

SYSTEM SUITABILITY:

The system suitability results showed that the developed RP-HPLC method was totally satisfactory in terms of satisfactory retention time, plate count, tailing factor & resolution values. The resolution value was 9.87 which implied that the method could separate the peaks of Remogliflozin & Teneligliptin well without any interference.

LINEARITY:

Linearity was assessed using standard stock solutions of teneligliptin & remogliflozin in the range of 5–15µg/ml and 50–150µg/ml, respectively. Concentration vs. peak area relationships were plotted and linear regression analysis was performed. The method was found to be suitable for quantitative analysis, as both analytes showed good linearity with r^2 values > 0.999, in line with ICH guidelines.

ACCURACY:

The accuracy evaluation showed good recovery rate of Teneligliptin & Remogliflozin at 50%, 100% & 150% concentration levels. The percentage recoveries obtained were within the acceptable range, which indicates that the developed RP-HPLC method is accurate, reliable & can be used for routine quantitative analysis.

PRECISION

The %RSD values of the peak areas of Remogliflozin & Teneligliptin obtained from 6 replicate injections were found to be in acceptable range which indicates that the RP-HPLC method has good system precision.

LOD&LOQ:

The Limit of Detection (LOD) & Limit of Quantification (LOQ) was determined by application of the following formulas under the guidelines of ICH:

$$LOD = \frac{3.3 \times \sigma}{S}$$

$$LOQ = \frac{10 \times \sigma}{S}$$

Where:

- σ = Standard deviation of the response
- S = Slope of the calibration curve

The limit of detection & limit of quantification of Remogliflozin are 0.0508 µg/ml & 0.1693 µg/ml & of Teneligliptin are 0.0208 µg/ml & 0.0695 µg/ml respectively.

ROBUSTNESS:

The robustness of the developed RP-HPLC method was checked with the drug solutions of 100µg/ml of Remogliflozin & 10 µg/ml of Teneligliptin. Chromatographic parameters were deliberately changed such as: ± 0.1 mL/min change in flow rate, ± 5% change in mobile phase composition & ± 2 nm change in detection wavelength. The effects of these changes on the following were investigated: retention time, peak area, plate count, the tailing factor & %RSD. The %RSD values obtained were less than 2%, and there were no significant changes observed in the chromatographic performance, showing that the method is robust, stable & reliable for routine pharmaceutical analysis.

FORCED DEGRADATION STUDIES:

Forced degradation data revealed that both Teneligliptin & Remogliflozin were affected by acid, base, peroxide, heat, sunshine & water degradation. Acid & heat stress conditions, the most critical, resulted in the highest amount of degradation while the water degradation studies resulted in minimal degradation, suggesting good stability in hydrolytic conditions. The percentage recovery obtained under all the stress conditions were in the acceptable limits which suggest that the developed RP-HPLC method is suitable as a stability indicating analytical method for both medicines even in the presence of the degradation products.

ASSAY RESULTS:

6 replicates analysis of standard & sample solutions was done to evaluate the assay performance of Remogliflozin & Teneligliptin. The purity of Remogliflozin was 99.26% & Teneligliptin was 98.69% indicating both drugs were of good quality in their dosage form. The present finding shows that the proposed RP-HPLC technology could accurately & reliably quantify both the medicines together in combination dose forms.

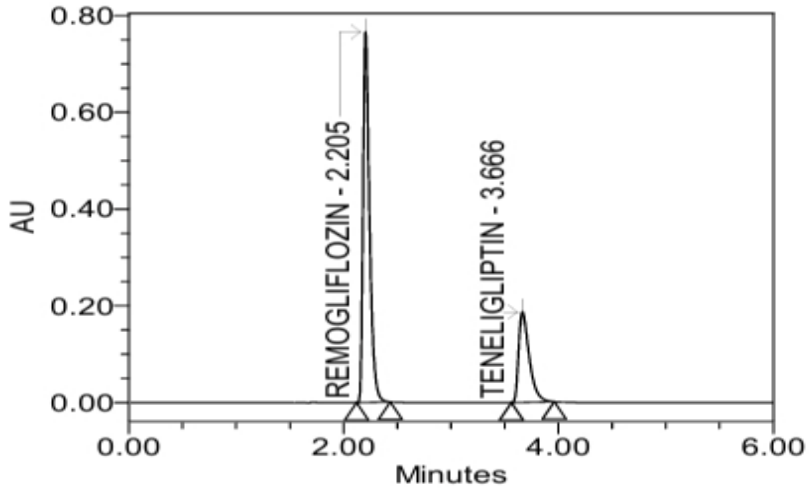

Figure 3 Optimized RP-HPLC Chromatogram

Table 1 Peak Characteristics of Remogliflozin & Teneligliptin

	Peak Name	Retention time	Peak Area	USP plate count	USP tailing
1	REMOGLIFLOZIN	2.205	3209695	6600	1.52
2	TENELIGLIPTIN	3.666	1285253	6765	1.70

Table 2 System Suitability parameters of Remogliflozin & Teneligliptin

	REMOGLIFLOZIN			TENELIGLIPTIN			
Inj	Retention time(min)	USP Plate Count	Tailing Factor	Retention time (min)	USP Plate Count	Tailing Factor	Resolution
1	2.204	6589	1.51	3.664	6752	1.69	9.86
2	2.205	6605	1.52	3.666	6765	1.70	9.87
3	2.205	6612	1.52	3.666	6768	1.70	9.88
4	2.206	6598	1.53	3.667	6772	1.71	9.87
5	2.206	6608	1.52	3.666	6764	1.70	9.86
6	2.207	6610	1.52	3.668	6775	1.71	9.88

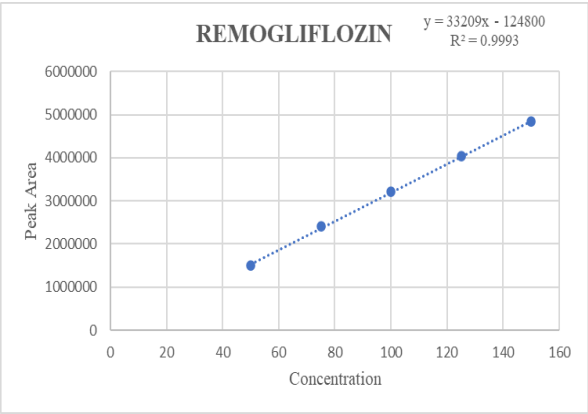

Figure 4 Calibration Curve of Remogliflozin

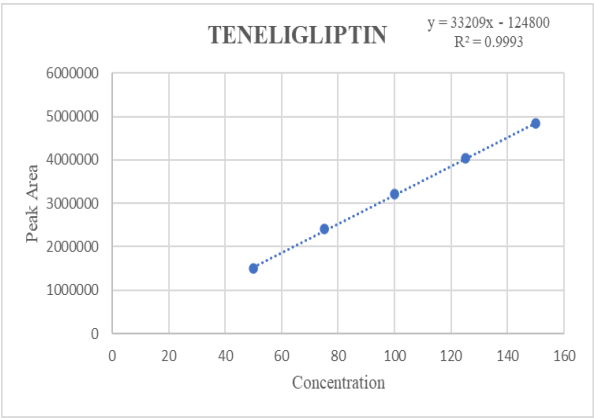

Figure 5 Calibration Curve of Teneligliptin

Table 3 Recovery data of Teneligliptin & Remogliflozin

% Level	Teneligliptin			Remogliflozin		
	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery
50%	4.950	4.96	100	49.500	49.31	100
50%	4.950	4.97	100	49.500	49.11	99
50%	4.950	4.98	101	49.500	49.33	100
Mean			**100**			**99**
100%	9.900	9.83	99	99.000	98.96	100
100%	9.900	9.83	99	99.000	99.41	100
100%	9.900	9.90	100	99.000	99.23	100
Mean			**100**			**100**
150%	14.850	14.89	100	148.500	149.29	101
150%	14.850	14.96	101	148.500	148.93	100
150%	14.850	14.98	101	148.500	149.07	100
Mean			**101**			**100**

Table 4 Precision data of Remogliflozin & Teneligliptin

S.No	Area of Remogliflozin	Area of Teneligliptin
1	3216179	1276193
2	3215428	1279303
3	3219570	1275251
4	3227568	1273380
5	3215407	1288263
6	3228164	1286457
Mean	3220386	1279141
S.D	5766.8	6516.2
%RSD	0.18%	0.48%

Table 5 Forced degradation data of Teneligliptin & Remogliflozin

Type of Degradation	Teneligliptin Area	% Recovered	% Degraded	Remogliflozin Area	% Recovered	% Degraded
Acid	1168237	90.08	9.92	2858084	88.09	11.91
Base	1188810	91.67	8.33	2990001	92.16	7.84
Peroxide	1241887	95.76	4.24	3050223	94.01	5.99
Thermal	1150260	88.70	11.30	2920643	90.02	9.98
UV/Sunlight	1213117	93.54	6.46	3100782	95.57	4.43
Water	1279574	98.67	1.33	3207111	98.85	1.15

Table 6 Assay data of Remogliflozin & Teneligliptin

S.No	Remogliflozin Std Area	Sample Area	% Assay	Teneligliptin Std Area	Sample Area	% Assay
1	3234793	3216179	99.13	1291658	1276193	98.41
2	3234793	3215428	99.10	1291658	1279303	98.65
3	3234793	3219570	99.23	1291658	1275251	98.33
4	3234793	3227568	99.48	1291658	1273380	98.19
5	3234793	3215407	99.10	1291658	1288263	99.34
6	3234793	3228164	99.50	1291658	1286457	99.20
Average	3234793	3220386	99.26	1291658	1279141	98.69
S.D	—		0.18	—		0.48
%RSD	—		0.19	—		0.48

CONCLUSION:

An effective RP-HPLC method has been successfully developed & validated for simultaneous quantification of Remogliflozin & Teneligliptin in pharmaceutical dosage forms. This technique had excellent linear range, accuracy, precision, robustness & sensitivity, which are all in compliance with International Council for Harmonisation (ICH). System suitability parameters were found to be within acceptable limits with excellent peak symmetry & resolution. Forced degradation studies were performed to confirm that RP-HPLC method was able to separate the API from degradation products under different stress conditions. Hence, the developed RP-HPLC method is considered as reliable, cost effective and suitable for routine quality control & stability study of the combined dosage form of Remogliflozin and Teneligliptin.

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