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HPLC AND UV-SPECTROPHOTOMETRIC ESTIMATION OF TENELIGLIPTIN FROM TABLET DOSAGE FORM

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ABSTRACT

The present work proposed precise, accurate and validated HPLC and UV-spectrophotometric methods for estimation of Teneligliptin from its tablet dosage form. The UV-spectrophotometric estimation includes Calibration curve, Area under curve (AUC) and First order derivative method based on measurement of absorbance at a selected wavelengths using UV-visible spectrophotometer with 1cm matched quartz cell and distilled water as a solvent. All UV-spectrophotometric methods obeyed Beer's-lambert's law in the concentration range of 10-70µg/mL, with correlation coefficient value less than 1. The chromatographic separation was achieved by isocratic mode with a mixture of methanol: phosphate buffer (pH 7.2) in the ratio of 70:30 v/v as the mobile phase using Shodex C_{18} column as stationary phase at flow rate of 1mL/min and detection wavelength of 244nm. The retention time was found to be 5.753min. The percent amount of drug estimated by all developed methods was nearly 100%, found to be in good agreement with label claim of marketed tablet formulation. The recovery study was carried out at five different levels and results were found to be satisfactory. The validation parameters like accuracy, precision, ruggedness, linearity and range were studied for all the developed methods and were found to be within limits. Stress testing under various conditions such as pH (acid/base), temperature, light, oxidation, humidity was carried out and % undegraded drug was calculated.

KEYWORDS

Teneligliptin, HPLC, Validation and Stress testing.

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INTRODUCTION

Teneligliptin is mainly use for the treatment of Type-II diabetes mellitus. It is a highly potent, competitive and long-lasting DPP-4 inhibitor that improves postprandial hyperglycemia and dyslipidemia. Teneligliptin chemically described as [(2S, 4S)-[-(3-Methyl-2-Phenylpyrozol-3-yl) Piperazin-1-yl] Pyrrolidin-2-[1, 3 Thiazolidin-3-yl) Methanone [Figure No.1] having molecular formula

426.58. It is white to pale white in color which is freely soluble in water, sparingly soluble in alcohol and methanol.

Literature survey indicated that the drug has been estimated from bulk and marketed formulation by HPLC and UV-spectroscopy including zero order method and first derivative method. The proposed work represents new simple, economical and rapid RP-HPLC and UV-spectrophotometric methods for the quantification of Teneligliptinin bulk and from its tablets. The developed methods were validated for accuracy, precision, ruggedness and sensitivity as per ICH guidelines.

MATERIAL AND METHODS

Chemicals and Reagents

Pharmaceutical grade Teneligliptin hydrobromide hydrate (TEHH) standard was obtained as generous gift from Micro Labs Ltd., Bangalore, India.

Instruments

HPLC: Shimadzu HPLC series 1100 and Jasco HPLC PU-2089 Plus

UV-Spectrophotometer: Jasco V-630 and Shimadzu-1700 double beam

Sonicator: PCi mumbai, Model No.3.5L 100H

Stability chamber: Thermo lab, Sr.No.00002008

DEVELOPMENT OF UV-SPECTROPHOTOMERTIC METHODS

Preparation of Standard Stock Solution

The standard stock solution was prepared by dissolving TEHH weight equivalent to 10.0mg of Teneligliptin in 10.0mL of distilled water to acquire a concentration of 1000µg/mL. The working standard solution of 30µg/mL was prepared by appropriate dilution of the stock solution with distilled water.

Selection of appropriate wavelengths for analysis for Teneligliptin

Method I (Zero order): The working standard solution of 30µg/mL was prepared and scanned in the UV range 400-200nm; Teneligliptin shows a maximum absorbance at 244nm.

Method II (Area under Curve): From the zero order spectrum of Teneligliptin, the AUC between a wavelength range 230-253nm was considered for the analysis.

Method III (First order derivative UV-spectrophotometry using amplitude), the zero order absorption spectrum of Teneligliptin was derivatized in first order and the amplitudes was recorded at 236.5nm.

The selection of wavelengths in all methods is shown in Figure No.2.

Preparation of Beer-Lambert's plot

Appropriate dilutions of standard stock solution were made to get final concentration in the range of 10-70µg/mL. Absorbance and area under curve of each prepared solution were measured at above selected wavelengths. The calibration curve was plotted between concentration *vs.* absorbance or AUC, shows correlation coefficient value less than 1 (Figure No.3).

Preparation of Sample Solution

To determine the content of Teneligliptin from marketed tablets; twenty tablets were weighed, powdered and average weight was calculated. An amount of tablet powder equivalent to 10.0mg of Teneligliptin was weighed accurately and transferred to a 10.0mL volumetric flask. Sufficient amount of distilled water was added and sonicated for 10 minute and the solution was diluted up to mark with the same solvent and filtered through whatmann filter paper (No. 41). From the filtrate, measured volume was taken and diluted with distilled water to get the final concentration of 30μg/mL. The absorbance's were measured at selected wavelengths as described above and concentrations in the sample were determined.

DEVELOPMENT OF RP-HPLC METHOD

Preparation of standard stock solution

An accurately weighed quantity of TEHH is equivalent to Teneligliptin (~10.0mg) was transferred in a 10.0mL volumetric flask, dissolved in sufficient quantity of diluent to get concentration of 1000µg/mL. The working standard solution of 30µg/mL was prepared by appropriate dilution of the stock solution with mobile phase. The chromatogram of standard was recorded as shown

in Figure No.4(a). The average retention time was found to be 5.424 min.

Preparation of mobile phase:

The mobile phase was prepared by mixing methanol and potassium dihydrogen phosphate buffer (pH-7.2) in ratio 70:30%v/v. The prepared mobile phase was sonicated and filtered through 0.45µm membrane filter.

Study of Calibration curve

Appropriate dilutions of standard stock solution were made to get final concentration in the range of 10-70µg/mL and peak area was measured of each prepared solution. The calibration curve was plotted between concentration vs. peak area, having correlation coefficient 0.991 (Figure No.5).

Preparation of Test sample

Weigh and finely powdered 20 tablets and transfer the quantity of tablet powder equivalent to 10.0mg of Teneligliptin to 10.0mL volumetric flask, sonicated for 15 min with sufficient quantity of mobile phase and volume was made up to mark with mobile phase. The content of the flask was filtered through 0.45µm membrane filter paper. From the filtrate, measured volume was taken and diluted with mobile phase to get the final concentration of 30μg/mL. After equilibration of stationary phase, such six sample solutions were injected separately and chromatograms were recorded and the content of Teneligliptin in each sample was determined.

Validation of Proposed Methods

The proposed method was validated as per ICH guidelines.

Accuracy

The accuracy of proposed method was ascertained on the basis of recovery studies. Weighed the pre-analyzed tablet powder equivalent to 5.0mg; a known amounts of standard drug was added at different levels 50-150%. The resultant solutions were then re-analyzed by the developed methods. At each concentration, each sample was analyzed thrice at each level to check repeatability and from the obtained data it was analyzed that the proposed methods were found to accurate.

Precision

The precision of the analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the methods can be studied as; intra-day variation, inter-day variation studies and results were expressed as SD and % RSD of series of measurements.

Ruggedness

Ruggedness of proposed methods was performed to examine effect of non-procedure related factors such as instruments and analysts. For this study, Teneligliptin (30μg/mL) was analyzed by proposed methods using two different analyst and two different instruments, restraining similar operational and environmental conditions.

Linearity

The linearity of an analytical procedure is the ability to obtain test results that are directly proportional to the concentration (amount) of an analyte in the sample within a given range. For linearity study, five solutions of Teneligliptin of different percent of label claim (80-120%) were prepared, analyzed by proposed methods and the using obtained data calibration plot was plotted (Figure No.6).

Robustness

It is the capacity of the method to remain unaffected by small but deliberate variations in method parameters. The analysis was performed by slightly changing in the pH, mobile phase composition, and detection wavelength and flow rate.

Force Degradation Study

Preparation of sample solutions

Solution state analysis

Accurately weighed quantities of tablet powder equivalent to 10.0mg Teneligliptin were transferred to series of 100.0mL volumetric flasks. To each flask 10.0mL of reagent (0.1N HCl/0.1N NaOH/ 3% H_2O_2/ Distilled water) was added and kept at 60^0C for a period of 180 min. The sample solutions were withdrawn after 180 minute for all stress conditions. The stressed samples were diluted to volume with distilled water. The content of each flask was sonicated for 15 minute and samples were

filtered separately. A 6.0mL portion of the filtered sample solution was further diluted to 10.0mL with distilled water (60µg/mL). After 180 minute, the spectra and chromatogram were recorded for unexposed standard, sample and exposed sample in acidic, alkaline, peroxide and neutral conditions.

Solid state analysis

Tablet powder of Teneligliptin was spread on petridish and kept in the oven at 80ºC, humidity chamber at 75% RH and in UV chamber (254nm). After 48 hours, an accurately measured quantity of tablet powder equivalent to 10.0mg of Teneligliptin was withdrawn and transferred to 100.0mL volumetric flask and volume was made up to the mark with distilled water. The content was sonicated for 15 min. and filtered. A 6.0mL portion further diluted to 10.0mL with distilled water (60µg/mL). UV-spectra and chromatogram of exposed sample in stress conditions were recorded.

RESULTS AND DISCUSSION

Teneligliptin was found to be highly soluble and stable in distilled water and methanol: phosphate buffer (pH-7.2) mixture. Using these solvents working standard solutions were prepared of desired concentration for UV-spectrophotometric and HPLC estimation of Teneligliptin respectively. The system suitability study was carried out and evaluated (Table No.1). The percentage amounts of Teneligliptin estimated from marketed tablet formulation by UV-spectrophotomertic methods I-III was found to be 100.86, 99.28, and 100.04 respectively (Table No.2) while 101.05% using RP-HPLC (Table No.3). The % estimation results indicates that there was no interference from excipients present in it. The linearity was followed over the concentration range 10-70µg/mL for proposed methods, showing the correlation coefficient value less than 1. The developed methods were validated for accuracy, linearity, precision, system suitability and robustness (Table No.4). All the results were satisfactory, within the limits as per ICH guidelines and hence the proposed analytical methods can be successfully employed for the determination of Teneligliptin for regular and routine analysis.

Analysis of Force degradation study

It was observed that degradation of drug in formulation was not very conclusive as no change in the spectral pattern was observed for the degraded samples but slight increase or decrease in absorbance was noted which indicates some sort of degradation might have taken place. Since Method I result indicates degradation in nearly all stress conditions but degradation especially in alkaline condition by method II and acidic condition by Method III was observed. Hence it was not clear from the spectrophotometric studies regarding the stability of drug in various stress condition considering the results of three methods. The drug was found to be susceptible in alkali which showed more than 20% degradation. The results obtained for humidity, UV and thermal study indicates that the drug is susceptible to degradation by method I but other two methods do not suggest much degradation, so formulation (drug) can be considered as stable.

The results obtained by hydrolysis and oxidative study using RP-HPLC were compared with the standard drug subjected to above mention conditions. It was observed that drug was degraded to large extent under alkaline condition as compared to other stress conditions. Moreover one additional peak was observed in the chromatogram of alkaline degraded sample (Table No.5).

Table No.1: Optimized chromatographic parameters

S.No	System	Shimadzu HPLC series 1100
1	Stationary Phase	Shodex C-18-4E (5µm), 250×4.6mm
2	Mobile Phase	Methanol : Phosphate Buffer with NaOH (70:30 % v/v)
3	Detection wavelength	244 nm
4	Flow rate	1.0mL / min
5	pH	7.2
6	Injection volume	20 µL
7	Mean peak area	595.67 mV

Table No.2: Results of % estimation by UV methods

S.No	Wt. of tablet powder (mg)	% Label claim		
		M-I	M-II	M-III
1	180.40	102.22	98.01	99.99
2	180.15	99.06	97.24	100.16
3	180.27	100.27	99.28	100.10
4	180.39	100.05	101.12	100.05
5	180.51	102.73	100.76	99.94
Mean		100.86	99.28	100.04
±SD		1.55	1.68	0.08
%RSD		1.54	1.70	0.09

Table No.3: Results of % estimation using RP-HPLC method

S.No	Wt. of tablet powder (mg)	AUC of sample (mV)	% Label Claim
1	179.88	602.347	100.22
2	179.76	590.920	99.06
3	179.30	583.501	100.27
4	179.21	593.808	100.05
5	179.25	596.633	102.73
6	179.32	588.654	100.40
Mean			101.05
±SD			1.0516
%RSD			1.04

Table No.4: Results of validation parameters

S.No	Parameters	Estimation of TENE by			
		UV			RP-HPLC
		M-I	M-II	M-III	
1	Accuracy (% Mean, %RSD)	102.21, 1.78	100.27, 1.48	100.07, 0.75	99.94, 1.23
2	Precision (%RSD)	1.54	1.70	0.09	1.04
3	Intraday (%RSD)	1.19	0.02	0.10	0.15
4	Interday (%RSD)	1.26	0.03	0.03	1.01
5	Linearity and range (r^2)	0.9940	0.9960	0.9730	0.9982
6	LOD (µg/mL)	-	-	-	1.80
7	LOQ (µg/mL)	-	-	-	5.40
8	Different analysts (%RSD)	1.61	0.03	0.04	0.71
9	Different instruments (%RSD)	1.34	0.36	0.44	-
10	Robustness (Mean, %RSD)	-	-	-	1.93

Table No.5: Results of forced degradation study

S.No	Condition	% Degradation with respect to unexposed sample			
		M-I	M-II	M-III	RP-HPLC
Solution state analysis					
1	Acidic hydrolysis	16.46	13.66	-3.96	2.11
2	Alkaline hydrolysis	11.89	5.66	23.14	75.94
3	Oxidative study	4.57	2.12	-3.73	-1.68
4	Neutral hydrolysis	16.06	6.19	3.91	-4.31
Solid state analysis					
5	Thermal study	11.56	1.91	-3.79	-1.77
6	Humidity study	11.81	2.01	-3.78	2.2
7	Photochemical study	12.53	2.59	-4.01	0.47

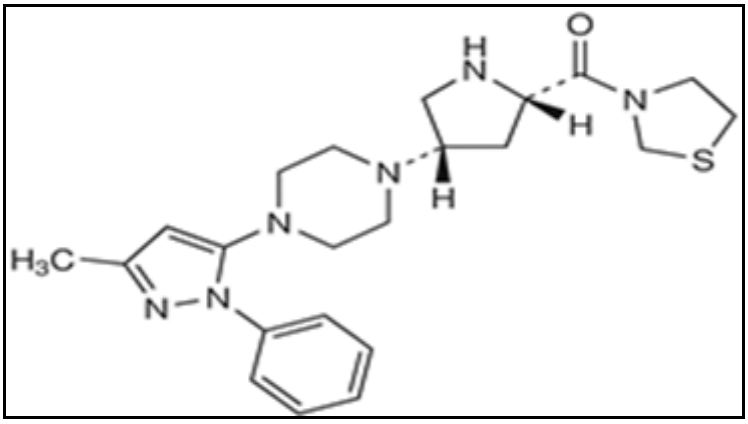
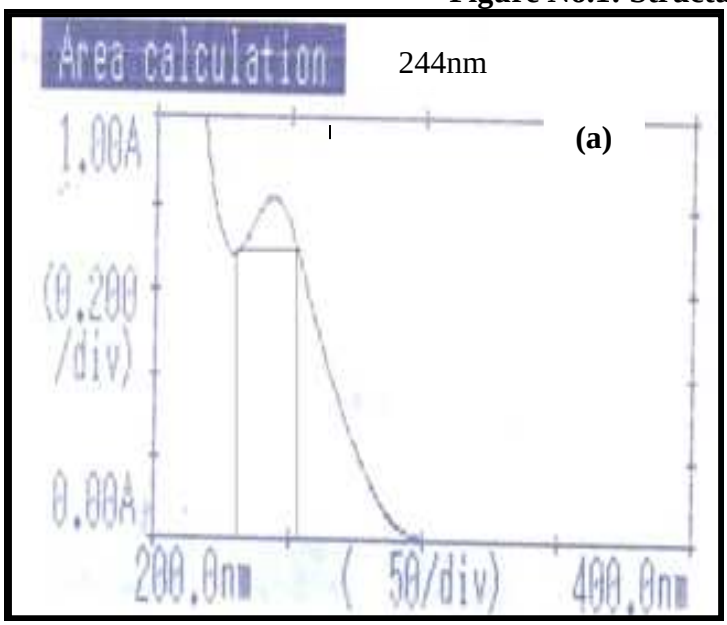


Figure No.1: Structure of Teneligliptin



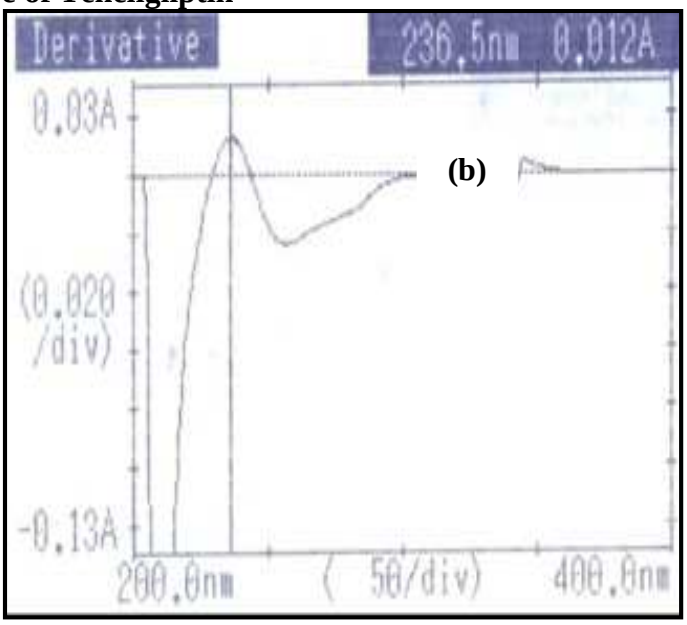


Figure No.2: (a) Zero order spectra showing AUC between selected wavelengths and (b) First order derivative spectrum of Teneligliptin

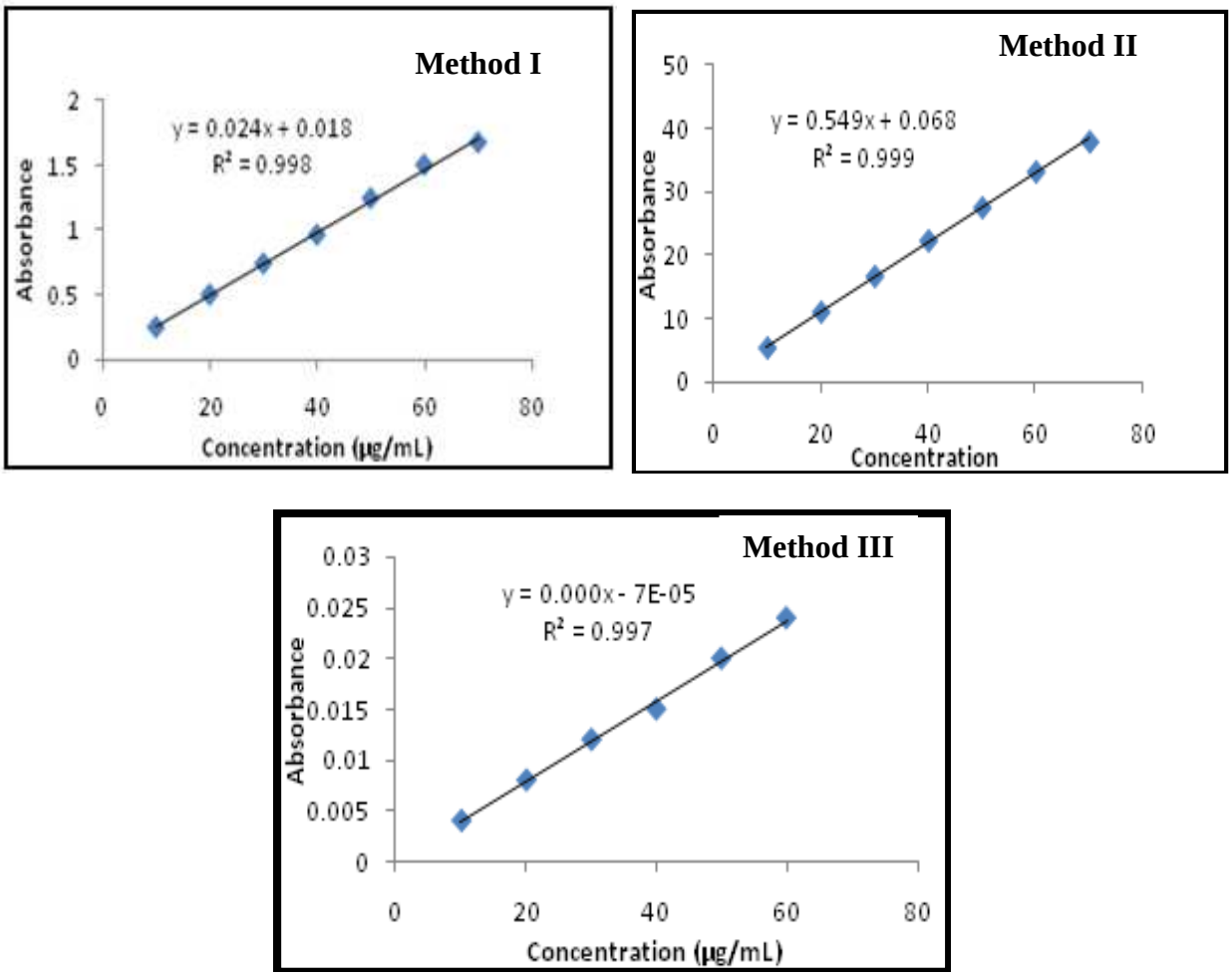


Figure No.3: Beer's Lambart's plots at selected wavelengths for method I-III

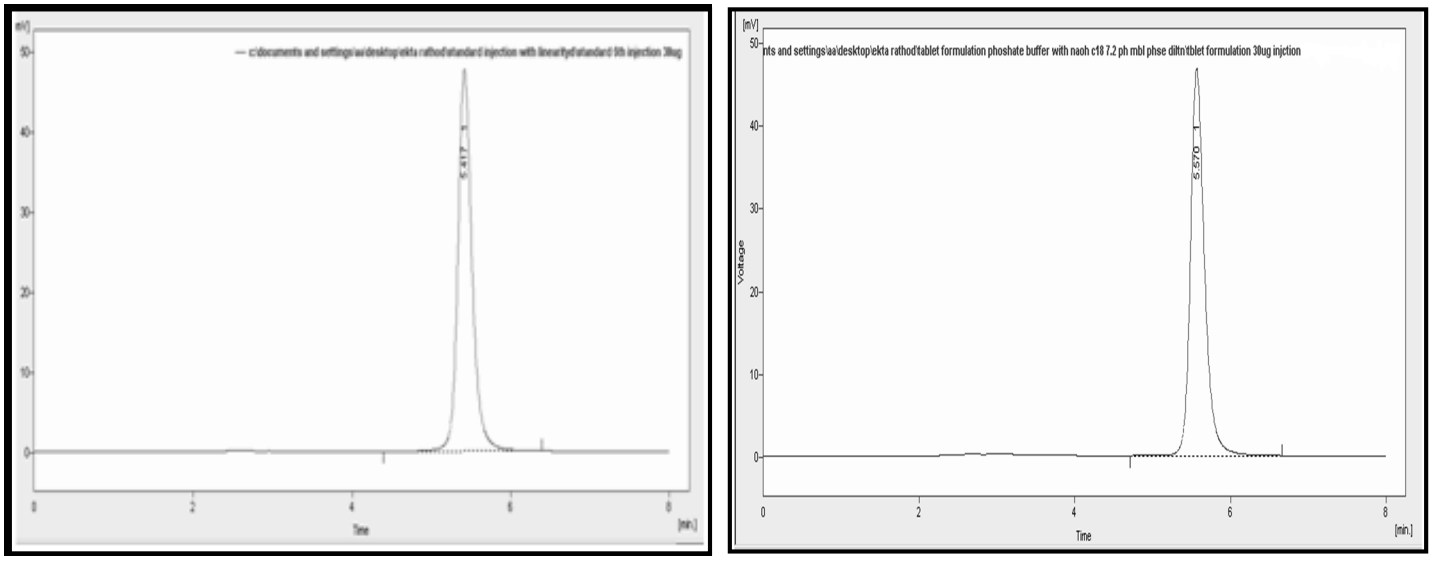


Figure No.4: (a) A typical chromatogram for standard Teneligliptin and (b) marketed formulation

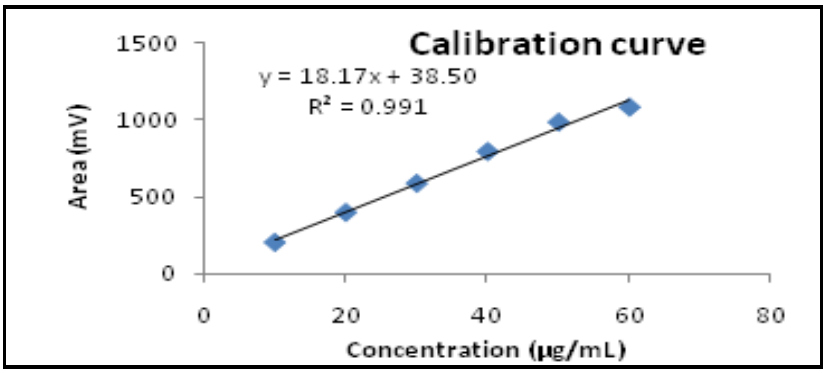


Figure No.5: Calibration curve of TENE

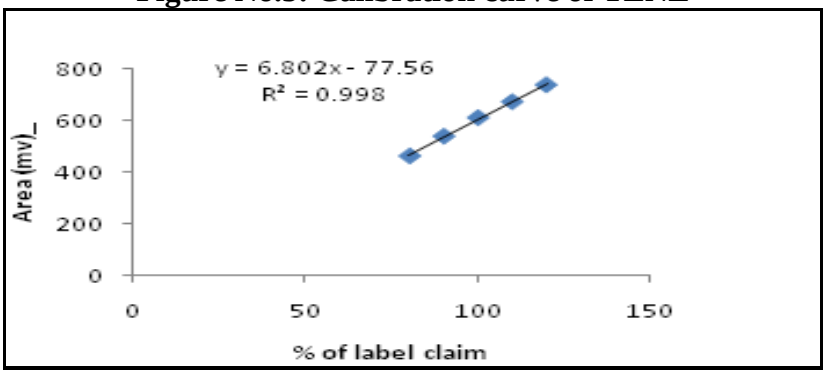


Figure No.6: Plot of linearity and range by RP-HPLC

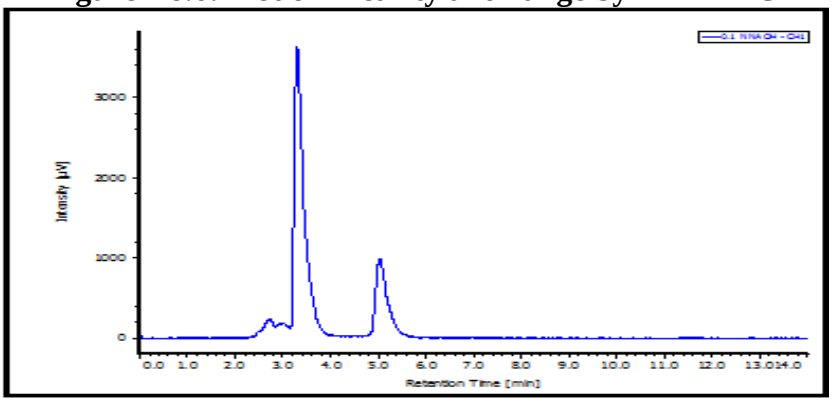


Figure No.7: Alkaline degradation peak of TENE by RP-HPLC

CONCLUSION

The results obtained by UV and RP-HPLC methods for determination of Teneligliptin are accurate and precise. The developed methods do not have any interference of excipients while determining drug from its formulation for its assay. The proposed methods are entirely new, simple, sensitive and economical as compared to some reported method in literature. Hence it can be employed for routine quality control analysis of Teneligliptin from tablet dosage form.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

ABBREVIATION USED

TENE: Teneligliptin, **TEHH:** Teneligliptin Hydrobromide Hydrate, **AUC:** Area under curve, **API:** Active pharmaceutical ingredient, **RSD:** Relative standard deviation, **SD:** Standard deviation.

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