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Development and Validation of Extractive Spectrophotometric Method for the Estimation of Teneligliptin in Its Pure and Dosage Form Using UV-Visible Spectroscopy

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ABSTRACT

A colorimetric method for the analysis of teneligliptin in pure and in tablets has been developed based on the formation of yellow colour complex. The method is based on the ion pair complex formation in the presence of phosphate buffer pH 2.6, between bromocresol green and primary amino group of teneligliptin. The complex formed was extracted using extractive solvent, chloroform. The complex was stable up to 25min and obeyed Beer's law over the concentration ranges of 4-20 µg/ml. Correlation coefficient was found to be 0.9989. The method was validated statistically. Recovery studies gave satisfactory results indicating that none of the common additives and excipients interfere the assay method. The proposed method was found to be simple, accurate, precise specific and reproducible that was successfully applied for the analysis of tablet formulation.

INTRODUCTION [1-2]

Teneligliptin is a recently developed oral dipeptidyl peptidase 4 inhibitor indicated for the management of type 2 diabetes mellitus (T2DM) in adults along with diet and exercise. Teneligliptin can be safely administered to patients with mild, moderate or severe renal impairment or end-stage renal disease without dose adjustment. Similarly, it can be used in patients with mild-to-moderate hepatic impairment. Diabetes is a noncommunicable disease and has reached to epidemic stage in many countries. It is a chronic disease that requires lifelong medical care and attention for multiple risk reduction and treatment approach beyond glycemic control. It affects many organs and complications due to high blood glucose level ,which is an important cause of disability, premature death.

As literature review reveals that there is lots of work done using uv –visible spectroscopy and chromatographic methods using teneligliptin in its dosage form as single and in combination with other drug. So our aim was to develop an extractive spectrophotometric method for the estimation of teneligliptin in bulk as well as in pharmaceutical dosage form. An extractive spectrophotometric method is a method where at a specific pH the ion pair complex formed is extracted into an organic solvent, which is immiscible with water and the concentration of the resulting ion pair in the organic layer is determined spectrophotometrically. Extractive spectrophotometric procedure are the best choice for quality control because of their high sensitivity, selectivity and low limit of detection in the assay of drugs. It also has an important advantage of determination of an individual component in the presence of routine excipients and filling materials. This aspect of spectrophotometric analysis is of great interest since it offers distinct possibilities for the assay of a particular component in a complex dosage formulation. Chemical structure of teneligliptin is shown in figure 1.

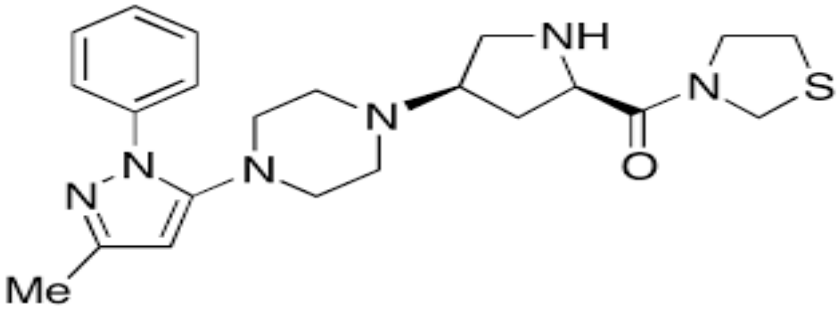

Figure 1. Chemical structure of Teneligliptin

MATERIALS AND METHOD [3-8]

SELECTION OF SOLVENT:

From the drug profile of teneligliptin, it was found that the drug was found to be freely soluble in distilled water, methanol, chloroform, DMSO. As distilled water is economic, cheap and easily available we chose it as a suitable solvent.

PREPARATION OF COLOURING REAGENT

125mg of bromocresol green was taken in 50ml standard flask. Made up the flask with distilled water to attain 2500 µg/ml concentration of BCG.

PREPARATION OF PHOSPHATE BUFFER (pH-2.6)

5gm of potassium dihydrogen orthophosphate was dissolved in distilled water and made up to 50ml. The pH of the resulting solution was adjusted to 2.6 using 0.1M HCl.

SELECTION OF WAVELENGTH:

20mg of teneligliptin was taken in 50ml standard flask and made up with distilled water to contain 400 µg/ml.0.3ml from the stock solution was pipetted to a separating funnel. To this add 0.5ml of BCG, 0.5ml of phosphate buffer and 10ml of chloroform. Shake the mixture for 2 min and then separate out the organic layer to 10ml standard flask. Keep it aside for 25 min and the solution were scanned between 400 – 800 nm in 1cm cell against blank solution. The absorption maxima (λ_{max}) of the drug was found to be 419nm.

PREPARATION OF STANDARD STOCK SOLUTION

Accurately weighed 20mg of teneligliptin was taken in 50ml standard flask. It was then made upto get 400 µg/ml of teneligliptin.

EXPERIMENTAL CONDITION

The standard stock solution of teneligliptin was further diluted. The diluted solution added with bromocresol green as colouring agent and phosphate buffer of pH 2.6 to maintain the pH. The bromocresol green is only reactive with amino group of teneligliptin. The ion pair complex only forms in a certain pH. The reaction is complexation reaction resulting in the formation of yellow coloured complex, shown in figure 2.

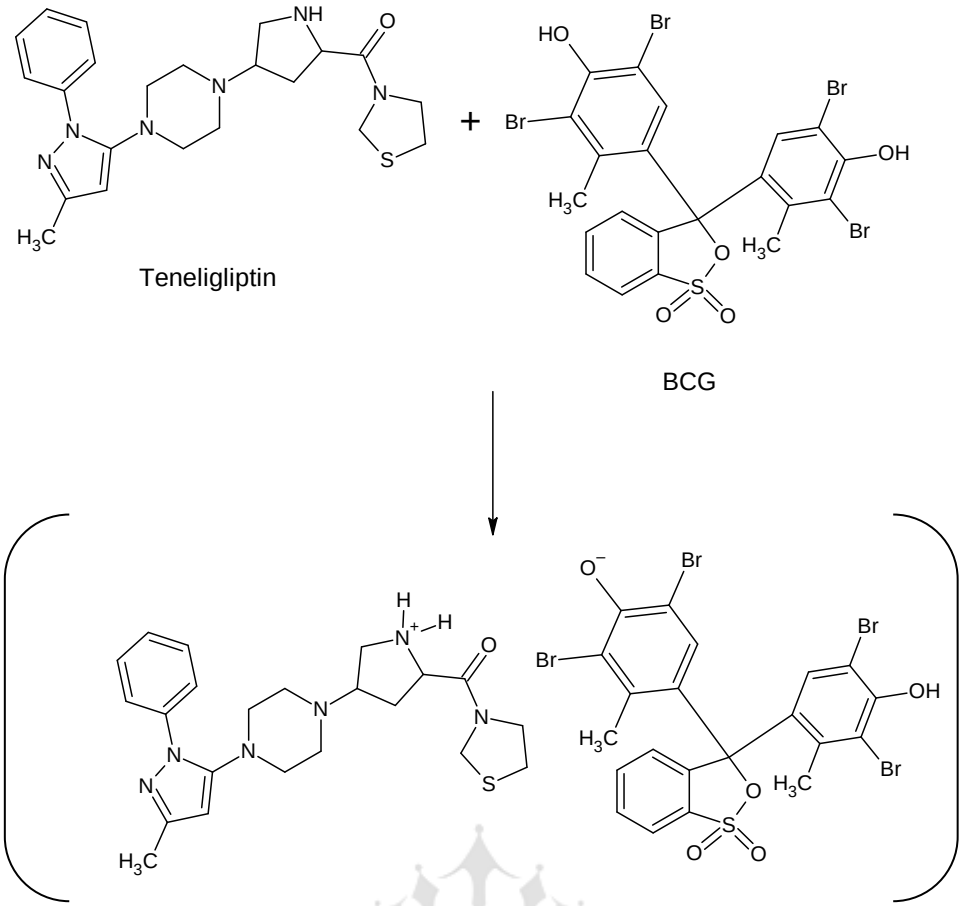

Figure 2. Teneligliptin-BCG ion pair complex (yellow colour)

For the overlain spectrum from the stock solution 0.1ml, 0.2ml, 03ml, 0.4ml, 0.5ml was pipetted out to a separating funnel, and mixed with 0.5ml of bromocresol green (2500µg/ml), 0.5ml of phosphate buffer pH-2.6 and 10ml of chloroform, the mixture was shaken for 2 minutes continuously, the organic layer was then separated to 10 ml standard flask and scanned in the range of 400 to 800nm at 25 minute and the spectrum was recorded.

ANALYSIS OF TABLET FORMULATION

Twenty tablets were accurately weighed and average weight was found. The tablets were triturated to a fine powder. An accurately weighed quantity of 102mg was transferred into a 50ml volumetric flask and add 20ml of distilled water for dissolving. The solution was sonicated for 1 minute and filtered through Whatman filter paper. Then the standard flask made up with distilled water. The stock contains 400µg/ml. From the clear solution, 03ml was pipetted out to a separating funnel, and mixed with 0.5ml of bromocresol green (2500µg/ml),0.5ml of phosphate buffer pH-2.6 and 10ml of chloroform ,the mixture was

shaken for 2 minutes continuously the organic layer was then separated to 10 ml standard flask and scanned in the range of 400 to 800nm at 25 minutes. Absorbance was noted.

METHOD VALIDATION [9]

The methods were validated using ICH guidelines by determining the following parameters:

Linearity, accuracy, precision, robustness, ruggedness, detection limit and quantification limit.

Linearity

Five concentrations of the standard teneligliptin (4, 8, 12, 16 and 20 µg/ml) were prepared and the regression coefficient were determined.

Accuracy

The accuracy of the method was determined by method of standard addition at three percentage levels, namely 50%, 75% and 100%.

Precision

To determine the precision of the proposed method, pure drug solutions (teneligliptin) at a concentration within the working range were prepared and analysed in three replicates during the same day on three consecutive days.

Robustness

To evaluate the robustness of the methods the concentration of the bromocresol green and the pH of the phosphate buffer was changed and the effect of these changes in the absorbance of the sample solution was studied.

Ruggedness

Method ruggedness was evaluated by performing the analysis following the recommended procedure by three different analysts.

Limit of detection (LOD) and the limit of quantification (LOQ)

LOD and LOQ values were calculated to check the sensitivity of the method by using following equation;

$$LOD=3.3\sigma/S$$

$$LOQ=10\sigma/S$$

Where σ the standard deviation and S is the slope of the curve.

RESULT AND DISCUSSION

Selection of wavelength

The detection wavelength was selected by preparing 12 µg/ml of teneligliptin from the stock solution and scanning in the visible range from 400 -800nm, as shown in the figure 3.

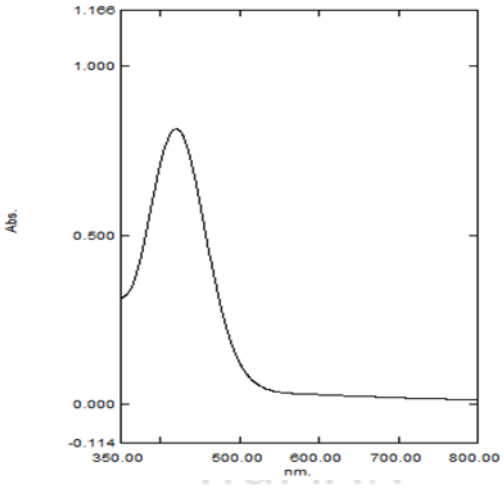

Figure 3: Visible Spectrum of Teneligliptin showing λ_{max} at 419nm

Assay of marketed formulation

The assay was performed in triplicate and the tabulated as shown in table 1.

TABLE 1: ASSAY OF MARKETED FORMULATION BY COLORIMETRY

METHOD VALIDATION

Marketed formulation	Drug	Label claim	*Estimated amount(mg) (mean±SD)	% purity	%RSD	
Tenlimac	Teneligliptin	20mg	19.97±0.057	99.9	0.17	

***Triplicate performance**

The overlay spectrum of teneligliptin is given in figure 4.

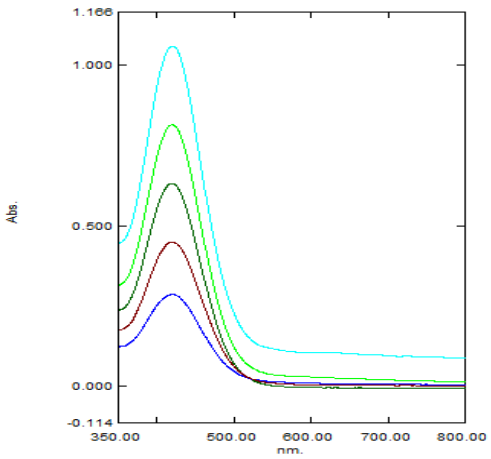

Figure 4: Overlay Spectrum of Teneligliptin at 419nm

Linearity: The solution obeyed Beer-Lamberts law in the range of 4-20mcg/ml with the regression of 0.9989, as shown in table 2 and figure 5.

TABLE 2: LINEARITY AND RANGE

SL.NO	Amount taken(µg/ml)	Absorbance
1	4	0.203
2	8	0.475
3	12	0.678
4	16	0.904
5	20	1.144

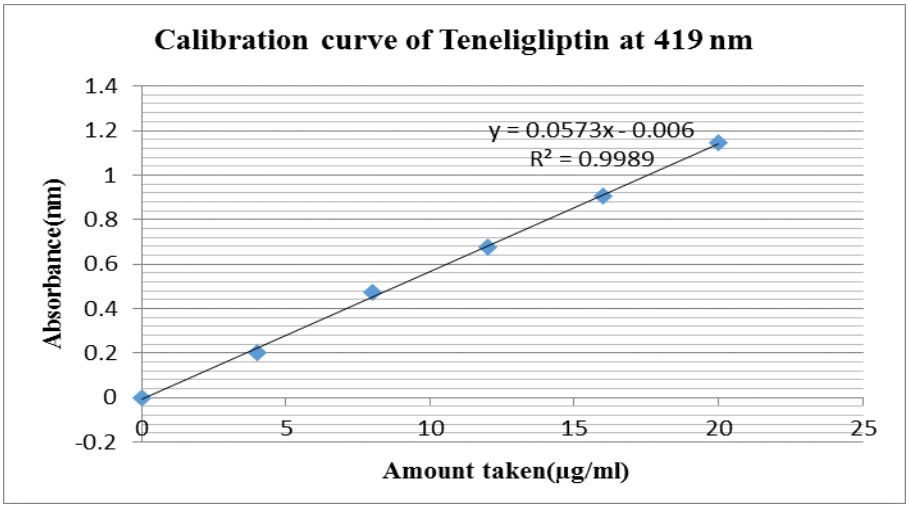

Figure 5: Calibration curve of Teneligliptin at 419 nm

Accuracy: the recovery studies were carried out three times and the percentage relative standard deviations was calculated and are shown in table 3.

TABLE 3: ACCURACY STUDIES OF TENELIGLIPTIN

Drug	Theoretical % target level	Amount added(µg/ml)	Amount recovered(mg)	% Recovery *mean	%RSD
Teneligliptin	50 75 100	6 9 12	20.8 19.9 21	104.32 99.70 105	0.71

***Triplicate performance**

Precision: Intra-day and inter-day precision were determined by evaluating 12 µg/ml and the percentage RSD was calculated, as shown in table 4.

TABLE 4: PRECISION STUDIES OF TENELIGLIPTIN

Drug	Amount(µg/ml)	*Intra-day		*Inter-day	
		%content	% RSD	%content	%RSD
Teneligliptin	12	100 101.57 102.5	1.03	108 107 107.3	0.47

***Triplicate performance**

Robustness: For the proposed method, robustness was carried by modifying the concentration of the bromocresol green and the pH of the phosphate buffer and the results were recorded as shown in table 5.

TABLE 5: ROBUSTNESS

Drug	Amount taken(µg/ml)	Parameter altered		Amount recovered(mg) (mean±SD)	%content	%RSD
Teneligliptin	12	**concentration of BCG (µg/ml)**	2400	0.0210±0.0001	105.36	0.78
			2600	0.0216±0.0001	108.03	0.38
		change in pH	2.4	0.0216±0.0001	108.05	0.38
			2.8	0.0212±0.0001	106.27	0.65

Ruggedness: To demonstrate ruggedness, three different analysts performed the proposed method and percentage RSD was calculated, as shown in table 6.

TABLE 6: RUGGEDNESS

Drug	Analyst	Amount taken(µg/ml)	Amount found(mg) (mean±SD)	%content	%RSD
Teneligliptin	Analyst 1 Analyst 2 Analyst 3	12	0.0205±0.0001 0.0209±0.0001 0.0199±0.0001	102 104.57 99.32	0.620

Limit of detection (LOD)and limit of quantification (LOQ): The LOD and LOQ for the method was found to be 0.926 µg/ml and 2.807 µg/ml, respectively, indicating the method is suitable for analysing in small quantities, as indicated in table 7.

TABLE 7: LOD AND LOQ RESULTS

Drug	LOD(µg/ml)	LOQ(µg/ml)
Teneligliptin	**0.926**	**2.807**

The proposed method for the estimation of teneligliptin in its pure and dosage form can be successfully utilized for routine quality control analysis. Analytical data parameters are shown in table 8.

TABLE 8: ANALYTICAL DATA

Parameter	Teneligliptin
Detection of wavelength	419nm
Beer's law limit	4-20 µg/ml
Regression equation	y = 0.0573x - 0.006
Correlation coefficient	R^2 = 0.9989
Slope	0.0573
LOD	0.926 mcg/ml
LOQ	2.807 mcg/ml

CONCLUSION

A simple, accurate, precise, specific and sensitive method was developed for estimation in tablet formulation by colorimetric method and find considerable application when sophisticated equipments like HPTLC, GC, HPLC and electrophoresis are not easily attainable. Extractive spectrophotometric procedures are the forerunner for quality control because of their high sensitivity and selectivity. Besides the proposed method have the advantages of low cost analytical reagents along with less reagent utilization leads to an environmental friendly procedure which construct a suitable method for routine quality control analysis work. For the determination of teneligliptin by visible spectrophotometric method, BCG was used to produce yellow colored complex. the reaction was carried out in a specific pH of 2.6 using phosphate buffer. The ion pair complex formed were extracted using chloroform and the developed yellow colour complex showed maximum absorbance at 419nm.

Linearity was found in the concentration range of 4- 20 µg/ml. The slope, intercept, and correlation coefficient values were found to be 0.0573, 0.006 and 0.9989 respectively. The developed colour was stable for about 25 min at room temperature. Low percentage relative standard deviation values show that the developed method is precise, robust and rugged. The

recovery studies were carried out at 50,75 and 100% levels. The method was successfully used for estimation of teneligliptin in bulk and pharmaceutical formulation.

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CONFLICT OF INTEREST - None to declare

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