A NEW SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF MICRO AMOUNTS OF THE DRUG TENELIGLIPTIN USING BROMO CRESOL GREEN (BCG)

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ABSTRACT:

A simple versatile Spectrophotometric method has been developed for the determination of micro amount of the drug Teneligliptin in pharmaceutical formulations and in the tablet dosage form using Bromo Cresol Green (BCG). The interaction of the drug with BCG results in the formation of an ion- pair complex, the composition of which is established as 1:1 (Drug: BCG) by Job's continuous variation method. The wavelength of maximum absorbance of the Ion-pair complex is found to be 400 nm. The absorbance values of the Ion-pair complex are found to increase linearly with the increase in the amount of the drug Teneligliptin up to 250 μg/ml. This suggests the suitability of the method for the determination of the drug in the range 10 μg/ml to 250 μg/ml. This also shows the verification of the Beer-Lambert's Law in this range. The method is successfully employed to evaluate the assay of commercial tablets in pharmaceutical formulations for Teneligliptin and the results agreed very well. The molar absorptivity and Sandell Sensitivity of the method are 1.168×10^4 lit/mole/cm and 0.0365 μg/ml/cm^2 respectively.

Keywords:

Bromo Cresol Green; Ion-pair complex; Pharmaceutical formulations; Spectrophotometry; Teneligliptin.

INTRODUCTION:

Teneligliptin is chemically {(2S, 4S)-4-[4-(3-methyl-1-phenyl-1H-pyrazol-5-yl) piperazin-1-yl] pyrrolidin-2- yl}(1, 3-thiazolidin-3-yl) methanone hemipenta bromide hydrate. Teneligliptin [1-6] is a novel oral Dipeptidyl Peptidase-4 (DPP-4) inhibitor developed by Mitsubishi Tanabe Pharma company and approved in Japan in September 2012 for the the management of type 2 Diabetes Milletus(T2DM). Currently Teneligliptin is marketed in Japan, Argentina and India. It is obtained with its characteristics from the local Pharma institute in Hyderabad. Teneligliptin is a recently developed new and relatively economic dipeptidyl peptidase-4 (DPP-4) inhibitor. It is a medicine to control raised blood sugar levels in patients with type 2 diabetes. It belongs to a class of anti-diabetic medicines called DPP-4[7-9] inhibitors or gliptins. Teneligliptin works by blocking an enzyme DPP-4 that degrades insulin thereby increasing insulin levels in the body and decreasing glucagon levels. Teneligliptin works by increasing the release of insulin from the pancreas and decreasing the hormones that raise the blood sugar levels. This reduces the fasting and post prandial sugar levels. Diabetes is a common non-communicable disease and has reached to epidemic stage in several countries. Considering the huge epidemic of type-2 diabetes mellitus newer therapies that improve efficacy, tolerability and long-term compliance and prevent complications associated with type 2 Diabetes Milletus (T2DM) are always required and preferred. The above prominent characteristics of the drug prompted the authors to take up this investigation relating to the method for the quantitative determination of the drug and its application in the analysis of the assay in the tablets available commercially in the market. The results obtained in the investigations are presented in the present paper. A survey of chemical and biochemical literature has shown that not much attention has been paid to the analytical estimations of the drug. However, a few methods have been reported [10-17].

MATERIALS AND METHODS:

(A) Instruments used

(i) Spectrophotometer:

A double beam UV-Spectrophotometer Model SP-UV200 with 1 cm matched quartz cuvettes is employed throughout the study for all absorbance measurements.

(ii) pH Meter: A digital ELICO-pH Meter Model LI-120 is used for pH measurements.

(B) Preparation of Reagents and Solutions:

(i) Teneligliptin solution:

50 mg of pure Teneligliptin is dissolved in methanol and the volume of the resulting solution is adjusted to the mark in the 50 ml standard flask with methanol. This is used as the stock solution of the drug. The working solution with concentration 100 µg/ml of the drug is prepared by suitably diluting the stock solution as and when required.

(ii) Bromo Cresol Green solution (0.5% w/v):

Bromo Cresol Green is prepared by dissolving 500 mg of Bromo Cresol Green in 100 ml of distilled water.

(iii) Buffer solution pH 3.5 (Potassium acid phthalate - HCl):

The potassium acid phthalate – HCl Buffer solution is prepared by diluting a mixture of 50 ml of 0.2M potassium acid phthalate and 8.4 ml of 0.2M HCl to 200 ml with distilled water and the pH is adjusted to 3.5.

All other chemical substances and reagents employed in the present investigations are of AR Grade only.

RESULTS & DISCUSSION:

Teneligliptin when treated with Bromo Cresol Green (BCG) forms an Ion pair complex. This Ion-Pair complex formation reaction is spectrophotometrically monitored to develop a method for the determination of purity of the drug. In this process a detailed investigation is done to know the optimization of various parameters such as wavelength of maximum absorbance (λ_{max}), the effect of concentration of Buffer solution (pH 3.5) and Bromo Cresol Green on the absorbance of Ion Pair complex are established and the procedures adopted in each case are described as follows:

Absorption Spectrum of Ion Pair Complex: -

The absorption spectrum of the Ion – Pair complex formed between Teneligliptin and Bromo Cresol Green is obtained in order to fix the wave length of maximum absorbance and its experimental procedure is as follows.

2 ml of Teneligliptin solution (100 µg/ml), 2 ml of BCG solution (0.5% w/v), 3 ml of Buffer solution of pH 3.5 and 2 ml of methanol are taken in a 10 ml standard flask the resulting solution is made upto the mark with distilled water. Then the absorbance values of the Ion- pair complex formed are measured in the wavelength range 340 nm to 460 nm against the reagent blank. The results obtained are used to draw a graph between the wavelength and the absorbance values. This graphical representation is called the absorption spectrum which is as shown in figure.1 below.

It is seen from the below graph of the absorption spectrum; the maximum absorbance is obtained at 400 nm. Hence for all further studies a wavelength of 400 nm is fixed.

Effect of Buffer solution of pH 3.5: -

The effect of Buffer solution of pH 3.5 on the absorbance of ion pair complex is studied by taking varying volumes of (x ml) Buffer solution of pH 3.5 in a series of 10 ml standard flask, keeping the volume of Teneligliptin solution fixed at 2 ml. To each flask 2.5 ml of BCG solution (0.5% w/v) and 1.5 ml of methanol are added followed by the addition of distilled water to make up each 10 ml flask to mark. The absorbance of each solution is recorded at 400 nm against the suitable blank. The results are as tabulated in Table .1 below.

From the above Table 1, it is observed that 1.5 ml of Buffer solution of pH 3.5 is necessary to achieve maximum absorbance. Hence for all further studies a volume of 1.5 ml of Buffer solution of pH 3.5 is fixed.

Effect of Bromo Cresol Green (BCG) concentration: -

The effect of Bromo Cresol Green on the absorbance of Ion – Pair complex is studied by taking varying volumes of (x ml) of BCG in a series of 10 ml standard flasks. After taking x ml (0.5 ml to 2.5 ml) of BCG in each flask, 1.5 ml of Buffer solution of pH 3.5, 2 ml of drug solution of Teneligliptin, 2 ml of methanol are added and the resulting solution is made upto 10 ml using distilled water. The absorbance of each solution is recorded at 400 nm against a suitable blank. The results obtained are mentioned in Table 2 below.

From the data presented in the below Table 2, it is clear that 2.5 ml of BCG solution is sufficient to give maximum absorbance. Therefore 2.5 ml of BCG solution is fixed.

Effect of time: -

The absorption is measured after preparing the solution after 10 minutes, 20 minutes and 30 minutes and it is observed that there is no change in the absorbance values after 30 minutes of preparation. So, it is concluded that, the stability of the complex formed after 30 minutes of sample preparation.

Effect of concentration of drug Teneligliptin: -

This study leads to the effect of the drug Teneligliptin concentration on the absorbance of Ion – Pair complex under established optimal experimental conditions. The recommended procedures for the

calibration curve and for the obedience of Beer-Lambert's law for the quantitative spectrophometric determination of the drug Teneligliptin is as follows.

Calibration Curve: Obedience of Beer-Lambert's Law: -

Various aliquots (xml i.e., 0.5 ml to 2.5 ml) of Teneligliptin solution (100µg/ml) are taken in a series of 10ml standard flask. To each flask, 1.5 ml of Buffer solution of pH 3.5, 2.5 ml of BCG solution (0.5% w/v), 2 ml of methanol followed by distilled water are added so as to make the total volume in each case at 10 ml. The contents of each flask are shaken well and allowed to stand for a minute for equilibration. The absorbance of each solution is measured at 400 nm against a suitable reagent blank which is prepared in a similar manner but devoid of drug solution. The results obtained are mentioned in Table 3 and figure 2 below.

It is obviously clear from the data presented in the above table, and from this calibration straight line, that the absorbance values increased linearly with the increase in the amount of the drug. This verifies the Beer-Lambert's law and suggests that the method can be successfully employed for the spectrophotometric quantitative determination of the drug Teneligliptin in the range 10 µg/ml to 250 µg/ml. The molar absorptivity and the sandell sensitivity of the method are found to be 1.168×10^4 lit/mole/cm and 0.0365 µg/ml/cm^2 respectively.

Stoichiometric composition of Ion-Pair Complex: Job's continuous variation method: -

The composition of the Ion – Pair complex between the drug Teneligliptin and the reagent BCG is established by the Job's continuous variation method. In this, the equimolar concentrations (5×10^{-4} M) of both the drug and BCG are varied continuously keeping the total volume of mixed solution as constant at 10 ml. In each case, the absorbance is measured at 400 nm against a suitable blank. The data obtained is presented in the Table. 4 and the fig. 3 below.

The data in the above table are plotted in the form of a graph between volume fraction of the drug i.e., (V_1/V_1+V_2) on X- axis and the absorbance values on Y-axis. The graph obtained is as shown in figure. 3 below.

From the graph shown above, it is found that one mole of the drug is reacting with 1 mole of BCG, there by establishing the stoichiometry of the Ion-Pair complex as 1:1 (Drug: BCG).

The proposed method offers an advantage over the methods already reported in literature. Since it is a direct method involving the formation of an ion-pair complex. when compared to a few methods involving redox reagents such as Bromate-Bromide mixture [loc. cit-ref-13], Ceric Ammonium Nitrate [

loc. cit-ref-14] and charge transfer complex using 2,3-Dichloro – 5,6-Dicyano-1,4-Benzoquinone (DDQ) [loc. cit -ref-15]. However, the method compares well and the results are in good agreement with the ion-pair complex reported with Bromo Phenol Blue [loc.cit-ref-16].From the results obtained on the various studies above, it is ascertained and discussed that the absorption maximum of the ion-pair complex is 400 nm in the presence of 1.5 ml of a Phthalic acid Phthalate-HCl buffer solution of pH 3.5.A study of variation of BCG indicated that 2.5 ml of BCG (0.5% w/v) solution exhibits maximum complexation. The complex is found to be quick stable without any change in the absorbance values for more than 30 minutes. These parameters are established before the quantitative estimation is carried out by studying the calibration curve obtained from the Beer-Lamberts law. Thus, the results are found to be very optimistic, encouraging and applicable to the assay of the pharmaceutical formation.

Assay of Teneligliptin drug in pharmaceutical formulations: -

The recommended procedure for the quantitative micro determination of Teneligliptin drug is applied for the assay of the drug in the dosage form of the commercial tablets and also in pharmaceutical formulations. The assay is carried out as follows:

20 tablets of Teneligliptin are weighed and finely powdered. An accurately weighed portion of the powdered sample equivalent to 50 mg of Teneligliptin is taken in a 50 ml volumetric flask containing 25 ml of methanol and is sonicated for about 20 minutes. The resultant solution is filtered through Whatman filter paper No.41 into another 50 ml volumetric flask. The filter paper is washed several times with methanol and the washings are added to filtrate. The final volume is made upto the mark with methanol. Now, 5 ml of filtrate of the sample solution is diluted to 10 ml with methanol and treated as per the recommended procedure of calibration. From this, the amount of the drug present in the sample is computed from the calibration curve. The results obtained are as shown in Table.5 below.

CONCLUSION:

The calibration curve is linear up to 250 µg/ml indicating the suitability of the proposed method for the spectrophotometric determination of Teneligliptin in the range of 10 µg/ml to 250 µg/ml. The standard deviation values are found to be low showing high accuracy and reproducibility of the method. The calculated `t' values are less than the `t' theoretical values with 4 degrees of freedom at 95% level of significance. This indicates that there is no significant difference between the proposed method and the standard method. Further, there is no effect of additives and excipients such as starch, calcium lactose and glucose in the concentration of those present in general pharmaceutical preparations. Thus, the proposed

method can be conveniently adopted for the routine analysis and estimation of Teneligliptin in pharmaceutical formulations.

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Data Availability: The data pertaining to the manuscript will be available to the public with prior intimation.

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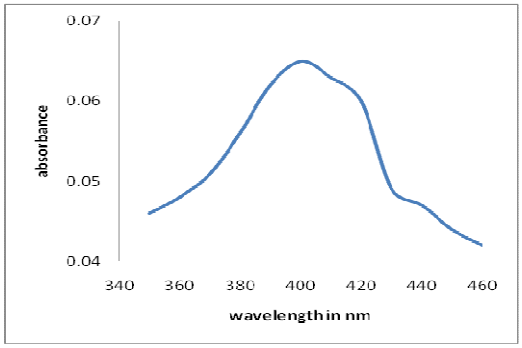

Fig.1 Absorption Spectrum of Ion-Pair complex of Teneligliptin with BCG

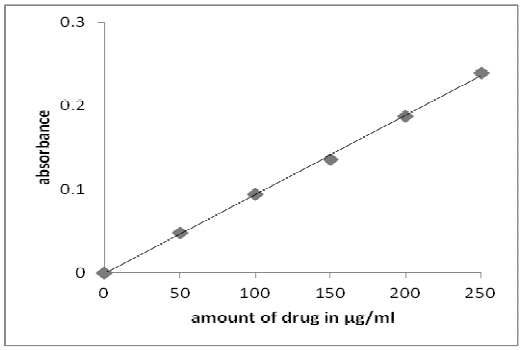

Fig 2: Calibration curve –Verification of Beer-Lambert's Law

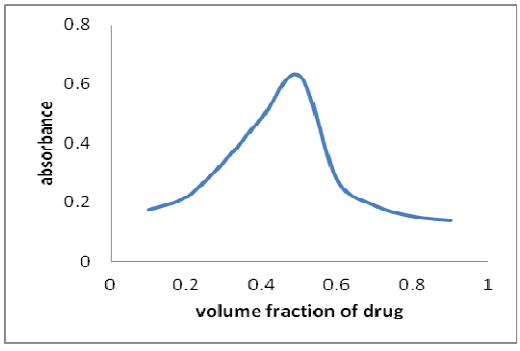

Fig. 3: Job's Continuous Variation Method

Table 1: Effect of Buffer Solution pH 3.5

S.No	Vol. of Teneligli-ptin (100 µg/ml) in ml	Vol. of BCG (0.5% w/v) in ml	Vol. of Buffer Solution(pH 3.5) x ml	Vol.of Methano-l in ml	Vol. of distilled water in ml (4-x)	Total Vol. in each flask in ml	Absorba-nce
1	2.0	2.5	0.5	1.5	3.5	10	0.223
2	2.0	2.5	1.0	1.5	3.0	10	0.216
3	2.0	2.5	1.5	1.5	2.5	10	0.233
4	2.0	2.5	2.0	1.5	2.0	10	0.196
5	2.0	2.5	2.5	1.5	1.5	10	0.200

Table 2: Effect of BCG on Ion- Pair complex

S. No	Vol. of Teneli-gliptin(100 µg/ml) in ml	Vol.of BCG solution (0.5% w/v) x ml	Vol.of Buffer Solution(pH 3.5) in ml	Vol.of Methanol in ml	Vol.of distilled water in ml (4.5-x)	Total Vol. in each flask in ml	Absorbance
1	2.0	0.5	1.5	2.0	4.0	10	0.057
2	2.0	1.0	1.5	2.0	3.5	10	0.081
3	2.0	1.5	1.5	2.0	3.0	10	0.107
4	2.0	2.0	1.5	2.0	2.5	10	0.131
5	2.0	2.5	1.5	2.0	2.0	10	0.162

Table 3: Calibration Curve – Obedience of Beer- Lambert's Law

S. No	Vol. in ml Teneligli-ptin (100 µg/ml) x ml	Amount of Teneligl-iptin in µg/ml	Vol.of Buffer Solution (pH 3.5)in ml	Vol.of BCG (0.5% w/v) in ml	Vol.of Metha-nol in ml	Vol.of distilled water in ml (4-x)	Total Vol. in each flask in ml	Absorb -ance
1	0.5	50	1.5	2.5	2.0	3.5	10	0.048
2	1.0	100	1.5	2.5	2.0	3.0	10	0.094
3	1.5	150	1.5	2.5	2.0	2.5	10	0.136
4	2.0	200	1.5	2.5	2.0	2.0	10	0.188
5	2.5	250	1.5	2.5	2.0	1.5	10	0.239

Table 4: Job's method of continuous variation:

S.No.	Vol. of Teneligliptin (5×10^{-4}M) V_1 in ml	Vol. of Buffer Solution of pH 3.5 in ml	Vol. of BCG (5×10^{-4} M) V_2 in ml	Vol. of Methanol in ml	Total volume in each flask in ml	Volume fraction (X) of the drug (V_1/V_1+V_2)	Absorba-nce
1	0.5	3.0	4.5	2.0	10	0.1	0.173
2	1.0	3.0	4.0	2.0	10	0.2	0.217
3	1.5	3.0	3.5	2.0	10	0.3	0.336
4	2.0	3.0	3.0	2.0	10	0.4	0.485
5	2.5	3.0	2.5	2.0	10	0.5	0.625
6	3.0	3.0	2.0	2.0	10	0.6	0.272
7	3.5	3.0	1.5	2.0	10	0.7	0.187

8	4.0	3.0	1.0	2.0	10	0.8	0.152
9	4.5	3.0	0.5	2.0	10	0.9	0.139

Table 5: Assay of Teneligliptin in Tablets

Sample	Labelled amount in mg	Amount found by present method ±SD*	Percentage of Label claim	*t_{cal}	% RSD
Tablet I	20	20.008±0.03	100.008	0.5110	0.17
Tablet II	20	20.002±0.08	100.002	0.0533	0.41

* Average of 5 determinations based on label claim.