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Development and validation of UV spectrophotometric method and high performance thin layer chromatographic (HPTLC) method for estimation of teneligliptin hydrobromide in pharmaceutical preparation

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ABSTRACT

Simple, rapid, sensitive, precise and specific UV spectrophotometric and High-performance thin layer chromatographic (HPTLC) methods for the determination of Teneligliptin Hydrobromide both in bulk drug and pharmaceutical dosage form were developed and validated. In UV spectrophotometric method, the solutions of Teneligliptin HBr were prepared in water. The standard solution of Teneligliptin HBr showed maximum absorption at wavelength 243.5 nm. The drug obeyed Beer–Lambert's law in the concentration range of 10– 90 µg/mL with coefficient of correlation (r^2) of 0.999. For HPTLC method, the method employed aluminium plates precoated with silica gel G60 F254 as the stationary phase. The solvent system consisted of toluene: chloroform: ethanol: diethyl amine in the proportion of 4:4:1:1, v/v/v/v. This solvent system was found to give compact spots for Teneligliptin HBr with Rf value 0.16 ± 0.01. Densitometric analysis of Teneligliptin HBr was carried out in the absorbance mode at 254 nm. Linear regression analysis showed good linearity (r^2 =0.998) with respect to peak area in the concentration range of 100–600 ng/spot. The developed methods were validated as per the ICH guidelines. Statistical analysis proved that the methods are repeatable and specific for the estimation of the said drug. These methods can be adopted in routine assay analysis of Teneligliptin HBr in bulk or tablet dosage form.

Keywords: Teneligliptin HBr, UV spectrophotometry, HPTLC, Method validation.

INTRODUCTION

A novel class of compounds which revolutionized the treatment of diabetes during the recent past are dipeptidylpeptidase-4 inhibitors (DPP-4). They are widely known as gliptins. Teneligliptin HBr hydrate is a novel, potent, peptidomimetic, and long acting DPP-4 inhibitor which got approval for the treatment of T2DM in Japan (2012) and Korea (2014). Teneligliptin,{(2S,4S)-4-[4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)piperazin-1-yl]pyrrolidin-2-yl}(1,3-thiazolidin-3-yl) methanone hemipentahydrobromide hydrate exhibits a unique structure that is characterized by five consecutive rings (Figure 1)[1,2,3]. Recent studies have revealed that this drug is unique in its nature and exhibits multiple pharmacological effects. It includes vasoprotective, neuroprotective effects.

The literature review revealed a liquid chromatography–tandem mass spectrometry method for estimation of Teneligliptin HBr in rat blood plasma [4], and a Stability indicating RP-HPLC method [5] for estimation of Teneligliptin Hydrobromide in pure and tablet dosage form. No official or draft monograph of Teneligliptin Hydrobromide Hydrate was published in any of the pharmacopoeia for compendia applications.

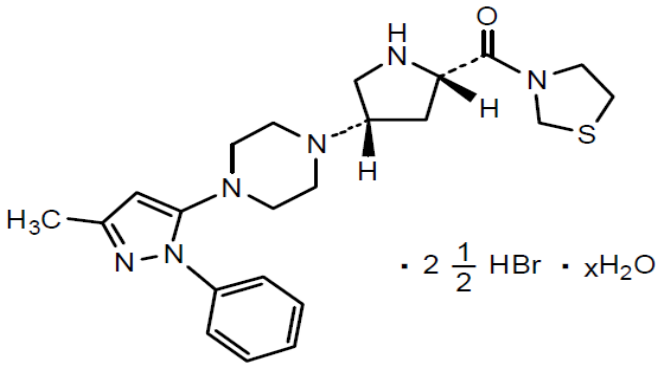

Figure 1: Chemical Structure of Teneligliptin HBr

The present work deals with the development of UV spectrophotometric method and high-performance thin-layer chromatographic (HPTLC) method and its validation as per International Conference on Harmonisation (ICH) guidelines. The developed methods can be adopted in routine analysis of Teneligliptin Hydrobromide in bulk and tablet dosage form. The methods involve relatively low cost solvents and no complex extraction techniques.

1. Method A: UV Spectrophotometry

1.1. Materials

Teneligliptin HBr bulk drug was obtained from Glenmark Pharmaceutical LTD, (Sinnar, India). The commercially available tablets of Teneligliptin HBr were purchased form Indian market (Ziten tablets B. No: 18150827 manufacture by Glenmark Pharmaceutical LTD). Water was obtained from a Milli-Q UF-Plus apparatus (Millipore) and was used to prepare all solutions for the method.

1.2. Instrument

Shimadzu UV - 1700 UV/VISIBLE spectrophotometer with UV probe 2.10 software and 1 cm matched quartz cells were used for absorbance measurements. Analytical balance used for weighing standard and sample was Make- Mettler Toledo, Model- X

MATERIALS AND METHODS

1.3. Experimental

1.3.1. Selection of Solvent

The solubility of Teneligliptin HBr was checked in water, methanol and Dimethyl sulfoxide (DMSO). It was found to be freely soluble in water, methanol, and DMSO Water was selected as the solvent for dissolving the drug.

1.3.2. Preparation of Standard Stock Solution

Accurately weighed Teneligliptin HBr working standard equivalent to 10 mg of Teneligliptin was transferred into a 100 mL volumetric flask. It was dissolved in 20 mL water by sonication for 10 minutes. Final volume was made up to 100 mL with water to give the solution containing 100 µg/mL of Teneligliptin.

1.3.3. Selection of Wavelength for Analysis

The standard stock solution was further diluted with water to obtain the solution of Teneligliptin with concentration 20 µg/mL. The solution was scanned between 200 and 400 nm using water as blank.

1.3.4. Preparation of the Calibration Curve

Aliquots of standard stock solution were further diluted with water to get the solutions of concentration within range 10 – 90 µg/mL. The absorbance was measured at 243.5 nm against water as blank. All measurements were repeated three times for each concentration.

1.3.5. Assay of Teneligliptin HBr in Tablet

Twenty tablets were weighed; their average weight was determined and finely powdered. Powder equivalent to 50mg Teneligliptin of was accurately weighed and dissolved in small amount of water in 50 mL volumetric flask and then the volume was adjusted with water to obtain the final concentration 1000 µg/mL. From this, 10 mL solution was taken and diluted up to 100 mL with the same solvent in a volumetric flask to obtain the solution of concentration 100 µg/mL. From this solution, aliquot of 2 mL was diluted to 10 mL using water. The absorbance of sample solution was measured at wavelength 243.5 nm. This procedure was repeated for six times.

1.3.6. Method Validation
The developed method was validated as per ICH guidelines for following parameters [6 – 7].

Linearity
Aliquots of standard stock solution were further diluted with water to get the solutions of concentration within range from 10 – 90 µg/mL. The absorbance was measured at wavelength 243.5 nm. Linear calibration graph was obtained by plotting the absorbance value versus concentration of Teneligliptin.

Specificity
The specificity of the method for determination of Teneligliptin in tablet dosage form was determined by comparing the spectrum of tablet solution with that of standard solution. The sample spectrum was checked for any interference from the excipients.

Recovery
To ensure accuracy of the method, recovery studies were performed by standard addition method at 80%, 100% and 120% level to pre-analyzed samples (20µg/mL) and subsequent solutions were reanalyzed. At each level, three determinations were performed. Accuracy is reported as % recovery which was calculated from the expression as equation given below,

$$\% \text{ Recovery} = \text{Observed value} \times 100 / \text{True value}$$

Precision
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision of the method was determined in terms of repeatability and intraday and interday precisions.

Repeatability
Repeatability of the method was determined by analyzing six samples of same concentrations of drug (20µg/mL**)**. Spectra were recorded, and the absorbance of each spectrum was measured.

Intraday and Interday Precision (Intermediate Precision)
Intraday precision was determined by analyzing the drugs at three different concentrations (10, 20 and 30 µg/mL**)** and each concentration for three times, on the same day. Interday precision was determined similarly, but the analysis being carried out daily, for three consecutive days.

Robustness
The robustness of developed method is its capacity to remain unaffected by small changes in altered conditions. To determine the robustness of the method, the wavelength of analysis was deliberately altered and assay was evaluated. The effect of detection wavelength was studied at ± 2 nm.

Solution Stability
The stability of the solution was studied by analyzing the standard solution at 1, 2, 3, 4 and 5 days intervals.

RESULTS AND DISCUSSION

1.4.1 Selection of Wavelength for Analysis
The UV spectrum of Teneligliptin HBr showed the maximum absorbance at the wavelength 243.5 nm. It was selected for the analysis of Teneligliptin HBr in bulk and tablet formulation (Figure 2)

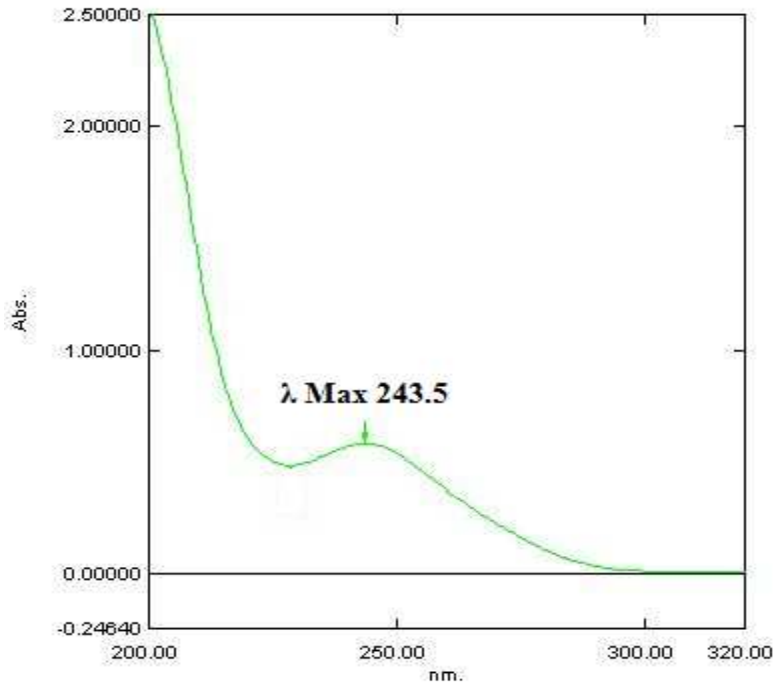

Figure 2: UV spectrum of standard Teneligliptin (20µg/mL)

1.4.2 Preparation of the Calibration Curve

The calibration curve was constructed by plotting absorbance against corresponding concentration. The calibration curve for Teneligliptin HBr is shown in Figure 3. The drug obeyed Beer–Lambert's law in the concentration range of 10–90 µg/mL with coefficient of correlation (r^2) of 0.999.

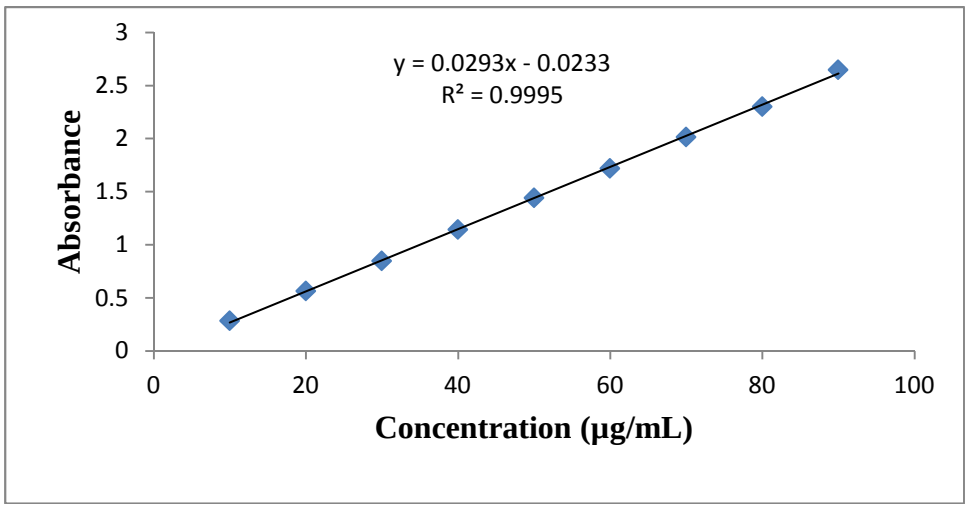

Figure 3: Calibration Plot for Teneligliptin by UV method

1.4.3 Assay of Teneligliptin HBr in Tablet

The amount of Teneligliptin HBr present in formulation was calculated by comparing the absorbance of sample with standard absorbance. Content of Teneligliptin HBr in tablet formulation determined by developed method was in good agreement with the label claim. The results obtained are shown in Table 1.

Table1: Assay of Tablet Formulation by UV method

Labelled claim (mg)	20 mg
Amount found* ± SD (mg)	20.24 ± 0.0031
% Assay	103.11
% RSD	0.54

**Mean of six determinations*

1.4.5. Method Validation

Teneligliptin HBr showed linear response in the concentration range of 10-90 µg/mL with the correlation coefficient of 0.999. The spectra obtained from tablet solutions were identical with that obtained from standard solution containing an equivalent concentration of Teneligliptin (Figure 4). This showed that there was no any interference from excipients. Therefore, it could be said that developed method is highly specific. The percentage recovery of standard drug, determined by developed method at 80, 100 and 120 % of sample concentration was ranged from 97.43 to 98.70%. The values of % recovery and % RSD shown in Table 2 indicate that the method is accurate. The % RSD values for repeatability and intermediate precision were found to be less than 2%.The low % RSD value indicate the precision of the method. The results are summarized in Table 3 and Table 4 respectively.

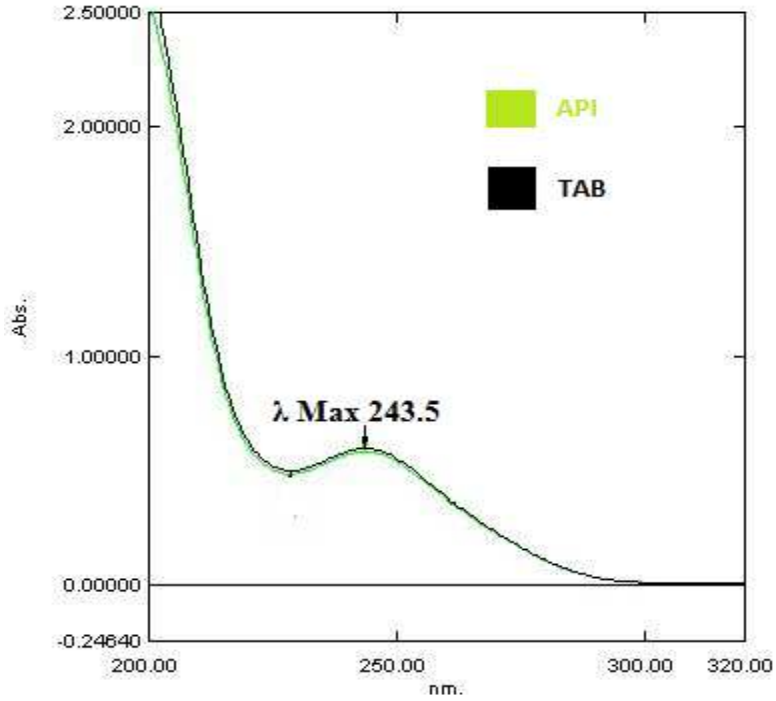

Figure 4: Overlain UV Spectra of Standard and Sample Teneligliptin HBr (20ppm)

Table 2: Results of recovery studies for Teneligliptin by UV method

Level of addition (%)	Amount of std drug added (µg/mL)	Amount of recovered * ± SD (µg/mL)	% Recovery	% RSD
80	16	17.17 ± 0.0079	97.43	0.733
100	20	21.31 ± 0.0127	98.65	1.060
120	24	25.27 ± 0.0145	98.70	1.104

**Mean of three determinations, SD- Standard Deviation*

Table 3: Results of repeatability studies by UV method

Concentration applied (µg/mL)	20
Concentration found* ± SD (µg/mL)	19.72 ± 0.0056
% RSD	0.97

**Mean of six determinations*

Table 4: Results of Intermediate Precision Studies by UV method

Concentration (µg/mL)	Intra-day precision		Inter-day precision	
	Concentration found* ± SD (µg/mL)	% RSD	Concentration found* ± SD (µg/mL)	% RSD
10	9.48 ± 0.0	0.0	9.82 ± 0.004	1.6
20	19.43 ± 0.003	0.62	19.44 ± 0.002	0.37
30	28.86 ± 0.002	0.25	29.00 ± 0.005	0.65

**Mean of three determinations*

Assay of Teneligliptin HBr for altered conditions was within 99.69 – 100.30 % as shown in Table 5, which indicates robustness of the method. Meanwhile, results of stability studies indicate that the solution was stable for 1 - 5 days at ambient temperature. The % assay was 102% after 5 days. The results are shown in Table 6.

Table 5: Result of Robustness Studies by UV method

Wavelength	% Assay* ± SD	%RSD
241.50	100.30 ± 0.002	0.33
245.50	99.69 ± 0.002	0.41

**Mean of three determinations*

Table 6: Result of Solution Stability Studies by UV method

Time (days)	% Assay* ± SD	% RSD
1	99 ± 0.70	0.86
2	98 ± 0.85	1.06
3	100.1 ± 0.10	0.78
4	99 ± 0.75	0.86
5	102 ± 0.90	0.92

**Mean of three determinations*

The summary of validation parameters of UV method is shown in Table 7.

Table 7: Summary of Results of Validation Parameters by UV method

Sr. No	Parameter	Results
1	Absorption maxima(nm)	243.5nm
2	Beers range (µg/ml)	10-90µg/ml
3	Standard Regression Equation	y = 0.029x - 0.023
4	Correlation Coefficient (r^2)	0.999
5	%Assay	103.11%
6	Precision	Repeatability: % RSD= 0.97
		Intermediate Precision**:** % RSD= Below 2%
7	Accuracy	% Recovery: 97.43 - 98.70%.
8	Robustness (%RSD)	Below 2 %

2 Methods-B: High performance thin layer performance chromatography (HPTLC)

2.1. Materials

The analytical grade methanol, toluene, chloroform, ethanol, diethyl amine were used.

2.2. Experimental

2.2.1. HPTLC Instrumentation and Chromatographic Conditions

The HPTLC plates were prewashed with methanol and activated at 110°C for 5 minute prior to chromatography. The samples were spotted in the form of bands of 8mm width with a Camag 100 microlitre sample syringe (Hamilton, Bonaduz, Switzerland) on silica gel precoated HPTLC aluminum plate G60 F254, [(20 ×10cm) with 250μm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai] using a Camag Linomat V applicator (Switzer-land). A constant application rate of 0.2 μL/s was used and the space between two bands was 16 mm. Linear ascending development was carried out in 20 cm×10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The mobile phase was consisted of toluene: chloroform: ethanol: diethyl amine (4:4:1:1 v/v/v/v) and 20 mL were used per chromatography run. The optimized chamber saturation time for mobile phase was 10 min using saturation pads at room temperature. The length of chromatogram run was 8 cm. Densitometric scanning was performed using a CAMAG TLC operated by CATS software (V 3.15, Camag). The slit dimension was kept at 6 mm×0.45 mm and the scanning speed was 100nm/s. The source of radiation used was a deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. All determinations were performed at detection wavelength of 254 nm.

2.2.2. Preparation of Standard Solution

Accurately weighed Teneligliptin HBr (equivalent to 100 mg of Teneligliptin) was transferred to a 100 mL volumetric flask and dissolved in and diluted up to the mark with methanol to obtain a standard solution of Teneligliptin (1000 μg/mL). The aliquot of 10 mL from this solution was diluted to 100 mL with methanol to obtain working standard solution of concentration 100µg/mL.

2.2.3. Method Validation

The HPTLC method was validated as per the ICH guidelines [6-8].

Linearity

The standard solution was spotted on the HPTLC plate (1µL to 6 µL) to obtain the spots in the concentration range of 100–600 ng/spot. Each concentration was spotted six times on the HPTLC plate. The plate was developed using the previously described mobile phase and scanned. The peak areas were plotted against the corresponding

concentrations to obtain the calibration graph. Linear calibration curve was generated using least-squares linear-regression analysis.

Precision

Precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analyses of the drug (500 ng/spot) in hexaplicate on the same day. The %RSD of six determinations was calculated Intermediate precision of the method was checked by repeating studies on two different days. The %RSD of twelve determinations was calculated

Limit of Detection and Limit of Quantitation

The sensitivity of the method was determined in terms of limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were calculated by using the formula, LOD = 3.3 × σ/S and LOQ = 10 × σ/S, where σ is residual standard deviation of regression line and S is slope of corresponding regression line.

Accuracy

Accuracy of the method was determined by standard addition method in which the known amount of standard Teneligliptin solutions were added to pre-analyzed sample solution. These amounts corresponded to 80, 100, and 120 % of the sample concentration. The amount of Teneligliptin was estimated by comparing the peak area of sample with that of standard. Accuracy study was performed in triplicate, and % recovery of Teneligliptin was calculated.

Specificity

Specificity of the method was determined by comparing the chromatogram of sample with the chromatograms of standard.

Solution Stability

The stability of standard solutions was tested after 1, 6 and 24 h of storage. The stability of the solutions was determined by comparing peak area with that of freshly prepared standard and peak purity at 500 ng/spot.

2.2.4. Analysis of Marketed Pharmaceutical Dosage Form.

To determine the content of teneligliptin in marketed pharmaceutical dosage form, twenty tablets were accurately weighed, their average weight was determined and they were finely powdered. The powder equivalent to 100 mg of teneligliptin was weighed and transferred into a 100 mL volumetric flask containing 50 mL methanol, sonicated for 15 minute, and diluted to 100 mL with methanol. The above solution was filtered through the whatmann no. 41 filter paper. From this solution 1 mL solution was transferred into 10 mL volumetric flask and diluted to volume with methanol. Aliquots of 3 µL were spotted for six times on the TLC plates followed by the development and measured at 254nm. The amount of teneligliptin was estimated by comparing the peak area of sample solution with that of standard

RESULTS AND DISCUSSION

2.3.1. Selection of Analytical Wavelength

The VU absorption spectrum of Teneligliptin showed maximum absorbance at 254 nm so it was selected as detection wavelength. (Figure 5)

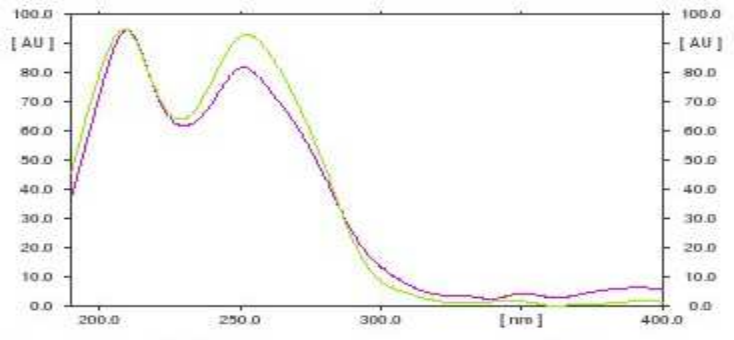

Figure 5: UV spectrum of standard Teneligliptin HBr

2.3.2. Optimization of the Chromatographic Conditions

The HPTLC procedure was optimized with a view to develop simple HPTLC method. The pure drug was spotted on HPTLC plates and run in different solvent systems. Initially, toluene: chloroform: ethanol: diethyl amine was tried in different ratios. The optimum mobile phase was found to be consisted of toluene: chloroform: ethanol: diethyl amine (4:4:1:1 v/v/v/v). The sharp peak was obtained with *Rf* value of 0.16 ±0.01 (Figure 6). In order to reduce the neckless effect, the TLC chamber was saturated for 10 minute using saturation pads. The mobile phase was run upto distance of 8cm, which takes approximately 20 minute for development of HPTLC plate.

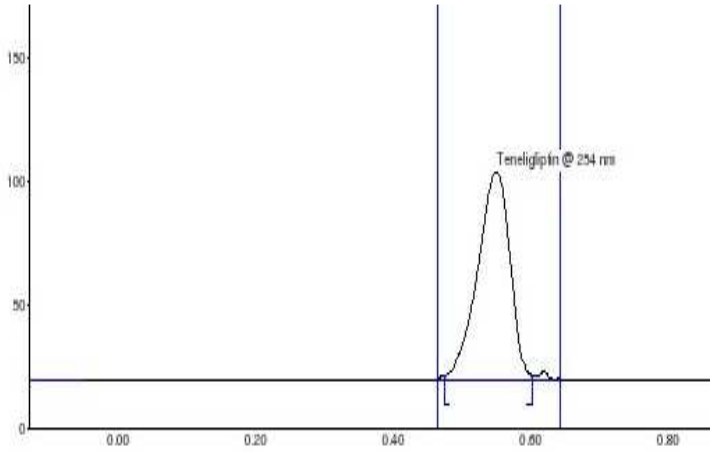

Figure 6: Densitogram of standard Teneligliptin HBr (*Rf*: 0.16 ± 0.01) toluene: chloroform: ethanol: diethyl amine (4:4:4:1:1 v/v/v)

2.3.3. Validation of the Method

Linearity

Linear relationship was observed by plotting drug concentration against peak areas. Teneliglitin showed linear response in the concentration range of 100–600 ng/spot (figure 7). The corresponding linear regression equation was Y = -207.8 + 6.264 * X with square of correlation coefficient (r^2) of 0.998 for Teneligliptin. Linear regression data is shown in table 8

Table 8: Linear regression data for Teneligliptin by HPTLC method

Parameter	Result
Linearity range	100-600 ng/spot
Regression equation	Y = -207.8 + 6.264 * X
Correlation coefficient (r^2)	0.998
Slope	6.264
Y-Intercept	- 207.8

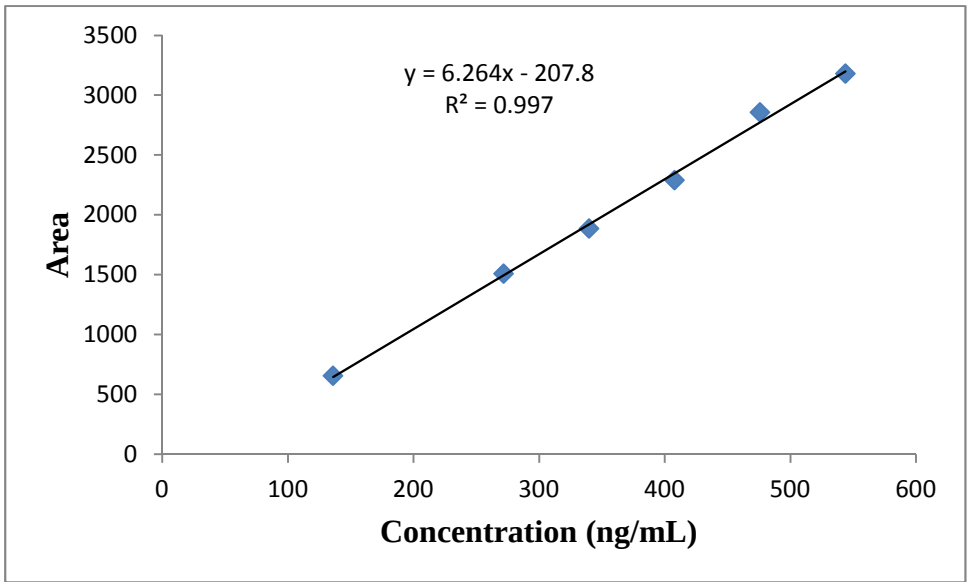

Figure 7: Plot of Concentration versus Peak area of Teneligliptin by HPTLC method

Precision

The results of the repeatability and inter-mediate precision experiments are shown in Table 9. The developed method was found to be precise as the % RSD values for repeatability and intermediate precision studies were < 2% respectively.

Table 9: Results of precision studies by HPTLC method

Concentration applied (ng/spot)	Repeatability (Intraday)		Intermediate precision (Interday)	
	Concentration found(ng/spot) ± SD (ng/spot)	% RSD (n=6)	Concentration found(ng/spot) ± SD (ng/spot)	% RSD (n=12)
300	285.777± 36.6	2.0	285.820± 0.38	0.02

n= number of determinations

Limit of Detection and Limit of Quantitation

The LOD and LOQ were found to be 136 ng/spot and 408 ng/spot respectively.

Accuracy

The developed method showed high and consistent recoveries at all studied levels. The results obtained from recovery studies are presented in Table 10. The mean % recovery ranged from 98.04 % to 100.77 %. Additionally, the obtained recoveries were found to be normally distributed with low % RSD at all concentration levels.

Table 10: Results of recovery studies by HPTLC method

Level	Standard Drug Added (ng/spot)	Drug Recovered* ± SD (ng/spot)	%Recovery	%RSD
80%	272	269.96 ± 1	99.25	0.018
100%	340	333.34 ± 1.15	98.04	0.020
120%	408	411.14 ± 1.73	100.77	0.03

**Mean of three determinations.*

Specificity

A single peak of teneligliptin in tablet solution was observed at R_f 0.16 (figure 8). No interference of excipients with the teneligliptin peak was observed.

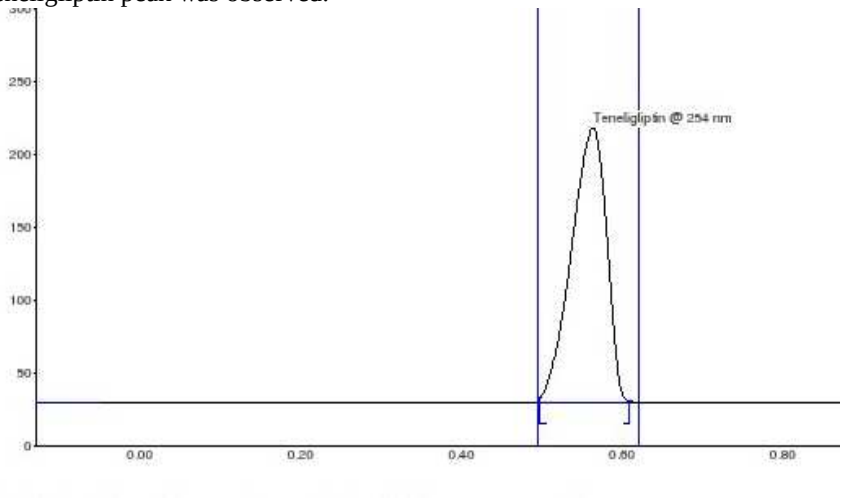

Figure 8: Chromatogram of tablet solution

Solution Stability

There was no indication of degradation in sample solutions of teneligliptin as revealed by peak purity data of solution stored at different times. The solution was found to be stable ambient temperature for 24h, and no unknown peaks were observed. The stability data is given in table 11.

Table 11: Stability data for Teneligliptin by HPTLC method

Time (h)	Concentration applied (ng/spot)	Concentration found* ± SD (ng/spot)	%RSD
1	600	597.23 ± 2.19	0.77
3	600	594.07 ± 5.20	1.70
6	600	594.91 ± 2.78	0.96

** Mean of three determinations*

The data of summary of validation parameters of HPTLC methodis listed in Table 12.

Table 12: Summary of validation parameters by HPTLC method

Parameters	Results
Linearity range	100-600 ng/spot
Regression equation	Y = -207.8 + 6.264 * X
Correlation coefficient (r^2)	0.998
Slope	6.264
Y-Intercept	- 207.8
Precision	Repeatability: % RSD = 2. 0
	Intermediate: % RSD = 0.02
Recovery	98.04 % to 100.77 %.

2.3.4. Analysis of Marketed Pharmaceutical Dosage Form

A single spot at *Rf* value of 0.16 was observed in the chromatogram of the drug samples extracted from tablet. There was no interference from the excipients that are commonly present in the formulations. The drug content was found to be 99.95%. The results are summarized in table 13. The good performance of the method indicated the suitability of this method for routine analysis of teneligliptin in pharmaceutical dosage form.

Table 13: Analysis of Tablet formulation by HPTLC method

Labelled claim(mg)	Amount found* ±SD(mg)	% Labelled claim	%RSD
20	19.85 ± 5.78	99.95	1.20

**Mean of six determinations*

CONCLUSION

Simple and reliable UV Spectrophotometric and HPTLC methods have been developed and successfully validated for estimation of Teneligliptin HBr in tablet dosage form. The results of the validation tests indicated that the developed methods were accurate, precise, robust and reproducible. Hence, the developed UV and HPTLC methods are suitable for routine determination of Teneligliptin HBr in pharmaceutical formulation in quality control laboratories, where economy and time are essential.

Acknowledgments

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