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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TENILIGLIPTIN IN PHRMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A reverse phase liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of Teneligliptin in Bulk and its pharmaceutical formulation. Separation was achieved with a Zodiac C18 column(250×4.6 mm I.D., 5µm particle size , methanol: acetonitrile (90:10) v/v as mobile phase at a flow rate of 1.0 ml/min , pH 5.2 adjusted with 0.10% orthophosphoric acid and the Column temperature was maintained at 25°C. UV detection was performed at 235 nm and sample temperature was maintained at 5°C with a run time of 10min . The method is simple, rapid, and selective. The described method of Teneligliptin is linear over a range of 25 to 150µg/ml with correlation coefficient of 0.9991 respectively .The method precision for the determination of assay was below 2.0% RSD. The method enables accurate, precise, and rapid analysis of Teneligliptin . It can be conveniently adopted for routine quality control analysis of Bulk and pharmaceutical formulations.

KEYWORDS: Teneligliptin, RP-HPLC, Method validation.

INTRODUCTION

Teneligliptin is chemically known as {(2*S*,4*S*)-4-[4-(3-Methyl-1-phenyl-1*H*-pyrazol-5-yl)-1-piperazinyl] - 2 -pyrrolidinyl}(1,3-thiazolidin-3-yl)methanone and its empirical formula is $C_{17}H_{27}NO_4$, with a molecular mass of 309.40 g/mol. Teneligliptin is a Type-2 diabetis drug.. It is a Dipeptidyl peptidase-4 (DPP-4) inhibitors have recently emerged as a new class of antidiabetic that show favorable results in improving glycemic control with a minimal risk of hypoglycemia and weight gain . The chemical structure was shown in Figure 1a.

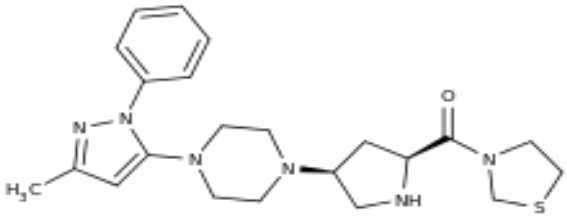

Fig. 1(a) Chemical structure of Teniligliptin

Teneligliptin inhibits human plasma DPP4 activity and recombinant human DPP-4

activity in a concentration-dependent manner with half-maximal inhibitory concentrations (IC_{50}) of 1.75 (95% CI, 1.62–1.89) nmol/L and 0.889 (95% CI, 0.812–0.973) nmol/L, respectively. However few methods could be found for development and validation for determination of assay of Teniligliptin in tablet formulation, attempt being made to develop sensitive, simple, accurate and rugged RP-HPLC method for determination of assay and related substances in film coated tablet formulation.

MATERIALS AND METHODS

Instrumentation

Chromatography was performed with PEAK HPLC, LC7000 UVdetector provided with Hamilton Syringe, auto sampler. All HPLC systems were equipped with a column compartment with temperature control and an on-line degasser. Sample acquisition, analysis and reporting were performed by Hitachi software.

Reagents and Chemicals:

Teneligliptin were procured from Merck Specialities private limited Mumbai. HPLC grade Water, Acetonitrile and water were obtained from Merck. Commercial Pharmaceutical preparations from HETERO PHARMA, which were claimed to contain 20mg was used in analysis.

Chromatographic condition

The isocratic mobile phase consisted of and Methanol and Acetonitrile in the ratio of(90:10) v/v at a flow rate of 1.0 ml/min, Zodiac C18 column(250×4.6 mm I.D., 5µm particle size Column was used as stationary phase. The detection wave length for both the drugs is 235nm.

Preparation of Standard solution:

Accurately weigh and transfer 10 mg of Teneligliptin working standard into a 10mL clean dry volumetric flask add about 7mL of diluents (Methanol and Acetonitrile in the ratio of(90:10) v/v) and sonicate to dissolve it completely and make volume up to the mark with the same solvent(1000µg/ml).From the solution pipette 0.75ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent (75µg/ml).

Preparation of Sample solution:

Accurately weigh and transfer equivalent to 10 mg(30mg) of Teniligliptin into a 10mL clean dry volumetric flask add about 7mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent(1000µg/ml).From the

solution pipette 0.75ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent t(75µg/ml).

METHOD VALIDATION

Linearity Study:

Linearity was performed by taking from stock solution aliquots of 2.5,5,7.5,10, 12.5and 15 ml were taken in10ml volumetric flasks and diluted up to the mark with diluent such that the final concentration of Teneligliptin of 25 to 150µg/ml. Volume of 20µl of each sample was injected in six times for each concentration level and calibration curve was constructed by plotting the peak area(y-axis) versus the drug concentration(x-axis).

Accuracy:

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of Teneligliptin (75µg/ml) were spiked with 50, 100, and 150 % extra teneligliptin standard and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate and recovery of the pure drug and% RSD were calculated.

Precision:

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision expressed as standard deviation or relative standard deviation.

The precision of the method was demonstrated by intra-day and inter-day variation studies.

Intra-day precision

In the intra-day studies, six injections of standard solution (75µg/ml) were injected into the chromatographic system in different time interval within a day and %RSD was calculated.

Inter-day precision:

In the inter-day variation studies, six injections of standard solution solutions Teniligliptin (75µg/ml) injected at different days. % RSD was calculated

Limit of detection:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Limit of quantification:

The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantification limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage**.** The mobile phase was varied by ±5%,wave length ±2,pH ±1.

Ruggedness:

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst. The standard solution was injected for five times and measured the area for all six injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

System suitability parameters:

Suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operation and sample to be analysed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

RESULTS AND DISCUSSION

Method development: The chromatographic conditions (composition of the mobile phase, its pH) were optimized through several trials to achieve the better sensitivity and good symmetric peak shape for Teniligliptin. Different combination ratios of Methanol and acetonitrile were tested. The best chromatographic separation was achieved with a Zodiac C18 column (250×4.6 mm I.D., 5µm particle size , methanol: acetonitrile (90:10) v/v as mobile phase at a flow rate of 1.0 ml/min,pH 5.2 adjusted with 0.10% orthophosphoric acid and the Column temperature was maintained at 25°C. UV detection was performed at 235 nm and sample temperature was maintained at 5°C with a run time of 10min.Under the above

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described chromatographic conditions, Teniligliptin was detected at retention time of 5.843min. The representative chromatogram of Teniligliptin is shown in the figure 2.

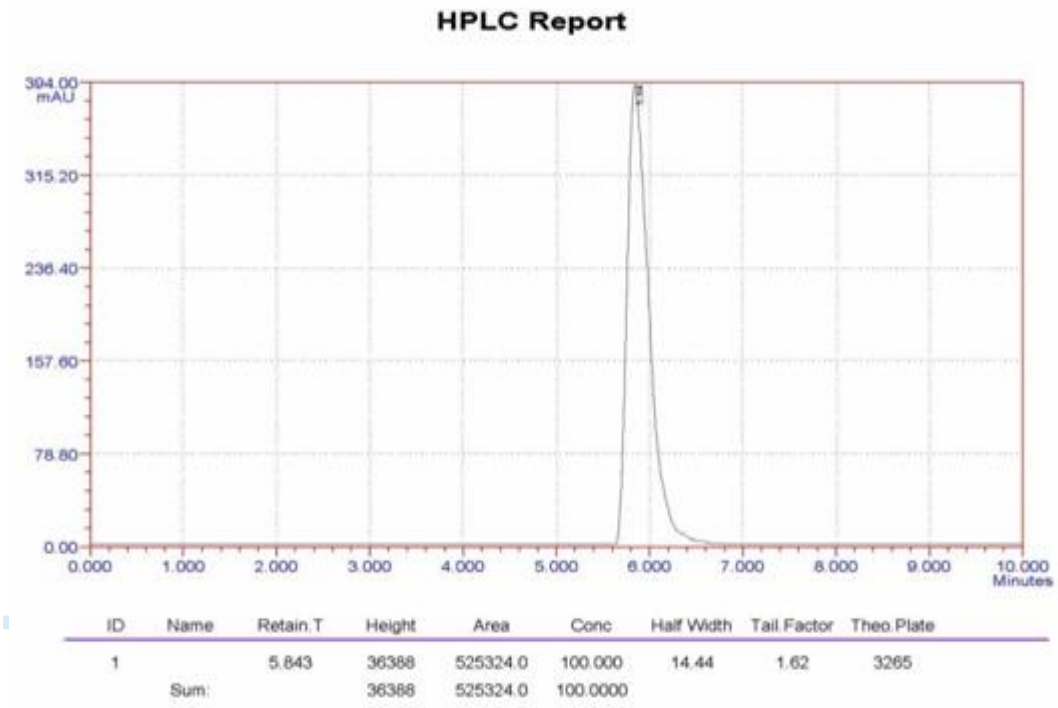
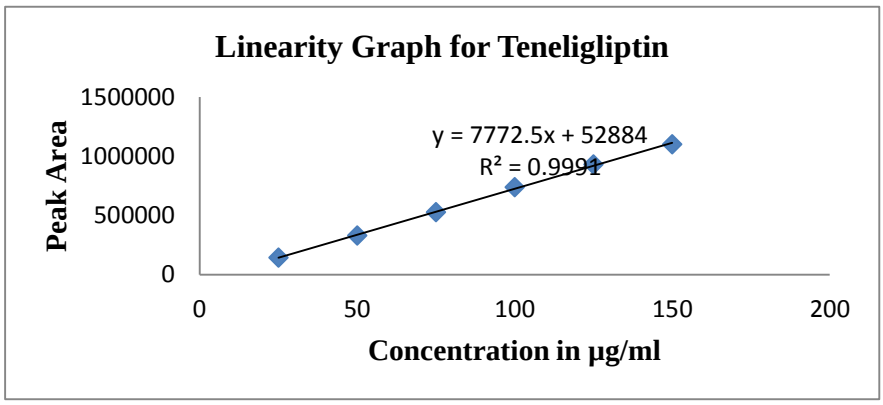


Fig. 2: Chromatogram of Teniligliptin

Method Validation

Linearity: The linearity response for Teniligliptin was between 25 to 150µg/ml (Figure 3) and the linearity were represented by the regression equation as shown below.

$$y = 7772.5x+52884 \ (R^2 = 0.9991)$$



Accuracy: Recovery studies were performed to validate the accuracy of developed method. A define concentration of standard drug solution was added to pre analyzed sample solution and recovery was studied. The results are as shown in the table 1.

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Table No:1 Accuracy data of Teniligliptin Recovery:

% Recovery	Target Conc., (µg/ml)	Spiked conc, (µg/ml)	Final Conc, (µg/ml)	Conc., Obtained	% of Recovery
50%	50	25	75	75.14	100.18
	50	25	75	75.73	100.97
	50	25	75	76.36	101.81
100%	50	50	100	99.82	99.82
	50	50	100	100.57	100.57
	50	50	100	100.51	100.51
150%	50	75	125	126.58	101.26
	50	75	125	126.42	101.13
	50	75	125	125.23	100.18

Precision

Intra-day precision: In the intra-day studies, six injections of standard solution (75µg/ml) were injected into the chromatographic system in different time interval within a day and results were found to be within acceptable limits (RSD <2) as shown in table 2.

Table No:2 For Intraday precision

S. No	Concentration in µg/ml	Peak area
1	75	516314
2	75	518917
3	75	533930
4	75	507525
5	75	520172
6	75	518598
% OF RSD		1.64

Inter-day precision: In the inter-day variation studies, six injections of standard solution solutions Teniligliptin (75µg/ml)injected at different days and results were found to be within acceptable limits (RSD <2) as shown in table 3.

Table No: 3 Interday Precision of Teneligliptin

S. No	Concentration in µg/ml	Peak area
1	75	522704
2	75	529617
3	75	531973
4	75	536162
5	75	517532
6	75	524264
% OF RSD		1.28

Limit of detection: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. inject the 0.075% solution into HPLC system and record the chromatogram.

Observation: LOD was found to be 0.6µg/ml. Here in this concentration it is not showing linearity and precision.

Limit of quantification: The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantification limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

Observation: LOQ value was found to be 1.5µg/ml. Here it shows precision and not linear.

Calculation:

LOQ = LOD x 3.3

0.6x3.3 = 1.98 µg/ml

Robustness: The robustness of the method was determined as per ICH guidelines under different conditions including change in Mobile phase, wavelength, and pH. The precision sample prepared was used for the robustness parameter. Variations in Mobile phase, wavelength, and pH were made to evaluate the robustness parameter. The retention times,

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theoretical plates and asymmetry factor values were recorded and these variations were found to be in the acceptable changes (table 4).

Table no :4 for Robustness

S.NO	Parameter	Change	Area	% of Change
1	Standard	……	525324	……
2	MP 1	Acetonitrile 5 Methanol 95	529617	0.18
3	MP 2	Acetonitrile 15 Methanol 85	527247	0.36
4	WL 1	233	521265	0.77
5	WL 2	237	516918	1.60
6	pH 1	5.1	535242	1.88
7	pH 2	5.3	528086	0.52

Ruggedness: The standard solution was injected for five times and measured the area for all six injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. Results are shown in Table 5.

Table No: 5 Ruggedness of Teniligliptin

S. No	Area
1	514127
2	526685
3	511407
4	503084
5	502689
6	513146
RSD	**1.72**

System suitability: System suitability parameters like number of theoretical plates, HETP and peak tailing were determined. The values for the parameters were shown in the table 6

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Table 6: **System suitability parameters**

Parameters	Results
Tailing factor	16.2
Theoretical plates per column	3265
Peak area	525324
%RSD Peak retention time	5.843

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Tablet analysis: Content of Teniligliptin was found in the commercial tablets by the proposed method and results were shown in Table 7.

Table 7: Results for HPLC Analysis of Tablets

Drug	Teneligliptin			
	Standard Conc	**Sample Conc**	**standard Area**	**Sample Area**
	75µg/ml	75µg/ml	525324	524349
% Purity	99.80%			

Applicability of the proposed HPLC method

From the results obtained after method validation, it is evident that the proposed method gave satisfactory results with the analysis of Teniligliptin in bulk. Therefore, commercial tablets containing Teniligliptin (20 mg/tablet) were subjected to the analysis by the proposed method. The percentage recovery was 99.80 ± 0.20%. This satisfactory value indicated the applicability of the developed method for the routine quality control of Teniligliptin tablets without interference from the excipients found in the tablet dosage form.

Conclusion

In the present work, a sensitive HPLC method with UV detection has been developed and validated for determination of Teniligliptin in bulk and in its tablet dosage forms. From the results of validation parameters, the proposed method was found to be sensitive, accurate,

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precise, robust, rugged and specific. The proposed method, hence, can be applied for routine quality control analysis of Teniligliptin in bulk and tablet dosage forms.

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