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RESEARCH ARTICLE

UV Spectrophotometric Method Development and Validation for Determination of Teneligliptin Hydrobromide Hydrate in API and in Pharmaceutical Dosage Form

Sanket A. Kshirsagar*, Sryesta B. Mane, Yogesh S. Hanchate, Aniket S. Katte, Kaushik V. Kulkarni

Department of Quality Assurance, D.S.T.S. Mandal's College of Pharmacy, Solapur, Maharashtra, India.

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ABSTRACT

Simple, rapid, sensitive, precise and specific UV Spectrophotometric for the determination of Teneligliptin Hydrobromide Hydrate (THH) in API and pharmaceutical dosage form were developed and validated. In this method solutions of Teneligliptin Hydrobromide Hydrate (THH) were prepared in Dimethyl Sulphoxide (DMSO). Teneligliptin Hydrobromide Hydrate (THH) 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 THH showed maximum absorption at wavelength 267.2 nm. The method obeys Beer's law in the concentration range from 20-100µg/ml. The correlation coefficient was found to be 0.999 and regression of the curve was found Y=0.012x+0.058 with excellent recovery 102-104%. Limit of detection and limit of quantification were found to be 4.1987µg/ml and 12.7233µg/ml respectively. The ruggedness and robustness were performed. 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 determination of the said drug. These methods can be adopted in the routine assay analysis of Teneligliptin Hydrobromide Hydrate (THH) in API and pharmaceutical dosage form.

KEYWORDS

Teneligliptin Hydrobromide Hydrate (THH) 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 Hydrobromide Hydrate is a novel, potent, peptidomimetic and long acting DPP-4 Inhibitor for the treatment of type -2 diabetes.

*Address for Correspondence:
Sanket A. Kshirsagar,
Department of Quality assurance, D.S.T.S. Mandal's College of Pharmacy,
Solapur, Maharashtra, India.
E mail ID: Skkshirsagar21@gmail.com

It is used to reduce hyperglycemia and dyslipidemia. The drug reduces the level of DPP- 4 enzyme responsible for degradation of incretin hormones. This hormone helps in adjusting the 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.

Teneligliptin slows the inactivation of incretin hormones, thereby increasing bloodstream concentrations and reducing fasting and postprandial glucose concentrations in a glucose dependent manner in patients with

type-2 diabetes mellitus. The inhibition of DPP-4 increases the amount of active plasma increatins, which helps with glycemic control.[2,3,4,5]

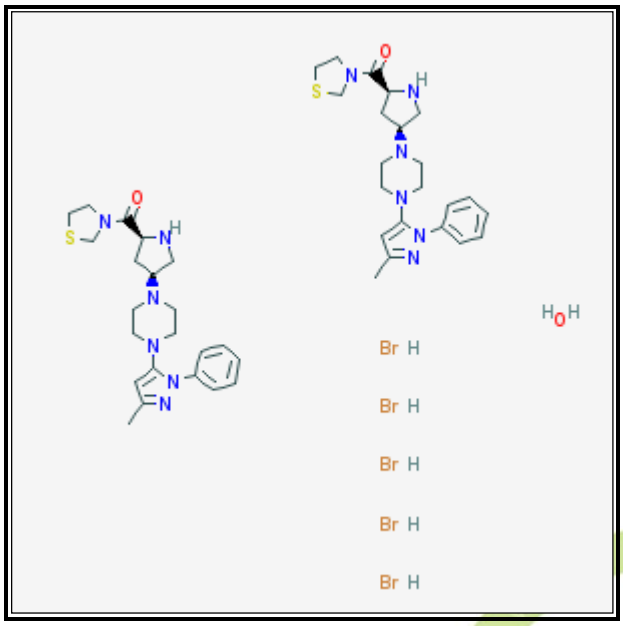

Figure 1: Chemical structure of THH[6]

THH It is a white fine powder which is freely soluble in water, methanol, Dimethyl Sulphoxide (DMSO) and insoluble in acetonitrile.

The literature review revealed a method development and validation of teneligliptin in pharmaceutical dosage form by UV Spectrophotometric methods.

Analytical method development and validation for the simultaneous estimation of metformin and teneligliptin by RP-HPLC in bulk and tablet dosage forms.

The present work is to Develop and Validate UV Spectrophometric Method for The Determination of THH in API and its Pharmaceutical Dosage Form with the help of DMSO solvent.

MATERIAL & METHODS

Instruments

For weighing, a calibrated weighing balance (Shimadzu) of 1 mg sensitivity was used.

A Systronic UV-visible double beam spectrophotometer- 2201 was used.

All other glasswares and apparatus were made up of borosilicate and were calibrated.

Chemicals

API- THH is pure drug was provided by Glenmark Pharmaceutical Ltd. India.

Tablets of 20 mg strength were purchased from the local pharmacy in Solapur under commercially available brand name DYNAGLIPT (Dicovery Mankind, Division of Mankind Pharma Ltd.), Dimethyl Sulphoxide (DMSO) 99% LR was used in this study.

UV Spectroscopic Method

Solvent Selection

Teneligliptin Hydrobromide Hydrate is soluble in Dimethyl sulphoxide (DMSO). In the present investigation DMSO was selected as a solvent.

Preparation of Standard Stock Solution

The standard stock solution of Teneligliptin Hydrobromide Hydrate (THH) was prepared by transferring, accurately weighed 10 mg of THH to 10 ml of volumetric flask containing 5ml DMSO. Dissolve drug properly. Then volume was made up to the mark by using DMSO to give concentration 1000 µg/ml. From this 2.5 ml of the solution was transferred to a 25 ml volumetric flask and make up the volume with DMSO to give a concentration of 100 µg/ml which is a standard stock solution and it is further diluted with DMSO to get concentration range of 20-100 µg/ml.

Determination of Absorption Maxima

The standard stock solution of 100µg/ml was scanned in the range of 400-200 nm to determine the wavelength of Maximum Absorption. The drug showed Absorption maxima at 267.2 nm.

Preparation of Calibration Curve

For the preparation of the calibration curve, the concentration of 20-100µg/ml were prepared by pipetting out 2, 4, 6, 8 and 10 ml of the 100 µg/ml solution into 10 ml volumetric flask and made up the volume with DMSO. The absorbance of each solution was measured at 267.2 nm against DMSO as a blank.

Calibration curve of the Teneligliptin Hydrobromide Hydrate (THH) was plotted by taking the absorbance obtained on the y-axis and the concentration of the solution on the x-axis. The curve showed linearity in the range of 20-100 µg/ml with correlation coefficient 0.999.

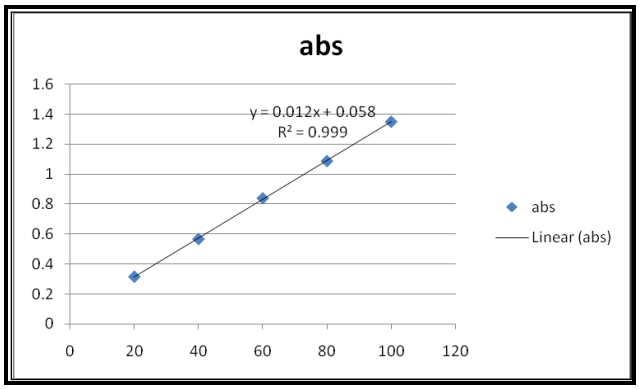

Figure 2: Calibration curve of Teneligliptin Hydrobromide Hydrate (THH)

Quantitative Analysis of Pharmaceutical Tablet Dosage Form

Twenty tablets were weighed accurately and powdered. Powder equivalent to 10 mg teneligliptin Hydrobromide Hydrate (THH) was weighed and transferred to a 10 ml volumetric flask. It was dissolved in 10 ml DMSO and sonicate for 15 minutes to get a homogeneous solution.

Then it was filtered through a 0.45 µ Whatman filter paper. A final concentration of 100 µg/ml of THH was prepared. This solution was filtered through filter paper to remove some un-dissolved excipients. After filtration, from this 6 ml was taken and diluted to 10 ml with DMSO which gives 60 µg/ml solution and the absorbance of the solution was measured at 267.2nm.

Table 1: Results obtained in the determination of THH in tablet dosage form

Tablet Form^{n}	Label claim	Amt Taken	Amt found	Assay %
DYNAGLIPT	20 mg	60 µg/ml	61.30 µg/ml	102%

Method Validation

The developed method was validated as per ICH guidelines for following Parameters

Linearity: 2, 4, 6, 8, 10 ml of Standard solution were transferred into a series of 10 ml volumetric flasks. The volume was made up to the mark with DMSO to obtain the concentration of 20, 40, 60, 80, 100 µg/ml. Then absorption of these solutions was recorded and the graph was plotted of absorption against concentration. The correlation coefficient (r^2) of least square linear regression of THH was calculated.

Range: The Range of the analytical method was decided from the interval between upper and lower level of calibration curve by plotting curve.

Accuracy: Recovery study was carried out by the standard addition method by adding a known amount of THH to the preanalyzed sample at three different concentration levels that is 80%, 100%, 120% of assay concentration and percent recovery were calculated.

2 ml of tablet solution was transferred to 4 different 10 ml volumetric flasks (labelled as blank, 80%, 100%, 120%) separately and 0, 1.6, 2, 2.4 ml of 100 µg/ml standard solution was added respectively and the volume was made up to the mark with DMSO. Absorbances were noted for these samples.

Standard deviation and %RSD was calculated. Accuracy is reported as % recovery, which was calculated from the expression as equation given below,

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

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 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 (60µg/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.

Repeatability: Repeatability of the method was determined by analyzing six samples of same concentrations of the drug (60µg/ml). Absorbance of each 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 deliberate and the assay was evaluated. The effect of detection wavelength was studied at ±5 nm.

Ruggedness: Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.

Limit of Detection: Detection limit was determined based on the standard deviation of absorbance of same concentration that is a standard solution of THH (60µg/ml) prepared six times and LOD calculated by

$$LOD = 3.3 (SD/S)$$

Where, SD- standard deviation; S= slope of the curve

Limit of Quantification: Quantification limit was determined based on the standard deviation of peak area of same concentration that is standard solution THH (60µg/ml) prepared six times and LOQ calculated by

$$LOD = 10 (SD/S)$$

Where, SD= standard deviation; S= slope of the curve

RESULTS

Determination of Wavelength of Maximum Absorption

The wavelength of maximum absorption was found to be 267.2 nm.

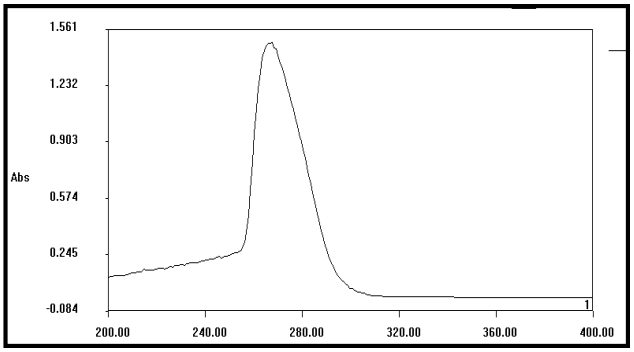

Figure 3: Wavelength of maximum absorption of THH

Linearity

The linearity of this method was determined at ranging from 20-100 µg/ml for THH.

The regression equation was found to be Y=0.012x+0.058 be, r^2=0.999.

Table 2: Linearity table

Sr. No	Concentration (µg/ml)	Absorbance
1	20	0.318
2	40	0.569
4	60	0.840
4	80	1.088
5	100	1.349

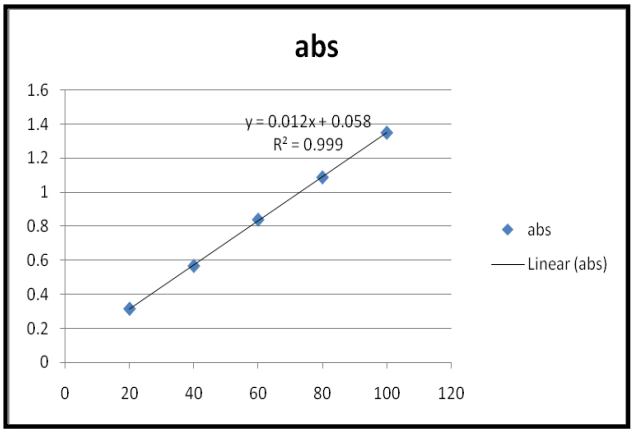

Figure 4: Linearity graph of THH

The linearity for THH was found to be linear in the range of 20-100µg/ml with r^2= 0.999 and the straight line equation as Y= 0.012x+0.058.

Accuracy

The accuracy of the analytical method for THH was determined at 80%, 100% and 120% levels of standard solution. Absorbance was measured at 267.2nm and results were expressed in terms of % recoveries.

Precision

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

Table 3: Table for accuracy

Sr. No	Level of % Recovery	Amount of Tablet sample (ml)	Amount of standard drug added (µg/ml)	Amount added µg	Amount found (µg/ml)	% Recovery
1	0	2	0	0	0	
2	80	2	1.6	36	36.92	102.5%
3	100	2	2	40	41.38	103%
4	120	2	2.4	44	45.84	104%

Intraday precision

Table 4: Intraday morning precision

Sr. no	Concentration (µg/ml)	Absorbance	SD	% RSD
1	60	0.951		
2	60	0.912		
3	60	0.920	0.015268	1.65%
4	60	0.911		
5	60	0.914		
6	60	0.915		
		ȳ=0.9205		

Table 5: Intraday afternoon precision

Sr. no	Concentration (µg/ml)	Absorbance	SD	% RSD
1	60	0.970		
2	60	0.970		
3	60	0.963	0.015639	1.60%
4	60	0.973		
5	60	0.967		
6	60	1.006		
		ȳ =0.9748		

Table 6: Intraday evening precision

Sr. no	Concentration (µg/ml)	Absorbance	SD	% RSD
1	60	0.943		
2	60	0.946		
3	60	0.940	0.017108	1.80%
4	60	0.945		
5	60	0.943		
6	60	0.985		
		ȳ =0.9503		

Interday Precision

Table 7: Interday morning precision study

Sr. no	Concentration (µg/ml)	Absorbance	SD	% RSD
1	60	0.970		
2	60	0.974		
3	60	0.966		
4	60	0.972	0.017378	1.77%
5	60	0.968		
6	60	1.012		
		ȳ =0.977		

Table 8: Interday afternoon precision study

Sr. no	Concentration (µg/ml)	Absorbance	SD	% RSD
1	60	0.930		
2	60	0.933		
3	60	0.929	0.015702	1.67%
4	60	0.933		
5	60	0.929		
6	60	0.969		
		ȳ =0.93716		

Table 9: Interday evening precision study

Sr. no	Concentration (µg/ml)	Absorbance	SD	% RSD
1	60	0.941		
2	60	0.944		
3	60	0.938		
4	60	0.943	0.016702	1.76%
5	60	0.941		
6	60	0.982		
		ȳ =0.9481		

Repeatability

Table 10: Repeatability study

Sr. no	Concentration (µg/ml)	Absorbance	SD	% RSD
1	60	0.951		
2	60	0.912		
3	60	0.920	0.015268	1.65%
4	60	0.911		
5	60	0.914		
6	60	0.915		
		$\bar{y}$ =0.9205		

Limit of Detection

Table 11: For Limit of Detection

LOD (µg/ml)	4.1987 µg/ml

Limit of Quantification

Table 12: For Limit of Quantification

LOQ (µg/ml)	12.7233 µg/ml

Robustness

Table 13: Robustness study

Sr. No	Wavelength (nm)	Absorbance	SD	% RSD
1	262.2	1.029		
2	263.2	1.041		
3	264.2	1.037		
4	265.2	1.023		
5	266.2	1.004	0.083828	8.79%
6	267.2	0.974		
7	268.2	0.944		
8	269.2	0.914		
9	270.2	0.876		
10	271.2	0.842		
11	272.2	0.805		
		$\bar{y}$ =0.95354		

Ruggedness

Ruggedness was determined by carrying out analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.

Table 14: For Ruggedness

Analyst-1		
Concentration (µg/ml)	**Absorbance**	**Statistical analysis**
60	0.972	
60	0.974	Mean=0.973
60	0.973	SD=0.000894
60	0.974	%RSD=0.091%
60	0.972	
60	0.973	
Analyst-2		
60	1.017	
60	1.017	Mean=1.0166
60	1.017	SD=0.000816
60	1.017	%RSD=0.080%
60	1.017	
60	1.015	

DISCUSSION

Preliminary Analysis of Teneligliptin Hydrobromide Hydrate (THH)

Preliminary analysis of THH such as description, solubility was performed.

UV-spctrophotometry for Teneligliptin Hydrobromide Hydrate (THH)

Teneligliptin Hydrobromide Hydrate (THH) being UV absorbing has been successfully employed for its quantitative determination by UV Spectrophotometric method. Being soluble in DMSO, stock solutions and working standards were made in DMSO. The maximum wavelength of absorption of a drug was determined by taking scan of the drug solution in the UV region (200-400 nm).the correlation of the standard curve for the drug was 0.999. The accuracy was from 102-104%at 267.2 nm.

The proposed method showed absorption maxima at 267.2 nm and obeyed Beer's law in the concentration of 20-100 µg/ml. The limit of detection (LOD) was found to be 4.1987 µg/ml and limit of quantification (LOQ) to be 12.7233 µg/ml respectively. All statistical data prove the validity of the proposed method, which can be applied for routine analysis of THH.

Assay of Tablet Formulation

Amount of drug present in tablet formulation was calculated using equation at 267.2nm, and Y=0.012x+0.058 and the amount of THH was found to be 102% of label claim respectively.

This method can be employed for routine analysis of THH.

SUMMARY AND CONCLUSION

Summary of UV Spectrophotometeric Method of Teneligliptin Hydrobromide Hydrate (THH)

Table 15: For Summary

Sr. No	Parameters	Values
1	Beer's law limit (µg/ml)	20-100
2	Absorption maxima (nm)	267.2
3	Standard Regression Equation	Y=0.012x +0.058
4	Correlation Coefficient (r^2)	0.999
5	Accuracy	102-104%

6	Precision (%RSD) Repeatability	1.65%
7	LOD	4.1987 µg/ml
8	LOQ	12.7233µg/ml
9	Robustness (%RSD)	8.79%
10	Ruggedness (%RSD)	0.0918807 and 0.0802675
11	Assay (%)	102%

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 Hydrobromide Hydrate (THH) in API and its pharmaceutical dosage form without any interference from the excipients. This method can be effectively applied to the routine analysis of Teneligliptin Hydrobromide Hydrate (THH) in API. Its advantages are the low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

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