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Original Article

Analytical method development and validation of teneligliptin hydrobromide in pure form by HPLC

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ABSTRACT

A Simple, rapid, sensitive, precise and cost effective High-performance liquid chromatographic (HPLC) method was developed for the Teneligliptin Hydrobromide in pharmaceutical dosage form. The separation was carried out on Protecol C18 ENDURO 250mm×4.6mm ID 5µm 120A column using with mobile phase comprising mixture of Methanol: Buffer (pH 3.5) in the ratio of 72:28 v/v, as the mobile phase at a flow rate 1 ml/min and the detection was carried out using UV-visible detector at 243.5 nm. The method was validated by evaluation of different parameters such as accuracy, precision, linearity, ruggedness, robustness, LOD and LOQ. The retention time were found to be 5.8. Calibration curves were linear with correlation coefficient (r2) 0.998 and concentration range of 10-90 µg/ml. The percentage recovery for Teneligliptin HBr was found to be in the range between 92.08- 100.30. Method was found to be reproducible with relative standard deviation (RSD) for intra and inter day precision less than 2%. The developed methods were validated as per the ICH guidelines.

Keywords: Teneligliptin Hydrobromide, HPLC, Method Validation.

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INTRODUCTION

Diabetes is the biggest cause of renal failure worldwide. Diabetes treatment is a very important factor in the overall survival of hemodialysis (HD) patients. While insulin therapy is the primary treatment for HD patients, impaired eyesight caused by diabetic retinopathy and aging-related dementia make multiple daily insulin injections difficult for many patients. Moreover, in HD patients, many diabetes oral medicines cause serious side effects such as hypoglycemia and lactic acidosis. Hence, the development of new diabetes oral medicines with little or no side effects is needed for these patients.

Dipeptidase-4 (DPP-4) inhibitors are the most highly used diabetic drugs and show both a lower incidence of hypoglycemia and good safety. In addition, they induce an ingestion control effect and may also prevent atherosclerosis and reduce cardiovascular events. Therefore, these medications are strongly expected to improve the quality of life and prognosis of diabetic HD patients.

As a new class of diabetic medications, sodium-glucose co-transporter 2 inhibitors both inhibit glucose reabsorption in renal tubules and increase glucose excretion, but cannot be administered to dialysis patients. G-protein–coupled receptor 40 agonist, GPR119 receptors agonist, and glucokinase activators are new antidiabetic medications currently in clinical trials and thus are not yet available. Therefore, DPP4 inhibitors have been the mainstay drugs during the past several years for HD patients with diabetes. Accordingly, a comprehensive research of the pharmacokinetics and pharmacodynamics of DPP-4 inhibitors in HD patients is important. Some reports have investigated the effectiveness of DPP-4 inhibitors in HD patients [. However, there has been no review of new treatment strategies for HD patients who have diabetes and limited choices for its treatment[1].

MATERIAL AND METHOD FOR HPLC

Preparation of standard stock solution: Accurately weighed Teneligliptin HBr equivalent to 10 mg of Teneligliptin working standard was transferred into a 100 ml volumetric flask it was dissolved with Water which further sonicated for 10 min. The volume was made up to 100 ml with Water to give the solution containing 100 µg/ml of Teneligliptin HBr.

Selection of λ max: The standard stock solution was further diluted with water to get a 20 µg/ml of concentration. The solution was scanned between 200 and 400 nm using water as blank. The UV spectrum of Teneligliptin HBr in water had shown λ max at 243.50 nm. The 243.50 nm was selected for the analysis of Teneligliptin HBr in bulk and tablet formulation[3]. (fig no.3)

Preparation of the calibration curve: Aliquots of standard stock solution were further diluted with water to get the solutions of concentration 2–90 µg/ml. The absorbance was measured at 243.50 nm against water as blank. All measurements were repeated three times for each concentration. The calibration curve was constructed by plotting mean of absorbance against corresponding concentration.

Preparation of the sample solution: Teneligliptin HBr bulk drug was obtained from Glenmark Pharmaceutical LTD, (*Sinnar*, India), the commercially tablets of Teneligliptin HBr were available in Indian market; hence we have purchased form Indian market. 20 mg Teneligliptin HBr equivalent to 20mg Teneligliptin was accurately weighed and dissolved in small amount of Water in 50 ml volumetric flask and then the volume was adjusted with Water, the resultant solution gives the concentration of 1mg/ml ie.1000 µg/ml (stock –I solution). From this 10 ml solution was taken and then diluted up to 100 ml with the same solvent in a volumetric flask and then the concentration of this stock will be 100 µg/ml (II stock solution). From this II stock solution, 2 ml solutions was pipette out and volume was made to 10 ml using water as a solvent to get concentration of 20 µg/ml.The absorbance of these solutions was measured at 243.50 nm. This procedure was repeated for six times. The amount of Teneligliptin HBr present in formulation was calculated by comparing it with standard absorbance.

Linearity: The standard solution was prepared by dilution of stock solution containing 1000 µg/ml. Linearity test solution for the method were prepared at different concentration level ranging from 2-90 µg/ml of analyte concentration. Linear calibration graph was obtained between absorbance versus concentration of Teneligliptin HBr drug. Linear regression data is shown in (Table 1) and (Fig 4).

Accuracy: To ensure accuracy of the method, recovery studies were performed by standard addition method at 80%, 100% and 120% level to reanalyzed sample and subsequent solution were reanalyzed. At each level, three determinations were performed. The developed method was found to be accurate, indicated by means of % recoveries ranging from 97.40 to 98.70% in (table 03).

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. The %RSD values for intraday and interday precision were found to be less than 2%. The results are summarized in (Table 04A, 04 B & 04 C).

Robustness: The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method, the experimental conditions were deliberately altered and assay was evaluated. The effect of detection wavelength was studied at ±2 nm. For changes of conditions, the sample was assayed in triplicate. The results are summarized in Table 05.

Stability: These results of stability studies indicate that the solution was stable for 5 day at ambient temperature. The % RSD of assay was 0.92 % after 5 day. The results are shown in (Table no. 06)

METHOD DEVELOPMENT

For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choice of stationary and mobile phases. The following studies were conducted for this purpose[4].

Detection wavelength: The spectra of diluted solutions of the Teneligliptin HBr in water were recorded on UV spectrophotometer. The peaks of maximum absorbance wavelengths were observed. The spectra of the Teneligliptin HBr showed that a balanced wavelength was found to be 243.50 nm[3].

Choice of stationary phase: Development trials have performed with Protecol C18 ENDURO 250mm×4.6mm ID 5µm 120 A finally the expected separation and shapes of peak were succeeded in same column[5].

Selection of the mobile phase: In order to get sharp peak and base line separation of the components, the author has carried out a number of experiments by varying the composition of various solvents and its flow rate. To effect ideal separation of the drug under isocratic conditions, mixtures of solvents like water, methanol and Acetonitrile with or without different buffers in different combinations were tested as the mobile phases on a C18 stationary phase. A mixture of Methanol and Buffer (ph 3.5) in the ratio of 28:72 v/v was proved to be the most suitable of all the combinations since the chromatographic peaks obtained were better defined and resolved and almost free from tailing[6].

Flow rate: Flow rates of the mobile phase were changed from 0.5 - 2.0 ml/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 1.0 ml/min flow rate was ideal for the successful elution of the analyte.

Mobile phase preparation: The mobile phase consisted of Buffer: Methanol (28:72) v/v, PH 3.5. Mobile phase was mixed properly and filtered through a 0.45 µm nylon membrane and degassed in an ultrasonic bath.

EXPERIMENTAL

Diluents preparation

Preparation of 0.1 n HCL: 8.5 ml conc. HCL transfer into 1000 ml volumetric flask then add 500 ml Water dilute it and make up volume up to 1000 ml.

Blank preparation: Mobile phase is used as a blank.

Buffer preparation: Accurately weigh 2.167 gm of octane sulphonic acid sodium salt and transfer into 1000 ml volumetric flask. Add 500 ml of diluents to dissolve the substance by sonication for one minute then dilute to volume with diluent.

Preparation of Standard stock solution: To prepare a stock solution (100µg/ml) for assay, accurately weigh Teneligliptin HBr equivalent to 10 mg of Teneligliptin reference standard and transfer into 100 ml volumetric flask. Add 70 ml of diluent to dissolve the substance by sonication for one minute and then dilute to volume with diluent. The concentration obtained is 100 µg/ml of Teneligliptin.

Preparation of sample solution: Teneligliptin HBr bulk drug was obtained from Glenmark Pharmaceutical Ltd, (*Sinnar*, India), the commercially tablets of Teneligliptin HBr were available in Indian market; hence we have purchased form Indian. Teneligliptin HBr equivalent to 50mg of Teneligliptin was accurately weighed and dissolved in small amount of diluents in 50 ml volumetric flask and then the volume was adjusted with diluents, the resultant solution gives the concentration of 1 mg/ml i.e. 1000 µg/ml the solution was filtered through Whatman filter paper. From this filtrate, 10 ml was transferred to a 100 ml volumetric flask and diluted with diluents to 100 ml in order to obtain the final concentration of 100 µg /ml. This solution was filtered through a 0.45 µm nylon syringe filter. The concentration obtained is 100 µg /ml of Teneligliptin[6 to16].(Table 7&8) (Fig no.5)

Linearity: The standard solution was prepared by dilution of stock solution containing test solution for the method were prepared at different concentration level ranging from 10-90 µg/ml of analyte concentration. Linear calibration graph was obtained between Areas versus concentration of drug. Linear regression data is shown in (Table 09) and (Fig 06).

Precision: Precision was performed by preparing three set of standard solution (Conc. 20 µg/ml 30µg/ml) Teneligliptin HBr and reproducibility of result. Precision of the method was determined in terms of repeatability and intraday and interday precisions which are given in (table 10A, 10B and 10C and 10D).

Accuracy: To ensure accuracy of the method, recovery studies were performed by standard addition method at 80%, 100% and 120% level to pre-analyzed sample and subsequent solution were reanalyzed. At each level, three determinations were performed.(table no. 11)

Robustness: Robustness of the method was determined by making slight changes in the chromatographic conditions as per ICH guidelines. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed and System suitability parameters were found to be within acceptable limits. Results are shown in Table indicating that the test method was robust for all variable conditions. Hence the method was sufficiently robust for normally expected variations in chromatographic conditions.(Table no.12)

LOD & LOQ: The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision, and variability. The LOD, LOQ were calculated as LOD = 3.3 σ Where σ is the standard deviation of the S is the slope of the standard curve.(Table no.13)

LOD = 3.3 × S.D. / SLOPE
= 3.3 × 470.69/ 65789
LOD = 0.023

LOQ = 10 × S.D. / SLOPE
= 10 ×470.69 / 65789
LOQ = 0.071

DISCUSSION AND CONCLUSION

The developed method was found to be linear over a range of 10µg/ml to 90 µg/ml with LOD of 0.023µg/ml and LOQ of 0.071µg/ml. It was found to be precise, accurate, robust and rugged (suggested by low value of % RSD) and can be used for determination of Teneligliptin Hydrobromide in pure form and pharmaceutical preparation successfully.

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Sr.No	Cone	**Absorbance**
1	2	0.063
2	4	0.114
3	8	0.227
4	10	0.285
5	20	0.564
6	30	0.848
7	40	1.143
8	50	1.443
9	60	1.721
10	70	2.013
11	80	2.300
12	90	2.649

Table 1:-Result of Linearity by UV Method

Sr. No	Parameter	Result
1	λ max (nm)	243.5
2	Beer's law limit (µg/ml)	2–90
3	Correlation coefficient (r^2)	0.999
4	Regression equation	y = 0.029x-0.010
5	Slope (m)	0.029
6	Intercept (c)	-0.010

Table 2: Linear regression data by UV Method

Level of addition (%)	Absorbance		Mean	Conc.	Amt of std drug added (µg/ml)	Amt. Recovered (µg/ml)	%Recovery
	R1	1.O87					
	R2	1.072					
	R3	1.075					
	R1	1.193					
	R2	1.188					
	R3	1.212					
	R1	1.327					
120	R2	1.298	1.313	45.27	24	25.27	98.70
	R3	1.313					

Table 03:- Results of Accuracy of Teneligliptin by UV Method

Sr.No.	Conc. (µg)	Absorbance	Mean	S.D.	%RSD
1		0.567			
2		0.575			
3	20	0.577	0.572	0.00558	0.98
4		0.564			
5		0.577			
6		0.569			

Table 04 A: - Result of Repeatability by UV Method

Sample Conc. (µg/ml)	SD	%RSD
10	0.00	0.00
20	0.0035	0.62
30	0.0031	0.25

Table 04 B: - Result of Intraday Precision by UV Method

Sample concentration (µg/ml)	%RSD			
	Day1	Day2	Day3	Mean
10	0.0052	0.0053	0.0050	0.0051
20	0.0075	0.0076	0.0072	0.0074
30	0.0055	0.0052	0.0050	0.0052

Table 04 C: - Result of Inter day Precision by UV Method

Method wavelength(nm)	Condition (nm)	%Assay	%RSD
243.50	241.50	99.12	0.040
	245.50	98.50	0.030

Table 05: Result of robustness studies by UV Method

Ingredient	Time(days)	%Assay	%RSD
Teneligliptin HBr	1	99	0.86
	2	98	1.06
	3	100.1	0.78
	4	99	0.86
	5	102	0.92

Table 06:- Stability of Teneligliptin HBr by UV Method

Sr.No.	Mobile Phase	Observation	Result
01	Methanol :Water (80:20)	Poor Resolution	Method Rejected
02	Methanol :Water (90:10)	Poor Resolution	Method Rejected
03	Methanol :Phosphate Buffer (80:20)	Poor Resolution	Method Rejected
04	Methanol :Buffer (75:25)	Resolution	Method Rejected
05	Methanol :Buffer (65:35)	Resolution	Method Rejected
06	**Methanol :Buffer (72:28)**	**Good resolution with acceptable PpppPikeSystem suitabilityparameters**	**Method accepted**

Table No.07: Selection of Mobile Phase

Parameter	**Value**
Mobile phase	Methanol :Buffer (72:28)v/v
Pump mode	Isocratic
pH of water	pH3.5
Diluent	0.1NHCL
Column	Protecol C18 ENDURO 250mm×4.6mm ID5µm 120A
Temp	Ambient
Wavelength	243.5nm
Injection Volume	20µl
Flow rate	1.0mL/min
Run time	12min
Typical RT	5.8min

Table 08: Optimized chromatographic conditions for estimation of Teneligliptin HBr by HPLC Method

Sr.No	Conc. (µg/ml)	Area
1	10	308.147
2	20	933.494
3	30	1377.182
4	40	1872.074
5	50	2307.582
6	60	2936.151
7	70	3292.438
8	80	3843.674
9	90	4232.915
Correlation Coefficient (r2)=0.998		
Slope (m)= 48.87		
Y-Intercept= 98.83		

Table 09: Result of Linearity by HPLC Method

Sr.No.	Conc.	Area
1	20	901.138
2	20	914.885
3	20	903.8
4	20	883.371
5	20	879.111
6	20	903.908
	Avg.	897.7022
	S.D	13.666
	%RSD	1.522

Table 10A - Result of Repeatability by HPLC Method

Sr.No.	Conc.	Replicate	Area	Mean	S.D	%RSD
1	20	R1	899.339	907.9837	8.137	0.63
		R2	915.494			
		R3	909.118			
2	30	R1	1284.489	1317.918	29.236	2.21
		R2	1330.555			
		R3	1338.71			

Table 10 B - Result of Inter Day Precision - Day –1 by HPLC Method

Sr.No.	Conc.	Replicate	Area	Mean	S.D	%RSD
1	20	R1	898.696	900.171	3.341	0.37
		R2	903.996			
		R3	897.822			
2	30	R1	1375.401	1372.485	8.811	0.64
		R2	1379.468			
		R3	1362.585			

Table 10 C –Result of Inter Day Precision-Day-2 by HPLC Method

Sr.No.	Conc.	Replicate	Area	Mean	S.D	%RSD
1	20	R1	915.494	907.984	8.137	0.89
		R2	899.339			
		R3	909.118			
2	30	R1	1338.71	1341.247	12.165	0.906
		R2	1354.48			
		R3	1330.55			

Table 10 D – Result of Intra Day Precision by HPLC Method

Level of Addition	Areas		Mean	Conc.	Amount. Of Std Drug added (µg/ml)	Drug Recovered (µg/ml)	%Recovery (µg/ml)
80%	R1	1851.775	1827.071	37.38	16	21.38	100.3
	R2	2005.854					
	R3	1623.585					
100%	R1	2105.046	1964.144	40.19	20	20.19	94.8
	R2	1890.456					
	R3	1896.931					
120%	R1	2167.355	2117.923	43.33	24	19.33	92.083
	R2	2114.37					
	R3	2072.044					

Table 11: - Result of Accuracy by HPLC Method

Parameter	Conditions and Sets	Variations	Parameter		
			Area	SD	%RSD
Flow rate (ml min-1) (±0.1mL)	**Low Flow**				
	Set-1	0.9	1399.502	14.127	0.99
	Set-2	0.9	1427.745		
	Set-3	0.9	1412.926		
		Avg	1413.391		
	High Flow				
	Set-1	1.1	1145.481	7.495	0.65
	Set-2	1.1	1143.986		
	Set-3	1.1	1131.816		
		Avg	1140.428		
M.P Composition (Methanol: Buffer)	**Low comp**				
	Set-1	74:26	1436.887	28.322	1.99
	Set-2	74:26	1436.021		
	Set-3	74:26	1387.404		
		Avg	1420.104		
	High comp				
	Set-1	70:30	1378.885	20.242	1.49
	Set-2	70:30	1356.602		
	Set-3	70:30	1338.473		
	Average		1357.987		

Table.12- Results of Robustness by HPLC Method

Sr.No	Conc. (µg/ml)	Area	Mean	S.D.
1.	0.1	86125	86076	470.69
2.	0.1	85965		
3.	0.1	86782		
4.	0.1	86395		
5.	0.1	85725		
6.	0.1	85468		

Table 13:- Result of LOD AND LOQ by HPLC Method

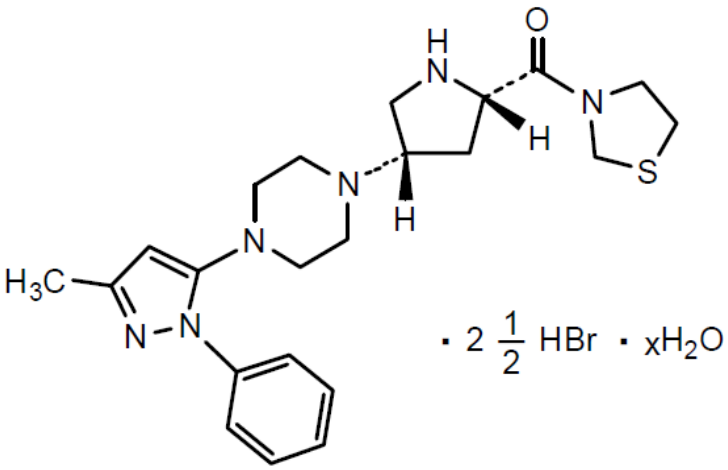

Fig 1: STRUCTURAL FORMULA

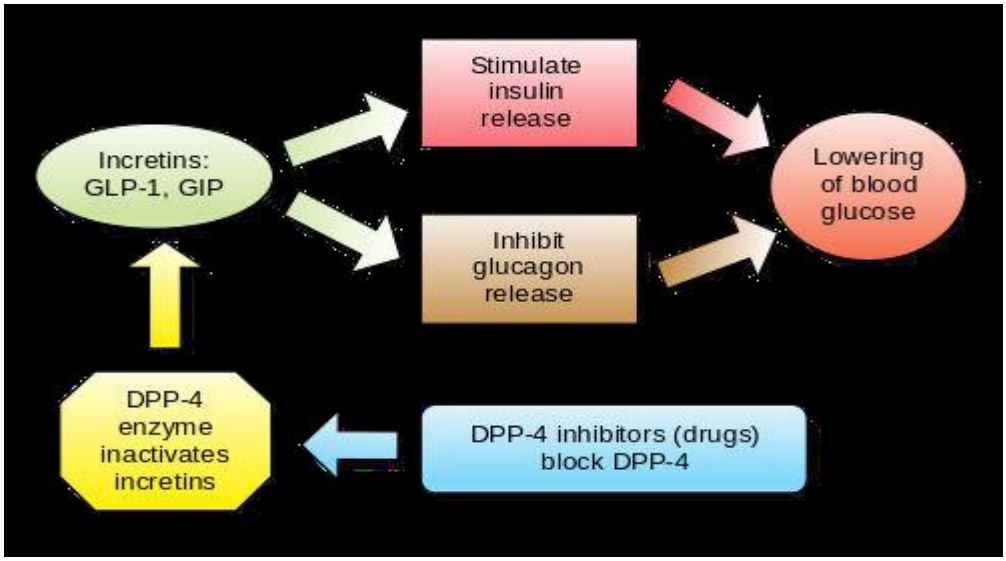

Fig 2: Mechanism of Action for Teneligliptin HBr

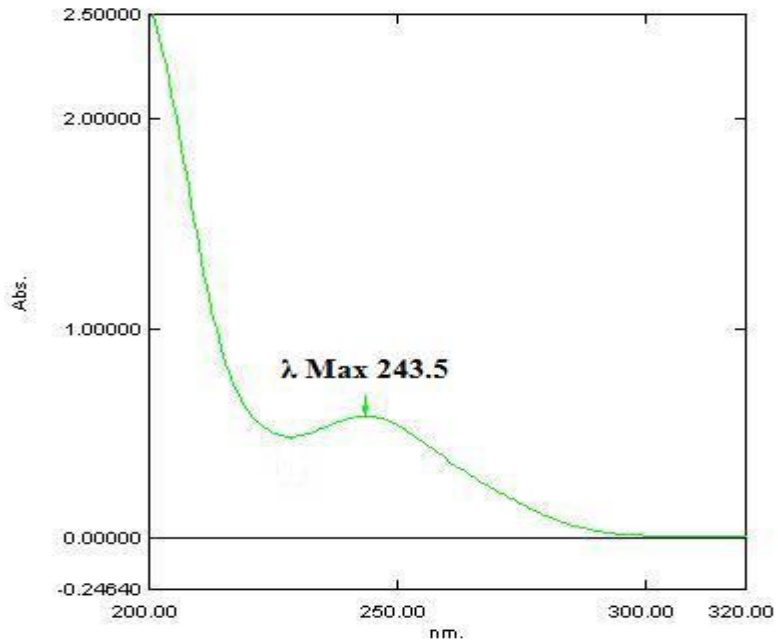

Fig3: UV spectrum of pure drug Teneligliptin HBr in water. (20 ppm)

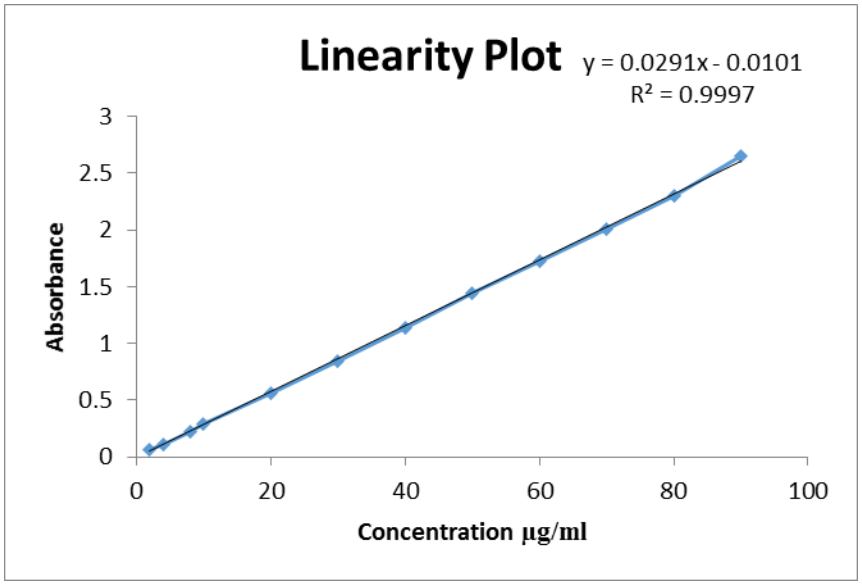

Fig 4: Linearity Plot of Teneligliptin HBr by UV Method

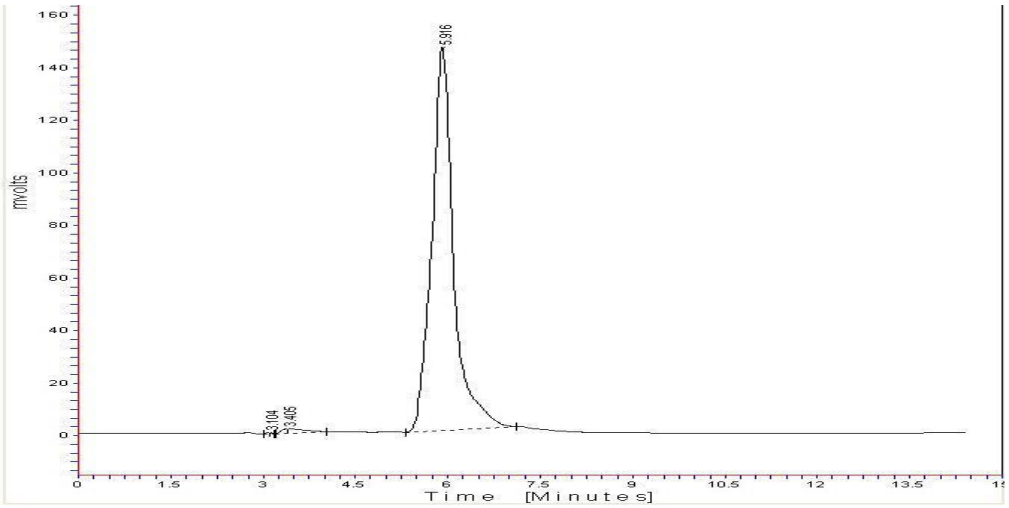

Fig 05;-Chromatograms of standard Teneligliptin HBr by HPLC Method (Method accepted)

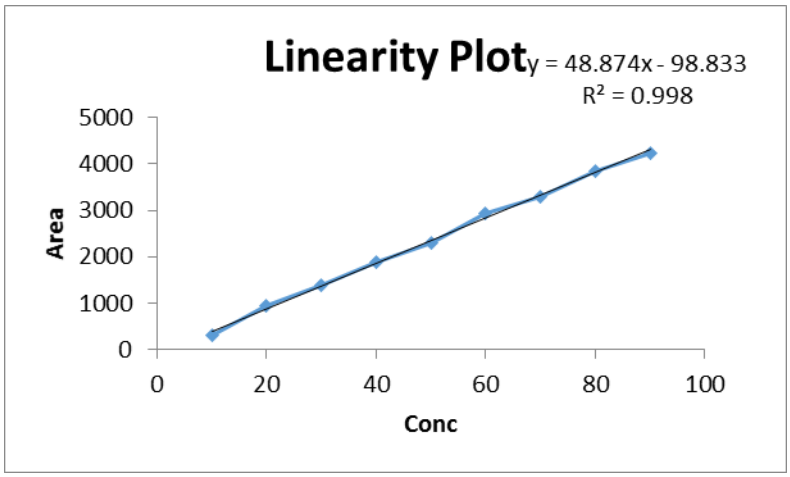

Fig 06: Linearity Graph of Teneligliptin HBr by HPLC Method

REFERENCES

1. Yoshida T. et al Discovery and preclinical profile of teneligliptin highly potent, selective, long-lasting and orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes, Journal of Bioorganic & Medicinal Chemistry (2012) 20; 5705–5719
2. Shantikumara S., Satheeshkumara N., Srinivasa R.,. Pharmacokinetic and protein binding profile of peptidomimetic DPP-4 inhibitor – Teneligliptin in rats using liquid chromatography–tandem mass spectrometry, Journal of Chromatography B, (2015) 1002:194–200
3. Shinde V. C., Aher K .B., Bhavar G. B., Kakad S. J. and. Chaudhari S. R. Development and validation of UV spectrophotometric method and high performance thin layer chromatographic (HPTLC) method for estimation of teneligliptin hydrobromide in pharmaceutical preparation, Scholars Research Library Der Pharmacia Lettre, 2016, 8 (8):291-301
4. Breda C.A., Bredam M., Frigerio, E. Bioanalytical method validation: A riskbased approach, Journal of Pharmaceutical and Biomedical Analysis, 2004; 35(4):887-89.
5. Stephen G., Schulman B., Vogt S., Munson W., *And* High Performance Liquid Chromatography: Theory, Instrumentation and Practice of application. (2001); Part B: 15 - 108.
6. Kasture A.V., Wadodkar S. G., Mahadik K. R., More H. M.,Pharmaceutical Analysis: Instrumental methods. (2002); 8 II: 48 –55.
7. Heftman D., Chromatography-Fundamentals and applications of Chromatography and Related differential migration methods; Elsevier, Amst. 69A, erdam. 2004;6: 253-291
8. Sharma B. K., Instrumental method of chemical analysis. (2002); 21: 310.
9. Chatwal G. R.,. Sham A. K, Instrumental methods of Chemical Analysis. (2002); 5: 256.
10. Stephen G., Schulman B., Vogt S., Munson W., *And* High Performance Liquid Chromatography: Theory, Instrumentation and Practice of application. (2001); Part B: 15 - 108.
11. Beckett H., Stenlake J. B., Practical Pharmaceutical Chemistry CBS Publishers and Distributors, New Delhi.(2004); 4(II) : 86-100.
12. Willard H. H., Merrit L. L., Dean J. A., Settle F. A.. Instrumental method of analysis. (1995); 7: 513.
13. Willard H. H., Merritt L.L., Instrumental methods of analysis 2001; CBS publishers and distributors New Delhi. 2001; 1-12, 97-106, 118-136, 513-534, 580-629.
14. Ledwig H.,. Stephan G. A, Diode Array Detection in HPLC, Marcel Dekker, Inc, (1993); 17.
15. Hubert P.H., Chiap, P., Crommena J., Boulanger B., Chapuzet E.., Mercier N., Bervoas-Martin S., Chevalier P., Grandjean D., Lagorce P., Lallier M., Laparra M.C., Laurentie M., Nivet J.C. The SFSTP guide on the validation of chromatographic methods for drug bioanalysis: From the Washington Conference to the laboratory. Analytical Chimica Acta, 1999; 391(3): 135-148.
16. ICH, Q2 (R1), Validation of analytical procedures: text and methodology, International Conference on Harmonization, Geneva, 2005; 1- 13.