Research Article

Development and Validation of RP-HPLC Method for Estimation of Teneligliptin and its Impurity in Tablet

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ABSTRACT

The objective of the study is a simple, precise and accurate stability RP-HPLC method has been developed and subsequently validated for the estimation of Teneligliptin and its impurity in tablet formulation. The adequate separation was carried out using Grace Smart C18 column (250mm x 4.6mm, 5µm particle size), mixture of 0.05M Potassium dihydrogen phosphate PH 4.0 and Acetonitrile 80:20 % v/v as a mobile phase with a flow rate of 1 ml/min and the effluent was monitored at 242 nm using PDA detector. The retention time of Teneligliptin, Impurity B and Impurity G were 7.443 min, 6.650 min and 8.473 min respectively. Linearity for Teneligliptin, Impurity B and Impurity G were found in the range of 500-3000 µg/ml (R2 = 0.998), 5-15 µg/ml (R2 = 0.994) and 5-15 µg/ml (R2 = 0.998) respectively. The accuracy of the present method was evaluated at 50%, 100% and 150%. The % recoveries of drug were found to be in range of 99.315 ± 0.283 for Teneligliptin. Precision studies were carried out and the RSD values were less than two. The method was found to be robust. The proposed method was found to be specific, accurate, precise and robust can be used for simultaneous estimation of these drugs in tablet dosage form.

Keywords: Teneligliptin, Impurity B, Impurity G, Reversed phase HPLC, Validation.

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INTRODUCTION

Teneligliptin (TEN) is designated chemically as [(2S,4S)-4-[4-(5-methyl-2-phenylpyrazol-3-yl)piperazin-1-yl]-(1,3-thiazolidin-3-yl) methanone (Figure-1), represents the class of Thiazoles, DDP-4 Receptor Blocker, reduce the glucose level in blood, used in treatment of type-2 diabetes mellitus[1-2]. Various analytical methods have been reported for the estimation of Teneligliptin as alone as well as in combination with other drugs. They include spectrophotometric methods HPLC[3-6], HPTLC[7-8], Ultra-fast liquid chromatography[9], UV Spectroscopy stability indicating UPLC method[13-14].

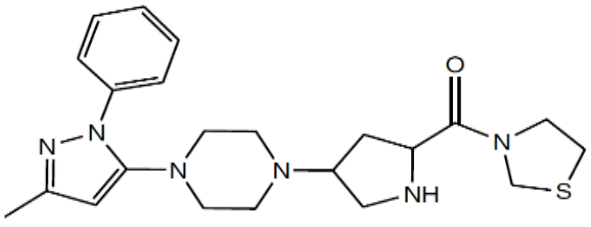

Figure 1: Chemical Structure of Teneligliptin

Teneligliptin impurity B is designated chemically as tert-butyl (2S,4S)-4-(4-(3-methyl-1-phenyl-1H-pyrazol-5-yl) piperazin-1-yl)-2-(thiazolidine-3-carbonyl) pyrrolidine-1-carboxylate (Figure-2)[15].

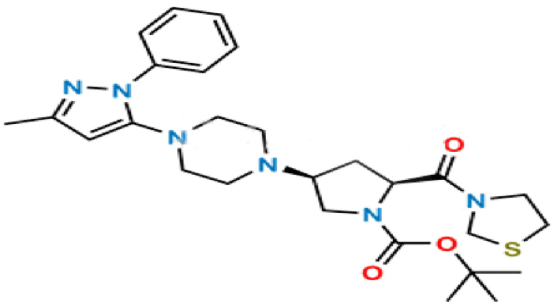

Figure 2: Chemical Structure of Teneligliptin impurity B

Teneligliptin impurity G is designated chemically as 1-(4-((3S,5S)-5-(thiazolidine-3-carbonyl) pyrrolidin-3-yl) piperazin-1-yl) butane-1,3-dione (Figure-3)[16].

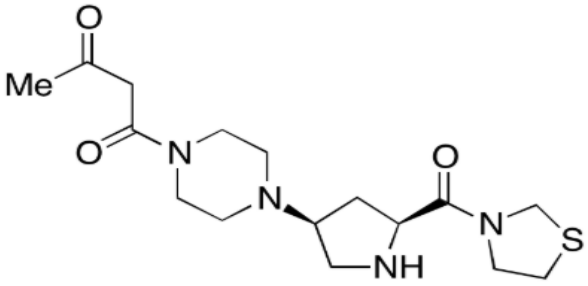

Figure 3: Chemical Structure of Teneligliptin impurity G

However, an extensive literature search didn't reveal any estimation method for both the drugs in their combined dosage form. Therefore, attempt was made to develop and validate simple, precise, and accurate RP-HPLC method for simultaneous determination of drug and its impurity in tablet dose form.

MATERIALS AND METHODS

Apparatus

Young lin HPLC system was used for method development and validation. Data acquisition was performed on YL 9100 HPLC software. The separation were achieved on Grace Smart C_{18} (250 × 4.6 mm, 5µm) column. Digital balance (SartoriousCP224S, Sensitivity: 0.1mg), Ultrasonic cleaner (PCi, 1.5L, 5H), pH meter (Systonic) and Pipettes and volumetric flask (Borosil) used during study.

Reagents and Materials

Teneligliptin dosage form tablets were purchased from local market. HPLC grade Acetonitrile, Water, Methanol and Potassium Dihydrogen Phosphate of analytical grade were obtained from SD Fine Chem Ltd.

Chromatographic Conditions

The column was maintained at room temperature and the eluent was monitored at 242 nm using PDA detector. The mixture of 0.05M Potassium dihydrogen phosphate PH 4.0 and Acetonitrile in proportion of 80:20 % v/v at a flow rate of 1.0 ml/min was used as a mobile phase. The injection volume was 20µl.

Preparation of stock Standard solution (Teneligliptin 10000 µg/ml, Impurity B 20 µg/ml and Impurity G 20 µg/ml).

An accurately weighed quantity of standard Teneligliptin (1000 mg), Impurity B (2 mg) and Impurity G (2 mg) Were transferred to 100 ml volumetric flasks and volumes were made up to mark with mobile phase individually.

Preparation of Mobile phase: (0.05 M KH_2PO_4 pH-4 Adjusted with 1%o-phosphoric acid: Acetonitrile (80:20 %V/V).

An accurately weighed 0.68 gm of potassium dihydrogen phosphate was transferred into 100ml volumetric flask, followed by addition of 95ml HPLC grade water, pH 4 was adjusted with 1% o-phosphoric acid, volume was made up to mark with HPLC grade water. Above solution filtered with vacuum filter using filter membrane. 80ml of buffer and 20ml Acetonitrile was mixed and solution was sonicated for degassing.

TEN, Impurity B and Impurity G Working Standard Solutions.

2 ml of standard stock solution of Teneligliptin (2000 µg/ml), 1 ml standard stock solution of Teneligliptin impurity B (20 µg/ml), and 1 ml standard stock solution of Teneligliptin impurity G (20 µg/ml) were transferred in to 10 ml volumetric flask and volume made up to the mark with methanol and mixed thoroughly.

Preparation of Sample Solution

Average weight of 20 tablets was determined and tablets were crushed into powder form. Accurately weighed amount of powder equivalent to 200 mg of teneligliptin was transferred into 100 ml volumetric flask. About 60 ml of methanol was added and solution was sonicated for 30 min. to ensure complete solubilization of drugs. Then solution was filtered through Whatman filter paper and then volume was made up to the mark with methanol (2000 µg/ml).

System suitability parameters

System suitability tests were performed to verify that the resolution and repeatability of the system were adequate for the analysis intended. The parameters monitored for system suitability includes retention time, theoretical plate number, peak area, tailing factor and resolution. The repeatability of these parameters was checked by injecting three times the test solution of 2000 µg/ml TEN, 20 µg/ml impurity B and 20 µg/ml impurity G. The results shown in Table 1 were within acceptable limits.

Method Validation[17]

Specificity

Specificity of method can be termed as absence of any interference at retention times of samples. Specificity was performed by injecting blank and standard preparations. Chromatograms were recorded and retention times from sample and standard preparations were compared for identification of analytes.

Calibration curve (Linearity)

A series of standard solutions 500-3000 µg/ml of TEN and 5-30 µg/ml of both impurities were prepared. An aliquot of 20µl of each solution was injected 3 times for each standard solutions and peak area was observed. Plot of average peak area versus the concentration is plotted and from this the correlation coefficient and regression equation were generated. The calibration data of TEN and both impurities is given in Table 3, while Figure 5, Figure 6 and Figure 7 represents linearity graphs of both drugs respectively.

Accuracy (% Recovery)

Accuracy was determined by calculating recovery of TEN and both impurities by the standard addition method. Known amounts of standard solutions of TEN (250, 500 and 750 µg/ml) were added to a pre quantified test solution of TEN (2000µg/ml). Each solution was injected in triplicate and the recovery was calculated by measuring peak areas. Results obtained are shown in Table 4.

Method Precision

The method was validated in terms of intra-day inter-day precision. The solution containing 2000µg/ml of TEN, 20 µg/ml of imp. B and 20µg/ml of imp. G was injected six times for repeatability study. Inter-day and Intra-day study were performed by injecting 1500, 2000 and 2500 µg/ml

of TEN and 15, 20, 25, µg/ml of both impurity solutions three times for each aliquot. The %RSD for precision study was found less than 2% as shown in Table 5.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations as per International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where σ = the standard deviation of the response and S = Slope of calibration curve.

Robustness

Robustness was carried by varying three parameters from the optimized chromatographic conditions. No significant change was observed.

Analysis Teneligliptin in tablet Dosage Forms

Pharmaceutical formulation of Teneligliptin in tablet dosage form was purchased from local pharmacy. The responses of tablet dosage form was measured at 242 nm for quantification of TEN by using RP-HPLC. The amounts of TEN present in sample solution were determined by the responses into the regression equation for TEN in the method. Results are given in Table 7.

RESULT AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of 0.05M Potassium dihydrogen phosphate pH 4 .0 and Acetonitrile 80:20 % v/v and 1.0 ml/min flow rate proved to be better than the other mixtures in terms of resolution and peak shape. The effluent was monitored at 242 nm using PDA detector. As it was shown in Fig. 3 the retention time of EN, imp B and imp G were 7.443 min, 6.450 min and 6.470 min respectively. The method was validated in terms of linearity, precision, accuracy, specificity, limit of detection and limit of quantification. Linearity of TEN and both impurities were in the range of 500-3000 µg/ml and 5-30 µg/ml respectively. The proposed method enables rapid quantification and simultaneous analysis of both drugs for commercial formulations without any excipients interference. The method can be used for routine analysis of marketed products of TEN in tablet formulation. System suitability test parameters for TEN and both impurity for the RP-HPLC method are reported in Table1. The optical and regression characteristics and validation parameters are reported in Table 2.

Table 1: Results for System suitability parameters

Parameters	TEN (mean ± SD)*	Impurity B (mean ± SD)*	Impurity G (mean ± SD)*
Theoretical plate	7701 ± 45.796	4274 ± 15.307	7675 ± 19.655
Tailing factor	1.245 ± 0.103	1.310 ± 0.034	1.232 ± 0.039
Resolution	2.381 ± 0.046		2.703 ± 0.085
	* = average of three determinations, SD=Standard deviation		

Table 2: Optical and Regression characteristics and validation parameters of HPLC method for analysis of TEN and Impurities

Parameter	TEN	Impurity B	Impurity G
Calibration Range	500-3000 µg/ml	5-30 µg/ml	5-30 µg/ml
Regression Equation	Y = 1.899x + 119.9	Y = 9.613x + 14.953	Y = 7.336x + 9.085
Slope (m)	1.899	9.613	7.336
Intercept (c)	119.9	14.953	9.085
Correlation co-efficient (R^2)	0.9983	0.9942	0.9980
Inter Day (%RSD)	0.063 – 0.116	0.203 – 0.697	0.265 – 0.416
Intra Day (%RSD)	0.052 – 0.114	0.425 – 0.764	0.540 – 0.780
Repeatability (%RSD)	0.327	2.023	1.691
Detection Limit(µg/ml)	9.539 µg/ml	0.410 µg/ml	0.406 µg/ml
Quantitation Limit(µg/ml)	59.210 µg/ml	1.245 µg/ml	1.232 µg/ml

Table 3: Linearity study data for TEN, Impurity B and Impurity G

Sr No	Teneligliptin			Impurity B			Impurity G		
	Conc (µg/ml)	Avg. area * ± SD	%RSD	Conc (µg/ml)	Avg. area * ± SD	%RSD	Conc (µg/ml)	Avg. area * ± SD	%RSD
1	500	1100.379 ± 11.115	0.998	5	71.195 ± 0.965	1.337	5	98.770 ± 0.964	1.973
2	1000	1990.491 ± 0.246	0.010	10	104.631 ± 1.133	1.072	10	79.185 ± 1.112	1.401
3	1500	2970.993 ± 12.567	0.422	15	156.538 ± 1.062	0.674	15	118.731 ± 0.880	0.736
4	2000	3960.910 ± 26.371	0.664	20	207.295 ± 0.900	0.434	20	157.473 ± 1.219	0.768
5	2500	4740.167 ± 10.046	0.211	25	247.875 ± 2.024	0.814	25	188.475 ± 0.950	0.504
6	3000	5900.652 ± 7.124	0.120	30	311.642 ± 1.101	0.353	30	232.231 ± 1.208	0.517
*= average of three determinations, RSD=Relative standard deviation									

Table 4: Recovery data for TEN and Impurities by HPLC method

Sr. No.	Accuracy Level %	Amount taken (µg/ml)	Amount Added (µg/ml)	Total Amount found* (µg /ml)	% Recovery	% Mean recovery ± S.D.	%R.S.D.
1.	50 %	500	250	248.241	99.296	99.502 ± 0.441	0.444
2.		500	250	248.006	99.202		
3.		500	250	250.023	100.009		
4.	100 %	500	500	501.012	100.202	100.215 ± 0.248	0.247
5.		500	500	502.345	100.469		
6.		500	500	499.873	99.974		
7.	150 %	500	750	751.132	100.150	100.110 ± 0.184	0.186
8.		500	750	749.320	99.909		
9.		500	750	752.034	100.271		
*= average of three determinations							

Table 5: Precision study for TEN and Impurities

Parameters	Conc.			% RSD		
	TEN (µg/ml)	Imp.B (µg/ml)	Imp.G (µg/ml)	TEN	Imp.B	Imp.G
Intra-day* precision	1500	15	15	0.114	0.764	0.780
	2000	20	20	0.023	0.539	0.953
	2500	25	25	0.052	0.425	0.540
Inter-day* precision	1500	15	15	0.063	0.697	0.416
	2000	20	20	0.116	0.635	0.265
	2500	25	25	0.071	0.203	0.337
Repeatability**	2000	20	20	0.327	2.023	1.691
*= average of three determinations; **= average of six determinations						

Table 6: Robustness

Parameter	Change Level	Peak Area		
		TEN	Imp. B	Imp. G
pH (±0.2)	3.8	3934.268	203.879	156.507
	4.0 #	3965.935	207.314	157.566
	4.2	3990.126	209.125	154.950
	Mean ± SD	3980.110 ± 12.620	206.772 ± 2.664	156.341 ± 1.315
	%RSD	0.317	1.288	0.841
Flow Rate (±0.02 ml/min)	0.98 ml/min	3963.615	204.373	154.328
	1.0 ml/min#	3947.135	207.147	156.985
	1.02 ml/min	3974.320	205.845	153.868
	Mean ± SD	3961.690 ± 13.694	205.788 ± 1.387	155.060 ± 1.682
	%RSD	0.345	0.674	1.085
Mobile phase Composition (±2.0 ml)	78:18	3924.985	206.417	158.182
	80:20 #	3938.526	207.814	156.438
	82:22	3968.689	209.312	159.343
	Mean ± SD	3944.067 ± 22.372	207.847 ± 1.447	157.987 ± 1.462
	%RSD	0.567	0.696	0.925
#= actual parameter as control standard				

Table 7: Analysis on marketed formulation

Teneligliptin		
Labelled amount (mg)	**Amount found (mg) (n = 3)**	**% Assay (n =3)**
20 mg	19.798	98.990
	19.889	99.445
	19.902	99.510
Mean ± SD	19.863 ± 0.056	99.315 ± 0.283
% R.S.D.	0.2852	0.285

Table 8: % of Known impurity of Teneligliptin impurity B & Teneligliptin impurity G by Proposed method

% Known impurity							
Impurity	**Area of known impurity in standard Preparation of impurity**	**STD Impurity Concentration (µg/ml)**	**Test Preparation Concentration (µg/ml)**	**Area of Known Impurity Present in Test preparation**	**% of Known impurity**	**Mean± SD**	**% R.S.D.**
Teneligliptin Impurity B	206.196	20	2000	70.080	0.340	0.340± 0.001	0.159
				70.144	0.340		
				69.927	0.339		
Teneligliptin Impurity G	156.593	20	2000	61.069	0.348	0.348± 0.001	0.169
				61.134	0.348		
				60.932	0.347		

Table 9: % of total unknown impurities of Teneligliptin test formulation by Proposed method

% of total unknown impurities						
Area of Teneligliptin Standard preparation	**STD Impurity Concentration (µg/ml)**	**Test Preparation Concentration (µg/ml)**	**Areas of Total Unknown Impurity Present In test preparation**	**% of Total Unknown impurities**	**Mean ± SD**	**% R.S.D.**
3960.910	20	2000	273.534	0.069	0.070 ± 0.002	1.803
			281.124	0.071		
			283.063	0.070		

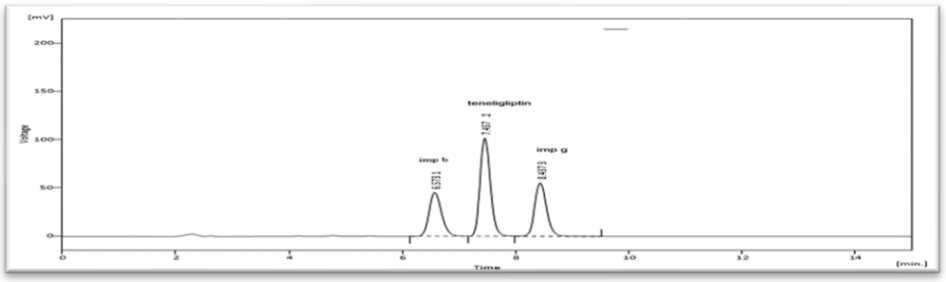

Figure 4: Optimized condition chromatogram of TEN, Imp B, Imp G

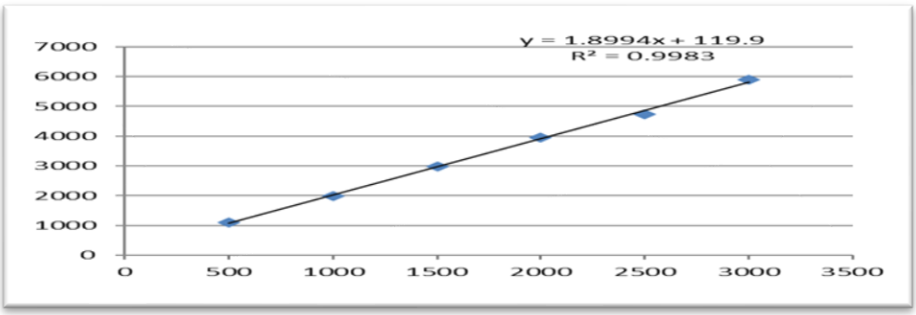

Figure 5: Calibration Curve of Teneligliptin (500-3000 µg/ml)

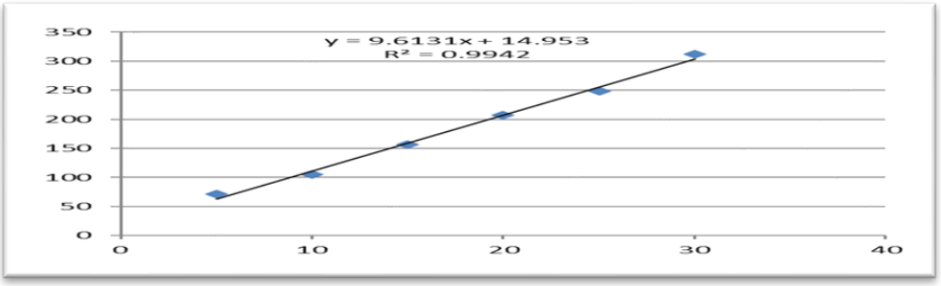

Figure 6: Calibration Curve of Impurity B (5-30 µg/ml)

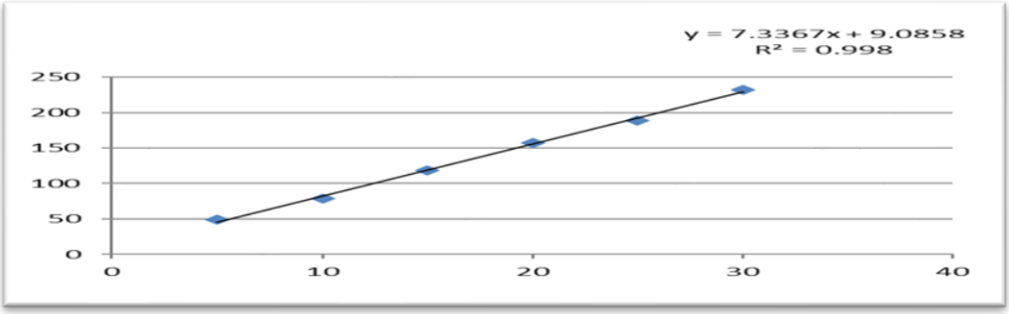

Figure 7: Calibration Curve of Impurity G (5-30 µg/ml)

CONCLUSION

Results for validation parameters are in good agreement with lable claim, which indicates that is no interference of exicipients in routinely used experiment. The proposed method is found to be accurate and precise, therefore proposed method can be used for routine analysis of Teneligliptin and both impurities in tablet dosage form.

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