IJPSR (2018), Volume 9, Issue 6 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 07 September, 2017; received in revised form, 20 November, 2017; accepted, 21 November, 2017; published 01 June, 2018

A NOVEL METHOD FOR ESTIMATION OF DIPEPTIDYL PEPTIDASE-4 INHIBITOR IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY VISIBLE SPECTROPHOTOMETRY

Vinutha Kommineni[*1], N. Prasanna Lakshmi [1], B. Ramya [1] and Chandra Bala Sekharan [2] and O. Ahmed [3]

Department of Pharmaceutical Analysis [1], Sri Venkateswara College of Pharmacy, Hyderabad - 500081, Telangana, India.
Department of Biochemistry [2], International Medical and Technological University, Dar Es Salaam, Tanzania.
Department of Pharmaceutical Chemistry [3], Deccan school of Pharmacy, Hyderabad - 500001, Telangana, India.

Keywords:

New spectrophotometric method, Teneligliptin, Sodium nitroprusside, Validation

Correspondence to Author:
Vinutha Kommineni

Assistant Professor,
Department of Pharmaceutical Analysis, Sri Venkateswara College of Pharmacy, Hyderabad - 500081, Telangana, India.

E-mail: vinutha08.ch@gmail.com

ABSTRACT: A new, simple and low cost spectrophotometric method for the determination of teneligliptin, a new DPP IV inhibitor drug in bulk and pharmaceutical dosage form using sodium nitroprusside was developed. The absorbance maximum (λ_{max}) of the resulted colored product was at 445 nm. As different solvents were tried methanol was found to be the most suitable solvent. The Beer's law has been obeyed in the concentration range of 4 - 20 µg/ml with the detection limits of 0.09µg/ml and limit of Quantification of 0.28µg/ml. The correlation coefficient was found to be (r = 0.9999). The reaction ratio between teneligliptin and sodium nitroprusside was studied and found to be 1:1. The method was validated and results obtained for the assay of two different brands of teneligliptin tablets were compared. The repeatability and reproducibility of the developed method were evaluated and the obtained results quoted.

INTRODUCTION: Teneligliptin, a novel DPP-4 Class 3 inhibitor is a long acting oral hypoglycemic agent. It is characterized by 5 consecutive rings namely - thiazole, pyyrolidine, piperazine, pyrazole and benzene [1]. DPP-4 inhibitors increase the levels of active GLP-1 and GIP by inhibiting DPP-4 enzymatic activity. These inhibitors improve hyperglycemia in a glucose-dependent manner by increasing serum insulin levels and decreasing serum glucagon levels [2, 3, 4].

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.9(6).2351-58

Article can be accessed online on:
www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(6).2351-58

Literature survey revealed many methods with different analytical techniques for individual or simultaneous determination of teneligliptin. The technique includes HPTLC [5], UPLC [6], HPLC [7 - 10], LC-MS-MS [11], UV [13, 14] and Visible [15, 16] spectrophotometric methods. The techniques other than spectrophotometry require lengthy procedures, highly sophisticated analytical instruments and some amount of the organic solvents contributing towards high cost of the analysis.

Spectrophotometry is the most common, convenient and favourite tool in analytical laboratories due to its inherent sensitivity and low costing. So, we aimed to report the original approach that allows a simple, rapid and improved spectrophotometric determination of teneligliptin avoiding lengthy procedure and minimizing the use of hazardous chemicals. Many spectrophotometric methods adapted SNP as an analytical reagent in their applications, including determination of bowel-specific aminosalicylate drug [17], selective angiotensin AT1 receptor blocker [18], piperazine antihistamine [19], antiprotozoal [20] aminoacid lysine derivative [21], aminoglycoside [22].

This paper deals with the use of Sodium nitroprusside as a chromogen for the spectrophotometric assay of Teneligliptin.

In the present work, simple and sensitive visible spectrophotometric method was developed and validated for the analysis of teneligliptin with broad linearity, good precision and accuracy. These methods could be applied for the quantitative determination of the teneligliptin in their tablet formulations.

MATERIALS AND METHODS:

Instrumentation:

- **A double Beam UV Spectrophotometer:** Lab India-3000 with UV Win software and 1cm quartz cell.
- **Weighing Balance:** Sartorius.

Preparation of Reagents and Solutions:

Standard Stock Solution of Teneligliptin: Teneligliptin working standard was procured from Hetero Pharma Labs Pvt. Ltd., Hyderabad, India. Standard stock solution of Teneligliptin was prepared by dissolving accurately weighed 10 mg of drug in 20 ml of 0.1 M NaOH in 50 ml volumetric flask and diluted upto the mark with distilled water.

Standard Solution of Sodium Nitroprusside: 0.4% w/v of sodium nitroprusside was prepared by dissolving 0.400g in distilled water in 100 ml volumetric flask and diluted upto the mark with water.

Standard Solution of Hydroxyl Amine HCl: 0.4% w/v of Hydroxyl amine HCl was prepared by dissolving 0.400 g in distilled water in 100ml volumetric flask and diluted upto the mark with water.

Standard Solution of Sodium Carbonate: 0.4% w/v of Sodium Carbonate was prepared by dissolving 0.400 g in distilled water in 100 ml volumetric flask and diluted upto the mark with water.

Preparation of Sample Solution:

General Procedure: Aliquots (0.2 - 1.0 ml) of Teneligliptin standard solution were transferred in to 10 ml volumetric flask. To each flask 1ml of Sodium nitroprusside, 0.9 ml of Hydroxyl amine HCl, and 1 ml of Sodium Carbonate solutions were added. The volume was then made upto the mark with water and absorbance of each solution was measured at 445 nm.

Preparation of Sample Solution: The tablet formulation of Teneligliptin labelled to contain 20 mg was purchased. Twenty tablets were accurately weighed and finely powdered in a mortar. A portion of tablet powder equivalent to 20 mg was weighed and transferred into 50 ml volumetric flask. 20 ml of 0.1 M NaOH was added and the mixture was sonicated for 15 min. The mixture was filtered through Whatman No. 1 filter paper. The solution was made up to the mark with distilled water and contents were analysed by the proposed method.

RESULTS AND DISCUSSION:

Method Development: The reaction of Sodium nitroprusside with amino group has been reported, these reactions demonstrate that Sodium nitroprusside is a valuable reagent in the development of simple spectrophotometric methods for the quantification of many pharmaceutical amines [17 - 22]. Tenegliptin acts as a donor due to the presence of cyclic secondary nitrogen. The mechanism is based on the electron transfer from donor Tenegliptin to acceptor Sodium nitroprusside. Sodium nitroprussside in the presence of hydroxylamine and alkali exists as aquoferrocyanide $(Fe(CN)_5.H_2O)^{3-}$. The electron transfer results in the formation of yellow colored complex characterized by high degree of covalency and electron delocalization.

The reaction was studied under various conditions of Sodium nitroprusside, Hydroxyl amine HCl, Sodium carbonate concentration and reaction time was studied to determine the optimum conditions for the method.

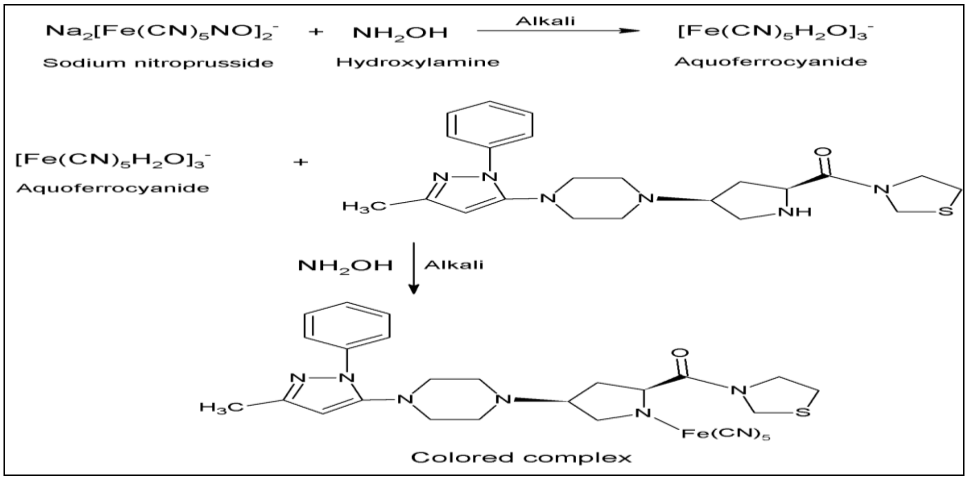

FIG. 1: PROPOSED REACTION PATHWAY BETWEEN SODIUM NITROPRUSSIDE AND TENELIGLIPTINE

The effect of the volume of 0.4% w/v Hydroxyl amine HCl on the absorbance of the yellow colored complex was studied in the range of 0.2 - 2.0 ml. The absorbance increases with the increase in the volume of Hydroxyl amine HCl up to 0.8 ml. Further addition of Hydroxyl amine HCl showed decrease in the absorbance. Therefore, 0.8 ml of 0.4% w/v Hydroxyl amine HCl was chosen as an optimum value **Fig. 2.**

The effect of volume of 0.4% w/v Sodium carbonate on the absorbance of yellow colored complex was studied in the range 0.2 - 2.0 ml. The absorbance increases with increase in the volume of Sodium carbonate and becomes constant at 1ml. Further addition of Sodium carbonate does not show any change in the absorbance. Hence 1ml of 0.4% w/v Sodium carbonate was selected as an optimum value **Fig. 3**.

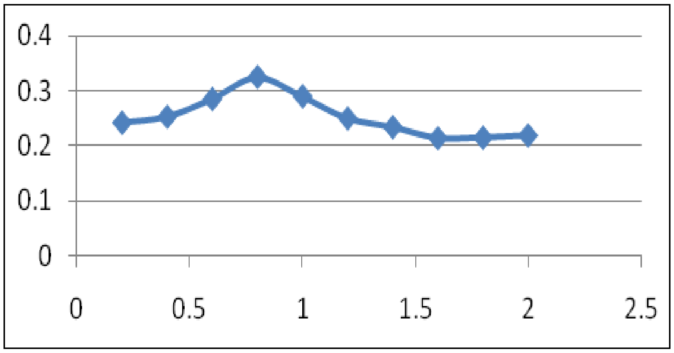

FIG. 2: EFFECT OF VOLUME OF HYDROXYL AMINE HCl

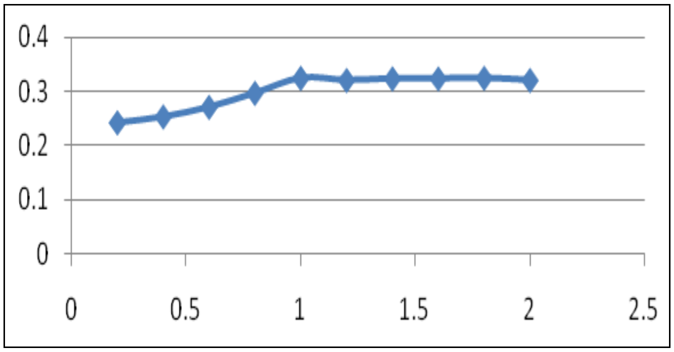

FIG. 3: EFFECT OF VOLUME OF SODIUM CARBONATE

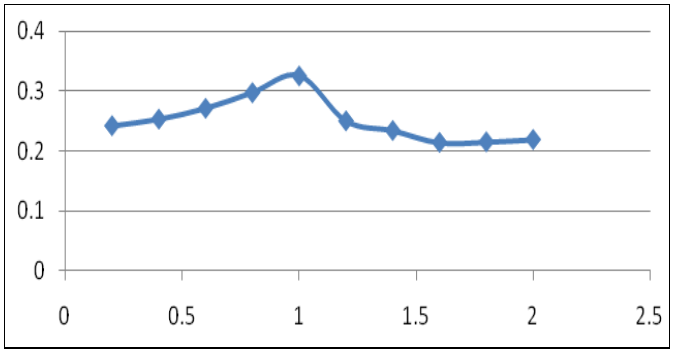

FIG. 4: EFFECT OF VOLUME OF SODIUM NITROPRUSSIDE

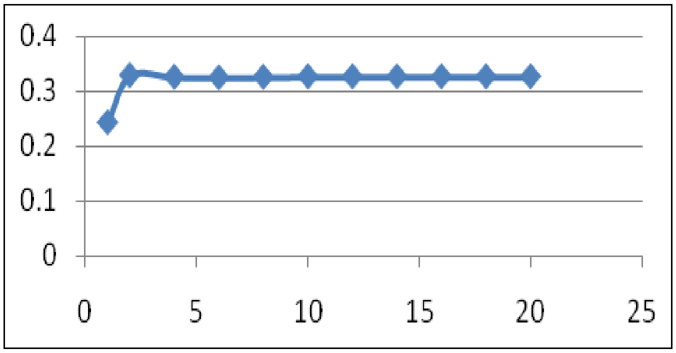

FIG. 5: EFFECT OF TIME

The effect of volume of 0.4% w/v Sodium nitroprusside on the absorbance of yellow colored complex was studied in the range 0.2 - 2.0 ml. The absorbance increases with increase in the volume of Sodium nitroprusside and becomes constant at 1ml. Further addition of Sodium nitroprusside does not show any change in the absorbance. Hence 1ml of 0.4% w/v Sodium nitroprusside was selected as an optimum value **Fig. 4**.

The effect of time on the formation of the yellow colored complex was also optimized. At room temperature, the color intensity of the product increased by the elapse if time, maximum absorbance was obtained at 2 minutes and remained constant for 20 minutes. The results indicated that the yellow colored product obtained was stable for 10 hrs **Fig. 5.**

In order to choose proper solvent for dilution, different solvents (methanol, dichloro methane, chloroform, isopropanol and Acetonitrile) were tested. The highest absorbance values were obtained when methanol was used as diluting solvent. In methanol medium, the absorbance values were found to be stable for 10 hrs. Hence methanol was chosen as dilution solvent.

Association Constant and the Free Energy Changes of the Complexes: The association constant of complex was determined by employing the Benesi - Hildebrand method [23], besides the association constant was calculated by using the following equation:

$$[Ao]/A\lambda=1/\varepsilon+ (1/Kc.\varepsilon).1/[Do]$$

Where;
[Do] = Concentration of the drug,
[Ao] = Concentration of the reagent,
Aλ = Absorbance of the complex at 445 nm,
ε = Molar absorptivity of the complex at 445 nm,
Kc = Association constant of the complex.

The ΔG° (the standard free energy of complexation) and the association constant Kc are related by the following equation [24]

$$\Delta G^\circ = -2.303\ RT \log Kc$$

Where;
ΔG° = Free energy change of the complex,
R = Gas constant (1.987 cal mol^{-1} degree^{-1}),
T = Temperature in Kelvin,
K = Association constant (L mol^{-1}) of the drug-reagent complex.

The results are summarized in the **Table 1**. The negative values of the standard free energy indicated that the complexes are stable and started to form spontaneously.

TABLE 1: THERMODYNAMIC STUDIES, LINEARITY AND SENSITIVITY OF THE PROPOSED METHODS

Parameter	Result
Beer's law limit (μg/mL)	4-20
Regression Equation (y = mx + c)	Y = 0.04545x – 0.0124
Slope (m)	0.04545
Intercept (x)	0.0124
Molar Absorptivity	1.94×10^2
Regression coefficient (r^2)	0.9999
Sandell's sensitivity (μg cm^{-2}/0.001 Absorbance unit)	0.022002
LOD (μg/mL)	0.09
LOQ (μg/mL)	0.28
Association constant (L mole^{-1})	4.7×10^{-8}
Free energy change	-7.8×10^2

Stiochiometry of Ion Pair Complex: Jobs method of continuous variation was employed to determine the composition of the ion pair complex [25]. For this, solutions of identical concentrations (1.1×10^{-3} M) of the drug and reagent were prepared and mixed in varying volume ratios such that total volume of each solution was constant. The absorbance of the resulting complex was measured at 445 nm. The absorbance values were plotted against mole fraction of drugs. The stiochiometry ratio observed for drug dye ion pair complex was 1:1 as evident from the results in **Fig. 6.**

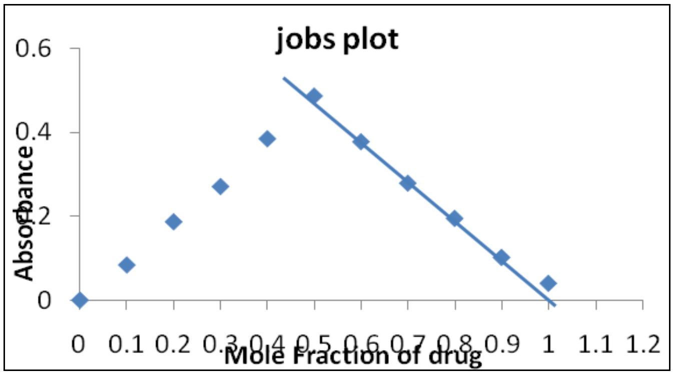

FIG. 6: MOLE FRACTION OF DRUG

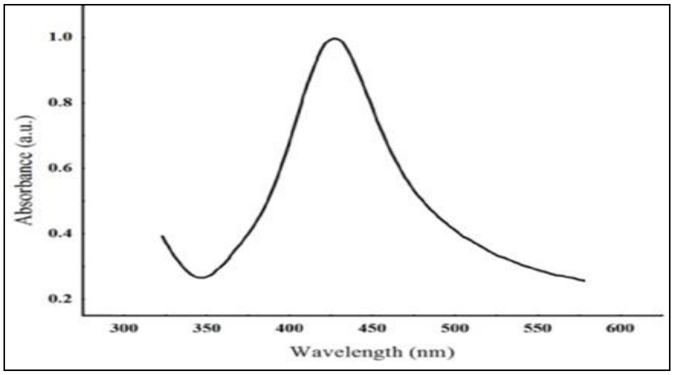

FIG. 7: λ_{max} OF COMPLEX

Method Validation:

Specificity: Specificity of proposed spectrophotometric method was evaluated by analyzing standard solution of Teneligliptine in the presence of common excipients of pharmaceutical preparations such as lactose, sucrose, starch, mannitol and magnesium stearate. The results indicated that there is no interference from excipients **Fig. 7**.

Sensitivity: According to the ICH guidelines, the sensitivity parameters like molar absorptivity, Sandell's sensitivity, Limit of Detection and Limit of Quantification were calculated and summarized in **Table 1**.

Linearity: The relation between the absorbance and final concentration of Teneligliptine was found to be linear over the concentration range of 4 - 20 µg/ml. Results are shown in **Fig. 8**.

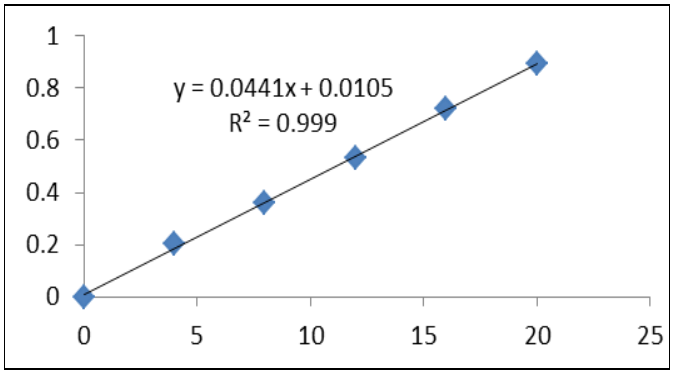

FIG. 8: LINEARITY GRAPH OF TENELIGLIPTINE

Precision: The repeatability (intra-day precision) of the proposed method was determined by replicate analysis (n = 5) of standard solutions at three concentration levels (2 µg/ml, 6 µg/ml and 10 µg/ml). The intermediate precision (inter-day precision) was conducted by repeating the analysis over a period of three consecutive days. The precision of the methods was expressed as standard deviation (SD) and percentage relative standard deviation (% RSD). The results are summarized in **Table 2**. The SD and % RSD obtained by both methods are found to be in the acceptable range. Therefore, it can be considered to be satisfactory.

Accuracy: The accuracy of the proposed method was established by performing intra-day and inter-day assays by determining at different levels of drug concentrations [lower concentration (50%), intermediate concentration (100%) and higher concentration (150%)] within 1 day and 3 consecutive days, respectively. The accuracy of the methods is expressed as percentage recoveries and percentage error. The results obtained by both the methods are found to be in the acceptable range. Therefore, we can say it can be considered as satisfactory. In addition, accuracy and validity of the proposed methods were determined by standard addition technique. The pre analyzed samples were spiked with additional 50, 100 and 150% were once again analyzed by the proposed methods. The accuracy of the methods was evaluated by percentage recovery of the Teneligliptine.

The average recovery and percentage standard deviation values **Table 3** of the methods lying in the acceptable range show that the methods are accurate.

TABLE 2: ACCURACY AND PRECISION OF THE PROPOSED METHODS

Type of Assay	Concentration (µg/mL) Taken (n = 3)	Found	SD	% RSD	% Recovery	% Error
Intra- day	4	3.996	0.016	0.408	99.90	0.1
	12	11.951	0.029	0.243	99.59	0.4
	20	19.95	0.03	0.13	99.73	0.3
Inter- day	4	3.95	0.03	0.75	98.87	1.1
	12	11.90	0.05	0.39	99.19	0.8
	20	19.95	0.02	0.11	99.77	0.2

TABLE 3: RESULTS OF STANDARD ADDITION TECHNIQUE OF PROPOSED METHOD

S. no.	Concentration (mg) Tablet	Spiked	Found	SD	% RSD	% Recovery
1	20	10	9.990	0.041	0.408	99.90
2	20	20	19.84	0.08	0.39	99.19
3	20	30	29.66	0.22	0.75	98.87

Robustness: The robustness of the proposed methods was checked for each operational parameter and investigated. The operational parameters were:

Volume of Sodium Carbonate: 1 ± 0.1 mL
Volume of 0.4% SNP: 1 ± 0.1 mL
Volume of 0.4% HA HCl: 0.9 ± 0.1 mL

The robustness of the methods was assessed by analyzing the Teneligliptine at two different concentration levels (4 and 20 µg/mL). The percent recovery and % RSD of the method **Table 4** was found to be satisfactory, indicating that the method is robust.

TABLE 4: ROBUSTNESS OF PROPOSED METHOD

S. no.	Experimental Parameter	Volume (ml)	Taken (4µg/ml) Absorbance	% Recovery	% RSD	Taken (20 µg/ml) Absorbance	% Recovery	% RSD
1	Hydroxyl	0.8	0.205	99.52	1.29	0.881	99.55	1.09
	Amine HCl	0.9	0.202	97.58	0.75	0.887	100.23	0.45
		1	0.207	100	0.39	0.884	99.89	0.79
2	Sodium	0.9	0.206	99.52	0.37	0.883	99.77	1.21
	Nitropruside	1.0	0.203	98.07	0.40	0.885	100	0.51
		1.1	0.205	99.03	0.71	0.884	99.89	0.62
3	Sodium	0.9	0.204	98.55	0.28	0.882	99.65	0.45
	Carbonate	1.0	0.203	98.07	0.42	0.886	100.11	0.15
		1.1	0.206	99.52	0.68	0.881	99.59	0.49

Application of the proposed methods to analysis of Teneligliptine in tablet formulations: It is obvious from the above mentioned results that the proposed methods gave satisfactory results with Teneligliptine in bulk. Therefore, Teneligliptine tablet formulations were subjected to the analysis of their Teneligliptine contents by the proposed methods. The percent recovery and % RSD **Table 5** clearly showed no interference of any excipients of formulation, thus proving accuracy and precision in the quantification of Teneligliptine.

TABLE 5: RESULTS OF ANALYSIS IN TABLET FORMULATION

Formulation	Labelled claim (mg)	Found ± SD	RSD (%)	Recovery (%)
Tenglyn	20	19.96 ± 0.098	0.968	99.60
Ziten	20	20.04 ± 0.071	0.707	100.40
Tenepride	20	19.92 ± 0.863	0.863	99.92
Teniva	20	19.96 ± 0.722	0.722	99.96

Comparison of the Proposed M with the Reported Spectrophotometric Methods: Three UV spectrophotometric methods [5, 13, 14] and two visible spectrophotometric methods [15, 16] were reported for the assay of Teneligliptine. The comparison between the proposed method and reported spectrophotometric methods is summarized in **Table 7**. Most of the reported spectrophotometric methods were not fully validated according to the ICH guidelines [26].

TABLE 7: COMPARISON BETWEEN PROPOSED AND REPORTED SPECTROPHOTOMETRIC METHODS

Method	λ_{max} (nm)	Linearity (µg mL^{-1})	LOD (µg mL^{-1})	LOQ (µg mL^{-1})	% Recovery	Reference
UV Spectrophotometry	243.5	10-90	N R	N R	97.43-98.70	5
UV Spectrophotometry	243	10-50	0.556	14.79	99.45-104	13
UV Spectrophotometry	243.6	10-50	95.4	28.91	99-102	14
Visible Spectrophotometry	424	0.2-1.6	N R	N R	100.01-100.03	15
Visible Spectrophotometry	554 & 421	0.1-0.6 & 0.2-1.6	N R	N R	99.9-100.01	16

NR-Not Reported

CONCLUSION: A sensitive visible spectrophotometric method for the determination of Teneligliptine have been developed and validated. The present methods demonstrate that alkaline sodium nitroprusside can be used for the quantitative determination of Teneligliptine in bulk and in tablet dosage forms. The reagent used in the developed method is cheap and readily available. From the values of molar absorptivity, Sandell's sensitivity, LOD and LOQ, it was observed that the new method is more sensitive than the reported methods.

Tedious procedures, extractions and sophisticated instrumentation associated with chromatographic methods [6 - 10] are not involved in the proposed method. The proposed method is more selective, sensitive and accurate than the spectrophotometric methods reported [5, 13 - 16]. The proposed method is fully validated and found to be sensitive, accurate, reproducible, selective, robust and rugged. These advantages support the application of the proposed method in routine quality control analysis of Teneligliptine.

ACKNOWLEDGEMENT: I express my sincere thanks to Dr. M. Bhagavan Raju, principal for his support and encouragement throughout my research work. I am also thankful to Sri Venkateshwara College of Pharmacy for providing chemicals and instruments and Hetero Labs Pvt. limited Hyderabad for providing drug samples for research.

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Patil M, Harsha DJ, Suleman SK, Narmin AP and Shamim SK: A review on chemistry and pharmacological activity of metformin and tenegliptin in combined dosage form. Ph arma Tutor 2017; 5(3); 24-30.
2. Goda M and Kadowaki T: Teneligliptin for the treatment of type 2 diabetes. Drug today (Barc). 2013; 49: 615-29.
3. Ideta T, Shirakami Y, Miyazaki T, Kochi T, Sakai H, Moriwaki H and Shimizu M: The dipeptidyl peptidase4 inhibitor teneligliptin attenuates hepatic lipogenesis via AMPK activation in nonalcoholic fatty liver diseasemodel mice. Int. J.Mol Sci 2015; 16: 29207-18.
4. Bronson J, Black A, Murali Dhar TG, Ellsworth BA and Robert Merritt J: Teneligliptin (antidiabetic), chapter to market, to market- 2012. Annu Rep Med Chem. 2013; 48: 523-524.
5. Shinde VC, Aher KB, Bhavar GB, Kakad SJ and Chaudhari SR: Development and validation of UV spectrophotometric method and high performance thin layer chromatographic (HPTLC) method for estimation of teneligliptin hydrobromide in pharmaceutical preparation. Der Pharmacia Lettre. 2016; 8(8): 291-301.
6. Kumar TNVG, Vidyadhara S, Narkhede NA, Silpa YS and Lakshmi MR: Method development, validation, and stability studies of teneligliptin by RP HPLC and identification of degradation products by UPLC tandem mass spectroscopy. Journal of Analytical Science and Technology 2016; 7(18): 1-20.
7. Chitlange SS, Rawat DG and Chandani S: Estimation of anti- diabetic teneligliptin hydrobromide hydrate by rp-hplc and derivative spectroscopic method. Indo American Journal of Pharmaceutical Research. 2016; 6(7): 6144-6153.
8. Chandana M, Rao MP, Samrajyam B, Sireesha KSKD and Premi VVN: Analytical method development and validation of teniligliptin in pharmaceutical dosage form by RP-HPLC method. Journal of Health Sciences and Nursing 2016; 1(12): 1-12.
9. Luhar SV, Pandya KR, Jani GK and Narkhed SB: Simultaneous Estimation of Teneligliptin Hydrobromide Hydrate and its Degradation Product by RP-HPLC method. Journal of Pharmaceutical Science and Bioscientific Research 2016; 6(3): 254-261.
10. kommineni V, Chowdary KPR and Prasad SVUM: Development of a New Stability Indicating RP-HPLC Method for Simultaneous Estimation of Metformin Hydrochloride and Teneligliptin hydrobromide and its Validation as per ICH Guidelines. IAJPS. 2017; 4(05): 1109-1119.
11. Chunduri RHB and Dannana GS: Development and validation of lc-ms/ms method for quantification of Teneligliptin in human plasma and its application to a pharmacokinetic study. World journal of pharmacy and pharmaceutical sciences. 2016; 5(5): 838-850.
12. Sen AK, Hinsu DN, Sen DB, Zanwar AS, Maheshwari RA and Chandrakar VR: Analytical method development and validation for simultaneous estimation of Teneligliptin hydrobromide hydrate and Metformin hydrochloride from it's pharmaceutical dosage form by three different UV spectrophotometric methods. J App Pharm Sci. 2016; 6(9): 157-165.
13. Ghuge BS, Pendhari SS, Malode PA and Anantwar SP: Development and Validation of Simple UV Spectrophotometric Method for the Determination of Teneligliptin Hydrobromide Hydrate in API and its Bulk Dosage Form. IJPRS 2017; 6(4): 5219-5224.
14. Sonawane AM, Dhokale KK and Randhe VA: A Simple UV- Spectrophotometric Method Development and Validation of Teneligliptin in Tablet Dosage Form. Indo American Journal of Pharmaceutical Research 2016; 6(04): 5219-5224.
15. Sunitha PG, Karthikeyan R, Kumar RB and Muniyappan S: Quantitative Estimation of Teneligliptin by Validated Colorimetric and FTIR Spectroscopic Methods. World Journal of Pharmacy and Pharmaceutical Sciences 2017; 6(8): 1680-1685.
16. Sunitha PG, Karthikeyan R, Kumar RB and Muniyappan S: Validated Colorimetric Methods for the Estimation of Teneligliptin in Tablets. Journal of Drug Delivery & Therapeutics 2017; 7(4): 38-40.
17. Al-Sabh TN and Habeeb NN: Spectrophotometric determination of Mesalamine using sodium nitroprusside as chromogenic agent. European Chemical Bulletin 2015; 4(8): 384-388.
18. Rani KS, Babu KR and Srividhya M: Spectrophotometric determination of Olmesartan Medox omil using sodium nitroprusside as chromogenic agent. Indo American Journal of Pharmaceutical Research 2015; 5(12): 3822-3826.
19. Annapurna V, Jyothi G, Subbayyamma AV and Sailaja BBV: Spectrophotometric determination of Buclizine as Hydrochloride using various chromogenic agents. E - Journal of Chemistry 2010; 7(4): 1523-1529.
20. Basavaiah K, Nagegowdha P and Chandrasekhar U: Determination of Tinidazole by potentiometry, spectrophotometry and high performance chromatography. Indian Journal of chemical Technology 2005; 12: 273-280.
21. Shantier SW, Adam ME, Mohamed RE and Gadhkare EA: Development and validation of colorimetric determination of aminocaproic acid in bulk and pharmaceutical preparations. Journal of Pharmaceutical and Biological Sciences 2017; 4(3): 67-70.

22. Liu and Bing: A novel technique for the determination of propylthiouracil with sodium nitroprusside as chromogenic agent by spectrophotometry. Journal of Analytical chemistry 2015; 70: 328-333.
23. Benesi HA and Hildebrand JH: A Spectrophotometric Investigation of the Interaction of Iodine with Aromatic Hydrocarbons. Journal of the American Chemical Society 1949; 71(8): 2703-2707.
24. Dash S, Singh Z, Prasad R and Sood DD: The standard molar Gibbs free energy of formation of $Ba_3Zr_2O_7$. The Jou. of Chemical Thermodynamics 1994; 26(7): 737-744.
25. Renny JS, Tomasevich LL, Tallmadge EH and Collum DB: Method of Continuous Variations: Applications of Job Plots to the Study of Molecular Associations in Organometallic Chemistry. Angew Chem Int Ed Engl. 2013; 52(46): 11998-12013.
26. Validation of Analytical Procedures; Methodology, International Conference on Harmonization (ICH): Text and Methodology Q2 (R1): Complementary Guideline on Methodology dated 06 November 1996: incorporated in London 2005.

How to cite this article:
Kommineni V, Lakshmi NP, Ramya B and Sekharan CB and Ahmed O: A novel method for estimation of dipeptidyl peptidase-4 inhibitor in bulk and pharmaceutical dosage forms by visible spectrophotometry. Int J Pharm Sci Res 2018; 9(6): 2351-58. doi: 10.13040/IJPSR.0975-8232.9(6).2351-58.