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Research Article

Analytical Method Development and Validation for the Simultaneous Estimation of Teneligliptin and Metformin by RP-HPLC Method

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ABSTRACT

A new method was established for simultaneous estimation of Teneligliptin and Metformin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Teneligliptin and Metformin by using X BRIDGE C18 column (4.6×50mm)3.7µ, flow rate was 1ml/min, mobile phase ratio was (40:20:40 v/v) methanol : Acetonitrile : phosphate buffer($KH_2PO_4$and K_2HPO_4)phosphate pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 290nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.191 mins and 3.868 mins. The % purity of Teneligliptin and Metformin was found to be 104.4% and 103.39% respectively. The system suitability parameters for Teneligliptin and Metformin such as theoretical plates and tailing factor were found to be 993, 1.23 and 5775, 1.12, the resolution was found to be 10.18. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Teneligliptin and Metformin was found in concentration range of 50µg-250µg and 5µg-25µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.1 and 1.4, % RSD for intermediate precision analyst 1 was 0.5 and 0.6 and intermediate precision analyst 2 was 0.4 and 0.4 respectively. The precision study was precision, robustness and repeatabilty. LOD value was 0.39 and 0.7 and LOQ value was 1.18 and 2.12 respectively.

Keywords: X BRIDGE C18 column, Teneligliptin and Metformin, RP-HPLC, Methanol.

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CONTENTS

1. Introduction

Teneligliptin has been investigated for the treatment of Type 2 Diabetes Mellitus. Metformin is a biguanide anti-hyperglycemic agent and first-line pharmacotherapy used in the management of type II diabetes.

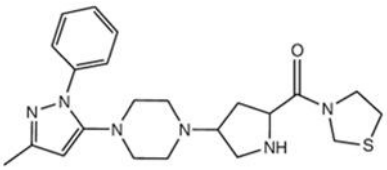

Fig.1.Teneligliptin

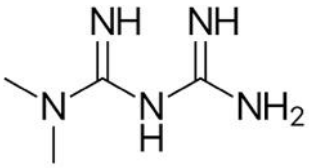

Fig.2.Metformin

2. Methodology

The instrument used was HPLC-auto sampler –UV detector Separation module2695, UV. Detector2487, Software - Empower-software version-2. The stationary phase used was Inertsil (250×4.6mm, 5µ) ODS C-18 RP-column Digital weighing balance-Model number BSA224SCW (Ascoset), Sonicator (Enertech)-SE60US, pH meter Model number AD102U.

Materials and reagents

Teneligliptin and Metformin were gift samples provided by Hetero Laboratories, Hyderabad, Ortho phosphoric acid, Potassiumdihydrogen, Methanol and Water for HPLC were supplied by Merck India Ltd, Mumbai.

Method development

Five trials were made by changing the mobile phase ratios and solvents MeOH: H_2O (60:40%v/v) ACN: Methanol (40:60%v/v) ACN: pH 4 buffer (70:30% v/v) ACN: pH 3 buffer (65:35% v/v) Methanol: Sodium acetate buffer (70: 30 % v/v). Finally, the mobile phase was optimized to Methanol: Sodium acetate buffer (70: 30 % v/v).

Chromatographic conditions

The chromatographic conditions were successfully developed for the separation of Teneligliptin and Metformin by using X BRIDGE C18 column (4.6×50mm) 3.7µ, flow rate was 1ml/min, mobile phase ratio was (40:20:40 v/v) methanol: Acetonitrile: phosphate buffer (KH_2PO_4 and K_2HPO_4) phosphate pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 290nm.

3. Results and Discussion

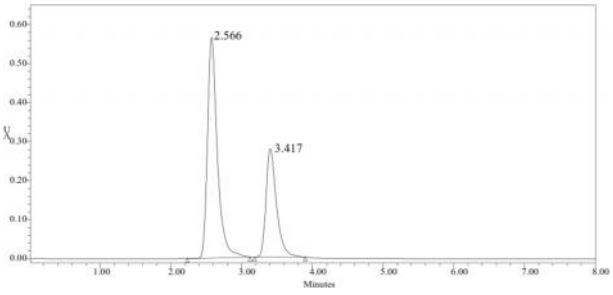

Figure 3 Chromatogram showing standard injection

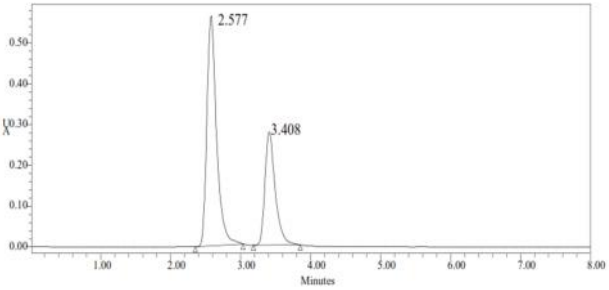

Figure 4 Chromatogram showing sample injection

Table 1 Linearity Results for Teneligliptin

S.No	Linearity Level	Concentration	Area
1	I	5 ppm	471543
2	II	10 ppm	656277
3	III	15 ppm	794999
4	IV	20 ppm	946124
5	V	25 ppm	1002139
Correlation Coefficient			0.999

Table 2 Linearity Results for Metformin

S.No	Linearity Level	Concentration	Area
1	I	5 ppm	441543
2	II	10 ppm	6562771
3	III	15 ppm	794899
4	IV	20 ppm	946134
5	V	25 ppm	1012139
Correlation Coefficient			0.999

Table 3 Showing accuracy results for Teneligliptin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	2630409	5	4.96	99.91%	99.56%
100%	5277055	10	9.98	99.18%	
150%	7514836	15	15.02	99.60%	

Table 4 Showing accuracy results for Metformin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1366666	0.5	0.99	99.53%	99.47%
100%	2777487	1.0	1.05	99.38%	
150%	4151234	1.5	1.495	99.52%	

Table 5 Showing% RSD results for Teneligliptin

Peak Name: Teneligliptin

	Peak Name	RT	Area	Height (µV) 1
	Teneligliptin	2.755	5223559	541538.3
2	Teneligliptin	2.687	5208511	485548.5
3	Teneligliptin	2.632	5323569	574440.4
4	Teneligliptin	2.612	5259147	557413.5
5	Teneligliptin	2.616	5273463	565020.1
Mean			5257650	
Std. Dev.			45206.4	
% RSD			0.86	

Table 6 Showing %RSD results for Metformin

Peak Name: metformin

	Peak Name	RT	Area	Height (µV) 1
	metformin	3.616	2742453	238643.4
2	metformin	3.634	2762750	271543.5
3	metformin	3.460	2797670	281711.6
4	metformin	3.446	2793578	274499.8
5	metformin	3.437	2778483	276713.0
Mean			2774987	
Std. Dev.			22806.9	
% RSD			0.82	

Table 7 Showing results for intermediate precision of Teneligliptin

Peak Name: teneligliptin

	Peak Name	RT	Area	Height (µV)
1	teneligliptin	2.756	5698542	539568.1
2	teneligliptin	2.688	5682534	536985.4
3	teneligliptin	2.633	5695846	539584.1
4	teneligliptin	2.613	5689452	534569.8
5	teneligliptin	2.617	5636591	534985.5
Mean			5600593	
Std. Dev.			203577.3	
% RSD			0.44	

Table 8 Showing results for intermediate precision of Metformin

Peak Name: metformin

	Peak Name	RT	Area	Height (µV) 1
	metformin	3.617	2624315	231325.6
2	metformin	3.635	2623598	231315.4
3	metformin	3.461	2623541	231250.1
4	metformin	3.447	2624987	231342.6
5	metformin	3.438	2635698	231765.2
Mean			2626428	
Std. Dev.			5215.78	
% RSD			0.19	

Table 9 Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Teneligliptin	373625.50	581075863	3.17
Metformin	5772.40	476579210	0.0172

Table 10 Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Teneligliptin	372727.80	574265980	5.80
Metformin	5761.30	478828490	0.212

Table 11 Showing system suitability results for Teneligliptin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5339	1.4
2	**1**	**4668**	**1.3**
3	1.2	5216	1.4

Table 12 Showing system suitability results for Metformin

S.No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	7036	1.3
2	**1**	**6089**	**1.2**
3	1.2	6998	1. 3

Table 13 Showing system suitability results for Teneligliptin

S.No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	6232	1.4
2	***Actual**	**4668**	**1.3**
3	5 % more	6387	1.4

Table 14 Showing system suitability results for Metformin

S.No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5437	1.3
2	***Actual**	**6089**	**1.2**
3	5 % more	4817	1.2

4. Conclusion

A new method was established for simultaneous estimation of Teneligliptin and Metformin by RP-HPLC method. The chromatographic conditions were success fully developed for the separation of Teneligliptin and Metformin by using ThermosilC18 column (4.0×125mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Sodium acetate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 252nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2690, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.566 mins and 3.417 mins. The % purity of Teneligliptin and Metformin was found to be 101.27% and 99.97% respectively. The system suitability parameters for Teneligliptin and Metformin such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Teneligliptin and Metformin was found in concentration range of 5µg-25µg and 50µg-250µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.44 and 0.19 respectively. The precision study was precise, robust, and repeatable. LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of

Teneligliptin and Metformin in API and Pharmaceutical dosage form.

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