 DOI: 10.18231/2394-2797.2017.0014

Method development and validation of Teneligliptin in pharmaceutical dosage form by UV spectrophotometric methods

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Abstract

Teneligliptin was estimated in tablet form by ultraviolet-spectrophotometric methods followed by distilled water as solvent. Here three methods were used for quantitative estimation of teneligliptin in tablet dosage form. Quantitative estimation of pure drug solution was done at λ_{max} of 244 nm for method I. For measurement of response in method II peak minima of 266.4 nm was selected. Wavelength range of 238.6-247.8 nm was selected for calculating area in area under the curve method. Validation of the methods was performed according to guidelines of International Conference on Harmonisation. The % assay of the formulation by the three methods was in the range of 100.17-100.74. Graph was linear in the range of 5-70 µg/mL for zero order and AUC techniques, while 5-80 µg/mL for first order derivative technique. Good correlation between response and concentration was found as the value of regression coefficient (R^2) was 0.999. The accuracy of the drug was ranged in between 98.54-101.80 for all ultraviolet-spectrophotometric methods and was in acceptable range. The percentage RSD values for method precision for all the methods were within the limit of ≤ 2. From the data it was concluded that the methods developed have scope to be applied for quantitative estimation of pure drug of teneligliptin in pharmaceutical dosage form.

Keywords: Ultra Violet-Spectrophotometry, Teneligliptin, Peak minima, AUC technique.

Introduction

Teneligliptin hydrobromide hydrate is an effective antidiabetic drug which inhibits dipeptidyl peptidase-4 enzyme (DPP-4). It is used to reduce hyperglycaemia and dyslipidemia.(1) The drug reduces the level of DPP-4 enzyme responsible for degradation of incretin hormone. This hormone helps in adjusting glucose level of blood. Teneligliptin hydrobromide hydrate is [(2s, 4s)-4-[4-(3-Methyl-1-phenyl-1H pyrazol-5-yl) piperazin-1-yl] pyrrolidin-2-yl] (1, 3-thiazolidin-3-yl) methanone hemi-penta hydrobromide hydrate (Fig. 1). The drug is white to off white in color having molecular weight of 628.86 g/mol while molecular weight of teneligliptin is 426.58 g/mol. Drug is water soluble, not soluble in acetonitrile and very less soluble in alcohol (methanol and ethanol). Teneligliptin melt in range of 188-190 ºC.(2-4)

From Literature review(5-10) it was observed that there are reported spectrophotometric and isocratic Reverse Phase HPLC methods for the quantitative estimation of TGN in different formulation. There is better possibility to develop simple and precise method by using distilled water as a solvent in place of methanol. This made the method more economical and results found were satisfactory.

It is a requirement to continuously develop new spectroscopic methods for estimation of coming medicines. The main objective of a method to develop is to make it easy to use, easily accessible, use of nontoxic and inexpensive solvents precise and accurate for quantitative estimation of TGN in formulation for routine analysis. Validation of the methods was done as mentioned in International Conference on Harmonisation guidelines.(11)

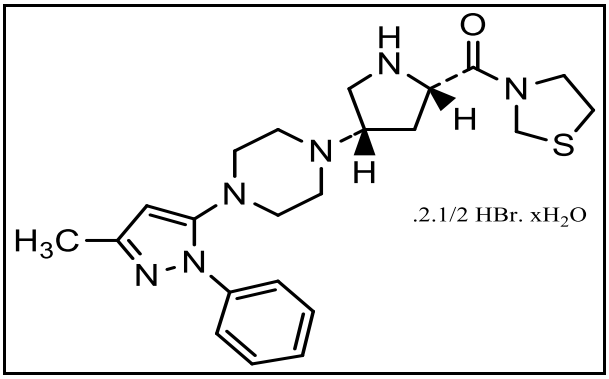

Fig. 1: Teneligliptin Hydrobromide Hydrate Chemical structure

Materials and Method

Reagents and Chemicals: Pure drug of Teneligliptin hydrobromide hydrate was procured from Molecule laboratory, Ahmedabad. Tenlima in tablet dosage form-Macleods pharmaceutical Ltd, Haridwar (Each tablet labeled to contain 20 mg of teneligliptin) were acquired from pharmacy. Distilled water was used.

Instrumentation: Shimadzu 1800 double beam UV-VIS spectrophotometer (Japan) with a resolution of 1 nm, having wavelength Range of 190- 1100 nm was used. Users can analyze data on a PC using software UV Probe (version 2.31). 10 mm cuvette made of quartz was used. Electronic balance (Schimadzu - 220h) having sensitivity of 0.001 g was used for

weighing.

Method development

Standard solution preparation: Teneligliptin (TGN) stock solution was prepared by weighing accurately 73.7 mg of teneligliptin hydrobromide hydrate (73.7 mg of teneligliptin hydrobromide hydrate is equivalent to 50 mg of TGN) and transferring into 50 mL graduated flask with the help of funnel. The drug was dissolved by adding sufficient quantity of distilled water and volume was made up to the mark with distilled water. To prepare working standard solution of 100 µg/mL, 5 ml stock solution was pipette out and transferred into a 50 mL graduated flask, volume was made up to the mark with distilled water. From this solution different desired concentrations of drug were prepared by transferring appropriate volume in different volumetric flask.

Preparation of calibration curve

Method I: zero order spectroscopic method: The standard solution (15µg/mL) was used to scan over a wavelength range of 210- 400 nm using distilled water as blank. The UV spectrum over scanned range showed λ_{max} at 244 nm (Fig. 2). Different concentrations of solution were scanned to find out linearity. By taking concentration on X-axis and absorbance on Y-axis, calibration curve was plotted and was found to be linear in a concentration range of 5-70 µg/mL. The R^2 value was obtained by analyzing regression equation.

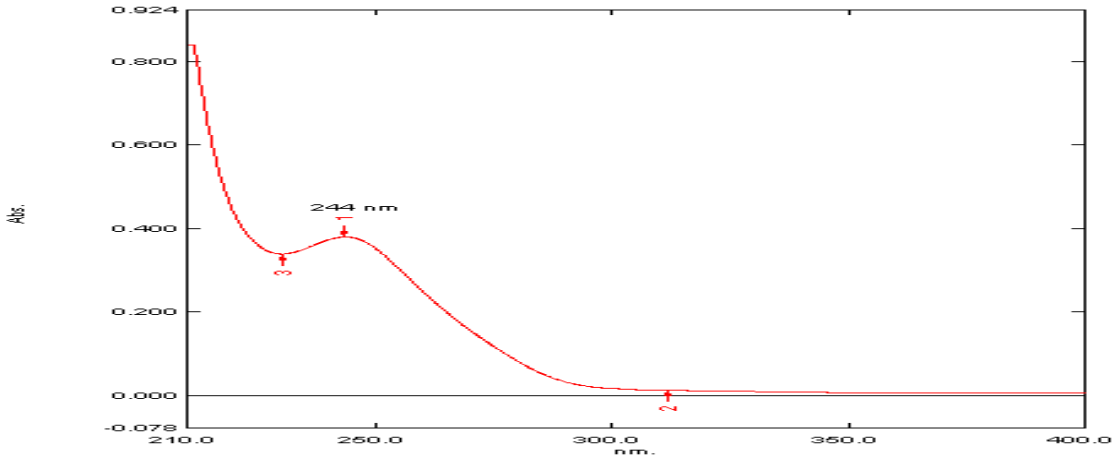

Fig. 2: Zero order spectra showing λ_{max} of Teneligliptin

Method II: first order spectroscopic method: Transformed basic spectra into first order derivative at wavelength interval of 2 ($\Delta\lambda = 2$) and scaling factor at 1. The response ($dA/d\lambda$) was measured at selected peak minima of 266.4 nm (Fig. 3).The calibration graph was plotted and was found to be linear over a concentration range of 5-80 µg/mL. The R^2 value was obtained by analyzing regression equation.

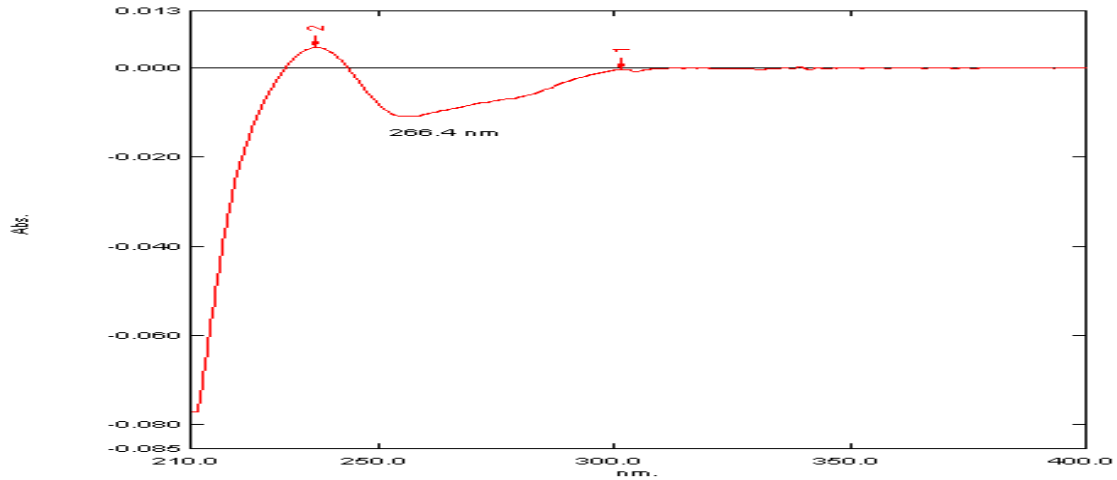

Fig. 3: First order derivative spectra showing peak minima of Teneligliptin

Method III: Area under curve method: It involves measurement of AUC between the selected wavelengths λ_1 and λ_2.Several trials were made to select the wavelength range so that the linearity could be obtained

between AUC and concentration. After selection of wavelength range zero order spectra of different concentration were changed into area under curve mode. Area under curve was measured between selected wavelength of 238.6 nm and 247.8 nm (Fig. 4). The calibration graph was obtained between AUC on Y axis and concentration on X axis and was linear in concentration range of 5-70 µg/mL. The R^2 value was obtained by analyzing regression equation.

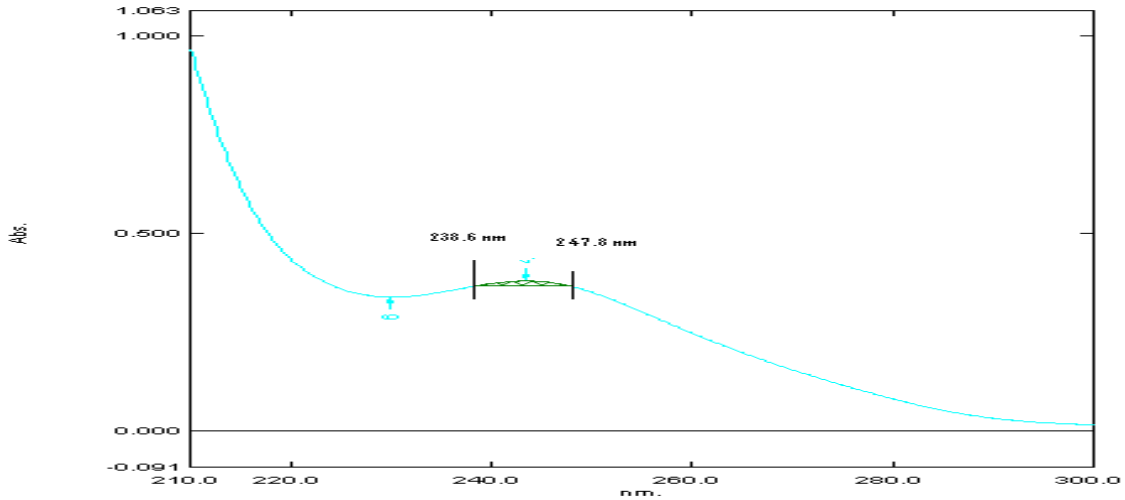

Fig. 4: Area under curve spectra of Teneligliptin

Estimation of Teneligliptin in tablet dosage form

Preparation of sample solution: Sample solution was prepared by accurately weighing 20 tablets (label claimed 20 mg), the average weight was calculated and the powder equivalent to 50 mg of TGN was transferred into a 50 mL graduated flask. Distilled water was added, sonicated, filtered through Whatman filter paper No. 41 and was diluted up to the mark with distilled water. To achieve a concentration of 100 µg/mL, 5mL stock solution of sample was pipette into 50 mL graduated flask and volume was made up to the mark with distilled water. Further dilution of the solution was made to get required concentration of 15 µg/mL for method I and III, while 25 µg/mL for method II independently in different volumetric flasks. The concentration of TGN in formulation was determined by above developed method. The assay procedure was repeated six five times (n= 6) for each method.

Validation of the Method: The methods were validated according to International Conference on Harmonisation guidelines[11] to study different parameters.

Linearity: To find out linearity range of TGN, linearity graphs were plotted by taking absorbance, response (dA/dλ) and AUC for method I, II and III respectively on Y axis against concentrations on X-axis. Graph was linear for zero order and AUC methods in the range of 5-70 µg/mL, first order derivative method was linear in range of 5-80 µg/mL. The R^2 value was calculated by analyzing regression equation.

Accuracy: The Accuracy was measured by adding standard solutions of TGN at 80%, 100% and 120% levels to previously determined tablet sample solutions. Total amount was calculated and percentage recovery of standard solution was determined. The results are reported in terms of % Recovery.

Precision: Precision was confirmed by measuring samples at different interval in same day (Intra-day) and at different days (Inter-day). To find out the intra-day and inter-day precision of the methods, absorbance values of TGN solution of three different concentrations were measured by all the three developed ultra violet-spectrophotometric methods of analysis. The precision of the developed methods were calculated in terms of % RSD.

Results and Discussion

Wavelength selected for quantitative analysis of TGN was 244 nm (λ_{max} for method I, Fig. 1), 266.4 nm (peak minima for method II, Fig. 2) and 238.6-

247.8 nm (working wavelength range for method III, Fig. 3). Solutions were prepared in distilled water, as drug was freely soluble and stable in it. Working linearity range was decided by making different concentrations of solution and measuring their response through developed methods. A broad working concentration range 5-70 µg/mL for zero and auc method and 5-80 µg/mL for derivative method were confirmed and are reported in Table 1. Percentage assay reported was in range of 100.17-100.74 for the spectroscopic methods and was within the limit of ± 2% given in Table 2. Percentage recovered amount of pure drug was in the range of 98.54-101.80 for the developed methods given in Table 3 & 4, which confirmed there is no interference of sample and its diluents in recovery of pure drug. The methods were precise as percentage relative standard deviation was within the limit of ± 2% given in Table 5, which shows all the measurements have less variation. Considering all the parameters of validation studies and % assay it was suggested that spectroscopic methods can be applied for quantitative estimation of TGN in tablet formulation.

Table 1: Linearity study of TGN by developed methods I, II and III

Parameters Observed	Method I	Method II	Method III
Wavelength (nm)	244	266.4	238.6-247.8
Linearity Range (µg/mL)	5-70	5-80	5-70
Regression equation (Y = mx+c)	y = 0.025x - 0.004	y = 0.0008x - 0.0001	y = 0.005x - 0.002
Correlation coefficient (r^2)	0.999	0.999	0.999
Slope (m)	0.025	0.0008	0.005
Intercept (c)	0.004	0.0001	0.005

Table 2: Assay results of TGN by the developed methods I, II and III

Analysis method	Label claim (mg/tablet)	% Label claim estimated*	SD	% RSD
I	20	100.20	± 0.83	0.83
II	20	100.17	± 0.82	0.82
III	20	100.74	± 0.58	0.57

*average of six determination

Table 3: Accuracy study of TGN by the developed methods I and III

Std. Drug level (%)	Amt. added (µg/mL)	*Amount found (µg/mL)		% Recovery	
		I	III	I	III
80	12	11.93	11.99	99.45	99.89
100	15	15.04	14.93	100.26	99.54
120	18	17.78	18.08	98.76	100.46

*average of three determination

Table 4: Accuracy study of TGN by the developed method II

Std. Drug level (%)	Amt. added (µg/mL)	*Amt. found (µg/mL)	% Recovery
		II	II
80	20	19.71	98.54
100	25	24.71	98.83
120	30	30.54	101.80

*average of three determination

Table 5: Precision study of TGN by developed methods I, II and III

Conc. (µg/mL)	Precision (Intra-day)						
	Method I		Method III		Conc. (µg/mL)	Method II	
	*mean ±SD	%RSD	*mean ±SD	%RSD		*mean ±SD	%RSD
10	0.248 ± 0.004	1.54	0.048± 0.001	0.80	25	0.020±0.0003	1.44
15	0.389 ± 0.005	1.29	0.072± 0.001	1.05	35	0.028±0.0005	1.86
20	0.512 ± 0.009	1.69	0.096± 0.002	1.60	50	0.040±0.0006	1.58

Precision (Inter-day)							
	Method I		**Method III**		**Method II**		
10	0.249 ± 0.004	1.57	0.048±0.001	1.19	25	0.020±0.0003	1.64
15	0.389± 0.006	1.55	0.073±0.001	0.79	35	0.028±0.0005	1.86
20	0.513± 0.009	1.76	0.094±0.002	1.62	50	0.039±0.0007	1.89

*mean of three replicates

Conclusion

The present work represents three ultra violet spectrophotometric methods which can be utilized for quantitative estimation of teneligliptin in formulation by using distilled water as solvent in place of methanol. Percentage assay is very near to the label claim which shows accuracy of the method. Analytical work can be performed in broad concentration range and shows good agreement between absorbance and concentration. All the results of validation confirmed that the parameters decided by ICH are within the limits. On the basis of these results it was concluded that the methods can be utilized by simple working, with accuracy, with precision and is inexpensive.

Acknowledgement

The authors are highly thankful to Molecule lab, Ahmedabad (India) to make available the pure drug of Teneligliptin Hydrobromide Hydrate and are also grateful to colleagues and authorities of PAHER University, Udaipur to support in this work.

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