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RESEARCH ARTICLE

Development and Validation of Simple UV Spectrophotometric Method for The Determination of Teneligliptin Hydrobromide Hydrate in API and Its Bulk Dosage Form

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ABSTRACT

Simple, rapid, sensitive, precise and specific UV spectrophotometric for the determination of Teneligliptin Hydrobromide hydrate in bulk drug and pharmaceutical dosage form were developed and validated. In this method solutions of Teneligliptin HBr hydrate were prepared in water. Teneligliptin HBr hydrate standard solution was scanned in the UV range (400-200nm) in a 1cm quartz cell in a double beam UV spectrophotometer. The standard solution of Teneligliptin HBrhydrate showed maximum absorption at wavelength 243.0 nm. The method obeys beers law in the concentration range from10- 50µg/ml. The correlation coefficient was found to be 0.998 and regression of the curve was found y = 0.0223x + 0.01 with excellent recovery 99-104%. Limit of detection and limit of quantitation were found to be 0.556 µg/ml and 14.79µg/ml respectively. The method was validated for several parameters like accuracy, precision as per 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 Hydrate in bulk or tablet dosage form.

KEYWORDS

Teneligliptin HBr Hydrate, UV Spectrophotometry, Absorbance Maxima, 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 and Argentina (2012), Korea (2014) and India (2015) Teneligliptin drug inhibit the enzyme dipeptidyl peptidase-4 which degrades incretin, a hormone adjusting blood glucose level.[11,12,13]

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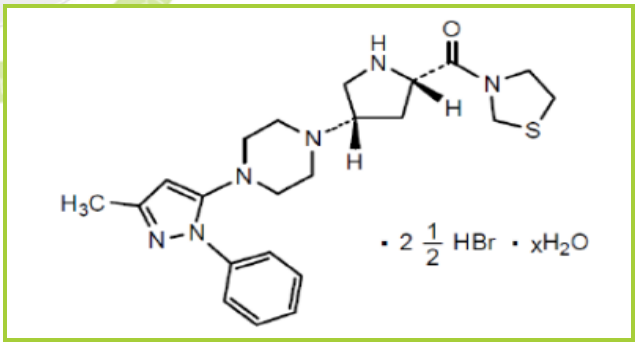

Fig. 1: Chemical Structure of Teneligliptin Hydrobromide Hydrate[1]

Teneligliptin,{(2S,4S)-4-[4-(3-methyl-1-phenyl-1H-pyrazol-5-yl) piperazin-1yl] pyrrolidin-2-yl}(1,3-thiazolidin-3-yl) methanone Hemi Penta hydrobromide hydrate exhibits a unique structure that is characterized by five consecutive rings (Figure 1)[11-12]. Recent studies have revealed that

this drug is unique in its nature and exhibits multiple pharmacological effects. It includes vasoprotective, neuroprotective effects. It is a white fine powder which is freely soluble in water and DMSO, sparingly soluble in methanol, slightly soluble in ethanol, and insoluble in acetonitrile.[7]

The literature review revealed a simple UV spectroscopic method development and validation of teneligliptin HBr hydrate in tablet dosage form, and a stability indicating RP-HPLC method for development and validation of teneligliptin HBr hydrate in pure and tablet dosage for analytical method development and validation for simultaneous estimation of teneligliptin HBr Hydrate and metformin hydrochloride from its pharmaceutical dosage form by three different UV spectroscopic methods. No official or draft monograph of Teneligliptin Hydrobromide Hydrate was published in any of the pharmacopeia for compendia applications.[8, 9, 10]

MATERIALS AND METHOD

Instruments:

For Weighing, a calibrated weighing balance (Make- Shimadzu) of 1mg sensitivity was used. For analytical purpose Shimadzu- 1800 UV Spectrophotometer and Shimadzu 2501 PC UV-Vis Recording Spectrophotometry was used. All other glasswares and apparatus were made of Borosilicate and were calibrated.

Chemicals:

API-Teneligliptin is pure drug gifted by MVPs College of pharmacy, Nashik. Tablets of 20 mg strength were purchased from the local pharmacy in Nashik under commercially available brand name Tenure (Glenmark pharmaceutical Ltd.), distilled water was used in this study.

Preparation of standard stock solution:

The standard stock solution of teneligliptin was prepared by transferring, accurately weighed 100 mg of teneligliptin to 100 ml volumetric flask containing 50ml distilled water. Dissolve drug properly. Then volume was made up to the mark by using distilled water to gives concentration 1000µg/ml. From this 10 ml of the solution was transferred to a 100 ml volumetric flask and make up the volume with distilled water to gives a concentration of 100µg/mL which is a standard stock solution and it is further diluted with distilled water to get concentration 10-50µg/ml.

Determination of Absorption Maxima:

The appropriate dilution of the standard stock solution with distilled water, solution contain 50µg/ml of teneligliptin HBr hydrate was scanned in the range of 400-200nm to determine the wavelength of maximum Absorption. The drug showed Absorption maxima at 243.0 nm.

Preparation of Calibration Curve

For the preparation of standard calibration curve, the concentration of 10-50 µg/ml were prepared by pipetting out 1, 2, 3, 4 and 5ml of the 100µg/ml solution into a 10ml volumetric flask and made up the volume with distilled water. The absorbance of each solution was measured at 243.0 nm against distilled water as a blank. Calibration curve of the teneligliptin was plotted by taking the absorbance obtained on the y-axis and the concentration of the solution on the x-axis (Fig. 2). The curve showed linearity in the range of 10-50 µg/ml with correlation coefficient 0.999.

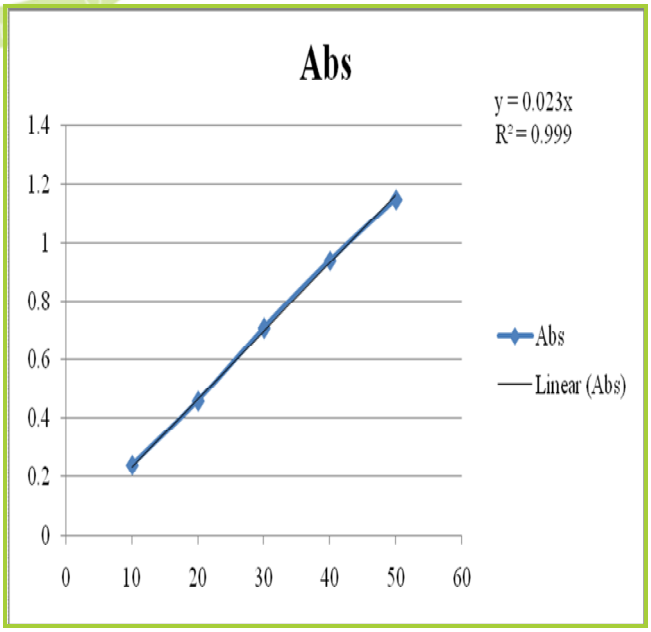

Fig. 2: Calibration Curve of Teneligliptin HBr Hydrate

Quantitative analysis of pharmaceutical tablet dosage form:

Twenty tablets were weighed accurately and powdered. Powder equivalent to 50 mg teneligliptin HBr hydrate was weighed and transferred to a 100 ml volumetric flask. It was dissolved in 100 ml distilled water and sonicate for 15 minutes to get a homogeneous solution. Then it was first filtered through a 0.45µ Whatman filter paper. A final concentration of 100 µg/ml of teneligliptin was prepared. This solution was filtered through filter paper to remove some un-dissolved excipients. After filtration, from this 5 ml was taken and diluted to 10 ml with distilled water which gives 50 µg/ml solution and the absorbance of the solution was measured at 243.0 nm.

Tablet Formulation	Label claim	Amount taken	Amount found	Assay%
Tenepure	20 mg	5 mg	4.98 mg	99.60 %

Table No 1: Results obtained in the determination of teneligliptin in tablet dosage form.

METHOD VALIDATION:

The developed method was validated as per ICH guidelines for following parameters [2, 3]

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.

Linearity:

Aliquots of standard stock solution were further diluted with water to get the solutions of concentration within a range from 10–50 µg/mL. The absorbance was measured at wavelength 243.00 nm. Linear calibration graph was obtained by plotting the absorbance value versus concentration of Teneligliptin.

Range:

The Range of the analytical procedure is an interval between upper and lower concentration of an analyte in the sample for which it has been demonstrated that the analytical procedure as a suitable level of precision, accuracy, linearity.

Precision:

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

Intraday and Interday Precision (Intermediate Precision):

Intraday precision was determined by analyzing the drugs at concentrations (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 two consecutive days.

Recovery:

The accuracy of the analytical method for teneligliptin was determined at 80%, 100% and 120% levels of standard solution. Absorbance was measured at 243 nm and results were expressed in terms of % recoveries. Standard deviation and % RSD was calculated. Accuracy is reported as % recovery which was calculated from the expression as equation given below,

$$\%\ Recovery = \frac{Observedvalue}{Truevalue} \times 100$$

Repeatability:

Repeatability of the method was determined by analyzing six samples of same concentrations of the drug (30µg/mL). Spectra were recorded, and the absorbance of each spectrum was measured.

Robustness:

The robustness of the 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 the assay was evaluated. The effect of detection wavelength was studied at ± 5 nm.

Solution Stability:

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

RESULT AND DISCUSSION:

Determination of wavelength of maximum absorption:

The wavelength of maximum absorption was found to be 243.0 nm

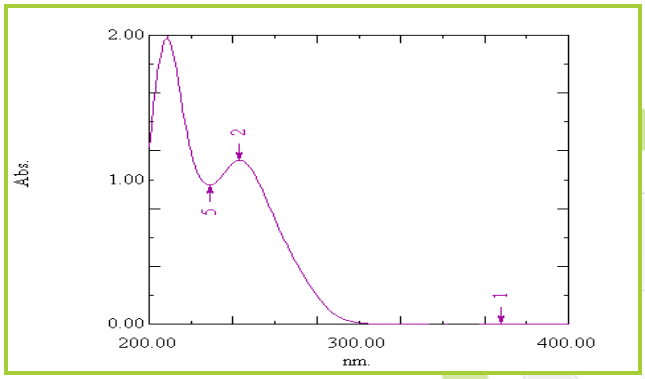

Fig 3: Wavelength of maximum absorption of Teneligliptin

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.

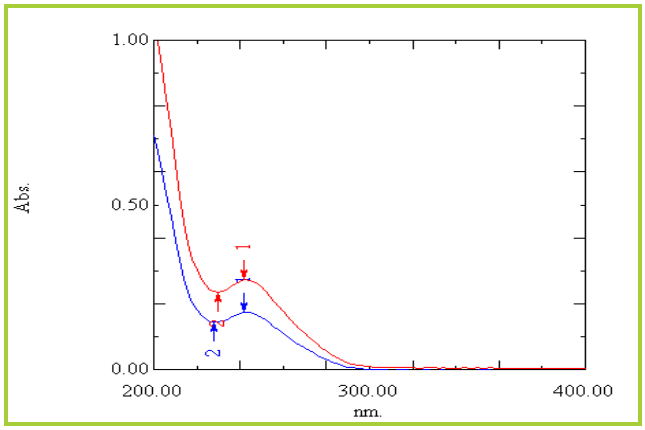

Fig: 4 Ultra-Violet spectra of Teneligliptin HBr Hydrate (API) and Tablet Dosage form

Linearity:

The linearity of this method was determined at ranging from 10-50µg/ml for teneligliptin HBr hydrate. The regression equation was found to $y = 0.023x + 0.01$ be, r^2= 0.999

Sr No	Concentration (ppm)	Absorbance
1	10	0.24
2	20	0.46
3	30	0.71
4	40	0.94
5	50	1.15

Table No.2: Linearity table

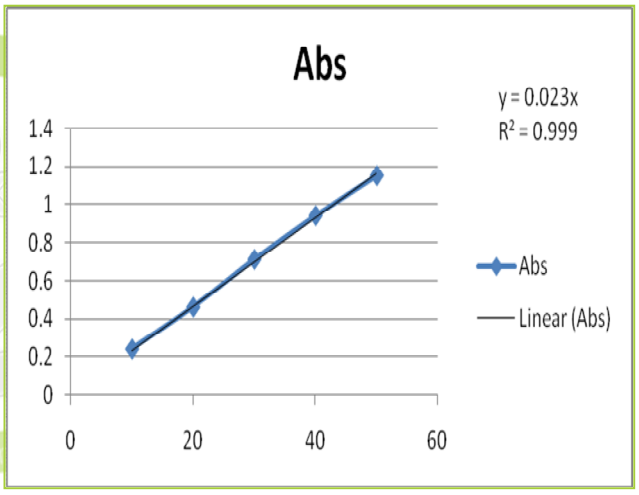

Fig 5: Linearity graph of Teneligliptin HBr Hydrate

The method for Teneligliptin HBr Hydrate was found to be linear in the range of 10-50 ppm with R^2 = 0.999and the straight line equation as y = 0.023x – 0.01

Precision: The precision (measurement of intraday, interday, repeatability) results showed good reproducibility with the percent relative standard deviation (% RSD) was below 2.0%. This indicated that method was highly precise.

Intraday precision:

Table No 3: Intraday morning precision

SN	Concentration	Morning absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	30	0.73215	0.00371	0.00001376	0.0093	1.27%
2	30	0.73217	0.00373	0.00001391		
3	30	0.71079	-0.01765	0.00003115		
4	30	0.72321	-0.00523	0.00002735		
5	30	0.74023	0.01179	0.00013900		
6	30	0.73214	0.0037	0.00001369		
		Ȳ=0.72844		Σ=0.00051923		

Table No 4: Intraday Afternoon precision

SN	Concentration	Afternoon absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	30	0.73239	0.00415	0.00001722	0.000093253	0.0128%
2	30	0.73214	0.0039	0.00001521		
3	30	0.71012	-0.01812	0.0003283		
4	30	0.72227	0.00597	0.00003564		
5	30	0.74041	0.01217	0.0001481		
6	30	0.73212	0.00388	0.00001505		
		Ȳ=0.72824		Σ=0.00055952		

Table No .5: Intraday Evening precision

SN	Concentration	Evening absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	30	0.71019	-0.11979	0.00009580	0.001892	0.32%
2	30	0.71129	-0.12089	0.014614		
3	30	0.69001	0.0996	0.0099201		
4	30	0.70019	0.10978	0.0120516		
5	30	0.71023	0.11982	0.014356		
6	30	0.72117	0.13076	0.017098		
		Ȳ=0.59048		Σ=0.0113		

Interday precision:

Table No 6: Interday morning precision Study

SN	Concentration	Morning absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	30	0.71019	0.0028	0.000078	0.0094	1.32%
2	30	0.71117	0.00386	0.0000148		
3	30	0.69002	-0.01729	0.000298		
4	30	0.7012	-0.00611	0.0000378		
5	30	0.71021	0.0029	0.0000841		
6	30	0.72112	0.013181	0.000173		
		Ȳ=0.7074		Σ=0.000539		

Table.7: Interday Afternoon precision Study

SN	Concentration	Afternoon absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	30	0.71014	0.007040	0.000049560	0.007950	1.13%
2	30	0.70027	0.00283	0.000008008		
3	30	0.69001	0.01309	0.0001713		
4	30	0.69721	0.00589	0.00003469		
5	30	0.71019	0.00709	0.000050268		
6	30	0.71119	0.00809	0.00006544		
		Ȳ=0.7031		Σ=0.0003792		

Table No 8: Interday Evening precision

SN	Concentration	Evening absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	30	0.71014	0.01354	0.0001833	0.0135	1.950 %
2	30	0.70029	0.000369	0.00001361		
3	30	0.69000	0.00066	0.00004356		
4	30	0.69721	0.00061	0.0000003721		
5	30	0.671029	0.002517	0.0006538		
6	30	0.71119	0.01459	0.0002128		
		Ȳ=0.6966		Σ=0.00018457		

Repeatability:

Table No 9: Repeatability study

SN	Concentration	Absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	30	0.73215	0.00374	0.00001398	0.0117	1.16%
2	30	0.73217	0.00376	0.00001413		
3	30	0.71072	-0.01769	0.0003129		
4	30	0.72319	0.01769	0.0003129		
5	30	0.74017	0.0045	0.00002025		
6	30	0.73209	0.01176	0.000138		
		Ȳ=0.72841		Σ=0.0008256		

3. Accuracy:

The accuracy of the analytical method for teneligliptin HBr hydrate was determined at 80%, 100% and 120% levels of standard solution. Absorbance was measured at 243.0 nm and results were expressed in terms of % recoveries

SN	% concentration	Concentration in ppm	Volume of API stock (ml)	Volume of Tablet stock (ml)	Absorbance (nm)	Mean
1	80	8	5	3	0.193	0.193
2					0.193	
3					0.192	

The concentration is calculated by using straight line equation:

$y = mx + c$

y = 0.023x 0.01

$x = (y - c)/m$

$$x = \frac{0.193 - 0.01}{0.023}$$

X= 7.95 ppm

% Recovery from 8 ppm solution: $\frac{x}{8} * 100$

= (7.95/8)* 100

= 99.45%

Table No 11: Recovery Study

%concentration	SN	Concentration in ppm	Volume of API stock (ml)	Volume of Tablet stock (ml)	Absorbance (nm)	Mean
100	1	10	5	5	0.240	0.240
					0.241	
					0.240	

The concentration is calculated by using straight line equation:

$y = mx + c$

y = 0.023x 0.01

$x = (y - c)/m$

$$x = \frac{0.240 - 0.01}{0.023}$$

X= 10 ppm

% Recovery from 10 ppm solution: $\frac{x}{8} * 100$

= (10/10)* 100

Table No12: Recovery Study

%concentration	SN	Concentration in ppm	Volume of API stock (ml)	Volume of Tablet stock (ml)	Absorbance (nm)	Mean
120	1	12	5	7	0.298	0.298
					0.298	
					0.297	

The concentration is calculated by using straight line equation:

$y = mx + c$

y = 0.023x 0.01

$x = (y - c)/m$

$$x = \frac{0.298 - 0.01}{0.023}$$

X= 12.5 ppm

% Recovery from 10 ppm solution: $\frac{x}{8} * 100$

= (12.5/12)* 100

= 104%

Limit of Detection and Limit of Quantification:

It is calculated by using slope and standard deviation from linearity and precision respectively:
Limit of detection (LOD):

$$LOD = 3.3 \times SD/Slope$$
$$LOD = 3.3 \times 0.033/0.0223$$
$$LOD = 0.556\ ppm$$

Limit of quantification (LOQ):

$$LOQ = 10 \times SD/Slope$$
$$LOQ = 10 \times 0.033/0.0223$$
$$LOQ = 14.79\ ppm$$

Robustness:

Table No 13: Robustness Study

SN	Wavelength(nm)	Absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	238	0.719	0.006	0.000036	3.5160	0.484
2	239	0.721	0.004	0.000016		
3	240	0.723	0.002	0.000004		
4	241	0.726	0.001	0.000001		
5	242	0.728	0.003	0.000009		
6	243	0.731	0.006	0.000036		
7	244	0.730	0.005	0.000025		
8	245	0.729	0.004	0.000016		
9	246	0.727	0.003	0.000009		
10	247	0.724	0.001	0.000001		
11	248	0.723	0.002	0.000004		
		Ȳ=0.725		Σ=0.0000136		

Result and Discussion: summary

SN	Validation Parameters	Results
1	Absorption maxima(nm)	243.00nm
2	Beers range (µg/ml)	10-50µg/ml
4	Standard Regression Equation	y = 0.023x + 0.01
5	Correlation Coefficient (r2)	0.999
6	Accuracy(8,10&12 ppm)	98.91%, 100%& 104%
7	Precision (%RSD)	0.53421
8	LOD &LOQ(µg/ml)	0.556 & 0.1479
9	Robustness(%RSD)	0.484
10	Assay (%)	99.60

CONCLUSION:

The UV-spectrophotometric method was developed and it is found to be simple, accurate, precise, highly sensitive, reproducible and inexpensive. The proposed method was found suitable for determination of Teneligliptin HBr Hydrate in API and its bulk dosage form without any interference from the excipients. The validation procedure confirms that this is a workable method for their quantification in the raw material and also in the formulations. Hence it can be effectively applied for the routine analysis of Teneligliptin Her Hydrate in bulk drug. Its advantages are the low cost of reagents, speed, and simplicity of sample treatment, satisfactory precision, and accuracy.

ABBREVIATIONS:

UV: Ultra Violet

µm: micrometer

Nm: nanometre

ml: milliliter

UV- Vis: Ultraviolet-Visible

API: Active Pharmaceutical Ingredient

% : Percentage

Ppm: Parts per million

API: Active Pharmaceutical Ingredient

HBr: Hydrobromide

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CONFLICT OF INTEREST:

The authors do not report any conflict of interest.

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