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Spectrophotometric Determination of Megagliptin and its Assay by Charge - Transfer Method using 2,3-Dichloro- 5,6 -Dicyano-1,4-Benzoquinone (DDQ)

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ABSTRACT

A simple, versatile and a new spectrophotometric method is proposed for the estimation of microgram quantities of the drug Megagliptin. The drug forms a charge transfer (CT) complex with 2,3-Dichlro-5,6-Dicyano-1,4- Benzoquinone (DDQ), the stoichiometry of which is established as 1:1 by Job's continuous variation method. The wavelength of maximum absorbance of the CT complex is found to be 385 nm. The absorbance values of the CT complex increased linearly with the increase in the amount of the drug Megagliptin upto 300 µg/ml. This suggests that suitability of the method for the determination of the drug in the range 10 µg/ml to 300 µg/ml. This also indicates the verification of the Beer-Lambert's Law in this range. The method is successfully applied to evaluate the assay of commercial tablets in pharmaceutical formulations for Megagliptin and the results agreed very well. The molar absorptivity and Sandell Sensitivity of the method are 2.346 x 10^4 lit. mol^{-1}. cm^{-1} and 0.01818 $\mu g.ml^{-1}.cm^2$ respectively.

Keywords: Spectrophotometry, Megagliptin, Teneligliptin, Charge Transfer Complex, DDQ, Pharmaceutical Formulations.

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INTRODUCTION

A novel class of compounds which revolutionized the treatment of diabetes in the recent past are Dipeptidylpeptidase-4 (DPP-4) inhibitors which are widely known as Gliptins. Megagliptin, which is Teneligliptin hydrobromide hydrate is a novel, potent peptidomimetric and long acting DPP-4 inhibitor is the approved drug for the treatment of type 2 Diabetes Milletus (T2DM). Megagliptin 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, the structure of which is as shown in Figure 1.

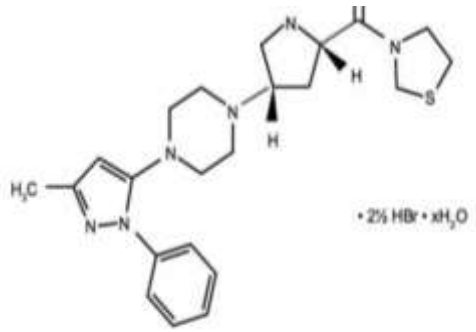


Figure 1: Structure of Megagliptin

It is a unique structure characterized by five consecutive rings. Recent studies have shown that the drug exhibits multiple pharmaceutical effects which include vasoprotective, neuro protective effects etc.

Megagliptin which inhibits the enzyme DPP-4 and degrades incretin, a hormone which adjusts blood glucose level and improves blood glucose control. A survey of chemical literature has shown that the drug Megagliptin is effectively used to treat Type 2 diabetes mellitus[1-7] (T2DM). Further it is noticed that only a very few methods on the development and validation for the estimation of Megagliptin are reported which include UV spectrophotometric methods[8-9] and High Performance Thin Layer Chromatographic (HPTLC) method[10]. Since not much attention has been given to develop newer analytical UV spectrophotometric methods for the quantitative determination of such an effective and potential anti diabetic drug in the dosage form and in the pharmaceutical formulations form, the authors are prompted to take up this study and develop suitable new, rapid, sensitive, precise and accurate method for the determination of Megagliptin. The results obtained in the present investigations are communicated in this paper.

MATERIALS AND METHOD

Instrumentation:

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

Preparation of Reagents and Solutions:-

Megagliptin solution:

50 mg of pure Megagliptin 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.

DDQ solution (0.1% w/v):

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone which is abbreviated as DDQ is prepared by dissolving 100 mg of it in 100 ml of Acetonitrile.

All other chemical substances and reagents employed in the present investigation are of is AR grade only.

RESULTS AND DISCUSSION

Megagliptin when treated with 2,3-Dichloro-5,6-Dicyano-1,4-Benzoquinone (DDQ) forms a charge transfer (CT) complex in which the drug Megagliptin acts as the n-electron donor and the DDQ as the electron acceptor. This charge transfer (CT) complex formation reaction is spectrophotometrically monitored to develop the method of determination of the drug. In the process of carrying out detailed investigations, first of all, the various required optimum parameters such as the wavelength of maximum absorbance (λ_{max}), the effect of the concentration of DDQ on the absorbance of the charge transfer complex are established and the procedures adopted in each case are described as follows:

Absorption Spectrum of CT Complex:

The absorption spectrum of the CT complex formed between Megagliptin and DDQ is obtained in order to fix the wavelength of maximum absorbance in the present study. The experimental procedure adopted is as follows:

1 ml of Megagliptin solution (100 µg/ml), 2 ml of DDQ solution (0.1% w/v) and 2 ml methanol are taken in a 10 ml standard flask. The resulting solution is made upto the mark with distilled water. The contents of the flask are shaken well and allowed to stand for a minute for equilibration. Then the absorbance values of the CT complex formed are measured in the wavelength range 330 nm to 430 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 shown in Figure 2.

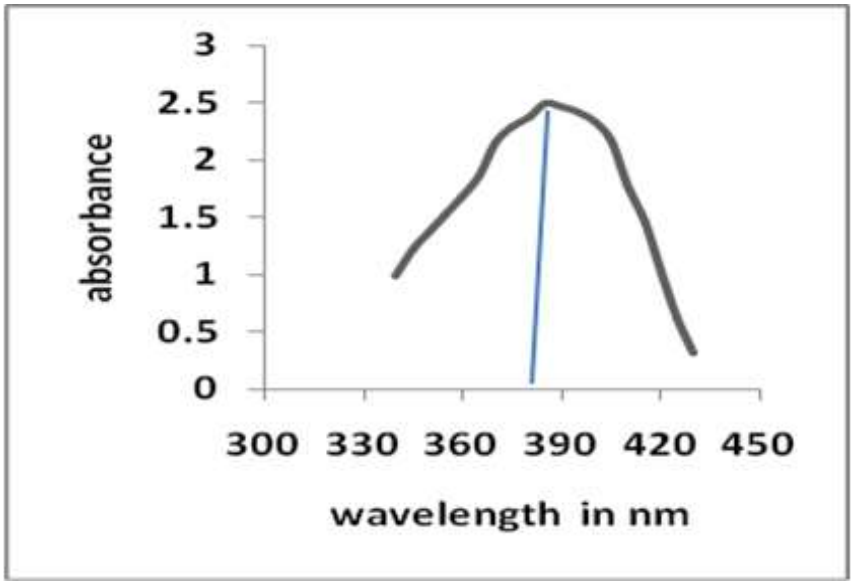


Figure 2 : Absorption spectrum of CT complex

It is seen from the Figure 2 of the absorption spectrum, that the maximum absorbance is obtained at 385 nm. Hence for all further studies, the wavelength 385 nm is fixed.

Effect of DDQ Concentration:-

The effect of DDQ on the absorbance of the CT complex is studied by taking varying volumes (x ml) of DDQ in a series of 10 ml standard flasks keeping the volume of Megagliptin solution fixed at 2 ml. To each flask 2 ml of methanol is added followed by the addition of distilled water to make up each 10 ml flask to the mark. The absorbance of each solution is recorded at 385 nm against a suitable blank. The results obtained are tabulated in Table 1. From the data presented in Table 1, it is clear that 2 ml of DDQ solution is required for maximum absorbance. Hence for all further studies a volume of 2.0 ml of DDQ solution is fixed.

Table 1: Effect of DDQ on CT complex

2 ml Megagliptin (100 µg/ml) + x ml of DDQ solution (0.1% w/v) + 2 ml of methanol + (6-x) ml distilled water = Total volume kept at 10 ml each λ_{max} = 385 nm.

S.No	Volume of DDQ solution X ml	Absorbance
1	0.5	1.020
2	1.0	1.069
3	1.5	1.349
4	2.0	1.508
5	2.5	1.478
6	3.0	1.452

From the data presented in Table 1 above, it is clear that 2 ml of DDQ solution is required for maximum absorbance. Hence for all further studies a volume of 2.0 ml of DDQ solution is fixed.

Effect of Concentration of Drug Megagliptin: Calibration Curve:

This study pertains to the effect of the drug Megagliptin concentration on the absorbance of the CT complex under the established optimal experimental conditions. The recommended procedure is as follows:

Recommended procedure for the determination of Megagliptin: Applicability of BEER LAMBERT'S Law:

Various aliquots (x ml i.e., 0.5 ml to 3.0 ml) of Megagliptin solution (100 µg/ml) are taken in a series of 10 ml standard flask. To each flask, 2 ml of DDQ solution (0.1% w/v), 2 ml of methanol followed by (6-x) ml of 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 385 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 2 and Figure 3.

Table 2: Calibration curve – Applicability of BEER LAMBERT'S Law

x ml (0.5 ml to 3.0 ml) of Megagliptin solution (100 µg/ml) + 2 ml DDQ solution (0.1% w/v) + 2 ml methanol + (6-x) ml distilled water = Total volume kept at 10 ml in each case. λ_{max} = 385nm

S.No	Volume in ml Megagliptin (100 µg/ml) x ml	Amount of Megagliptin in µg/ml	Absorbance
1	0.5	50	0.35
2	1.0	100	0.67
3	1.5	150	0.89
4	2.0	200	1.22
5	2.5	250	1.46
6	3.0	300	1.79

It is obviously clear from the data presented in Table 2 that the absorbance values increased linearly with the increase in the amount of the drug

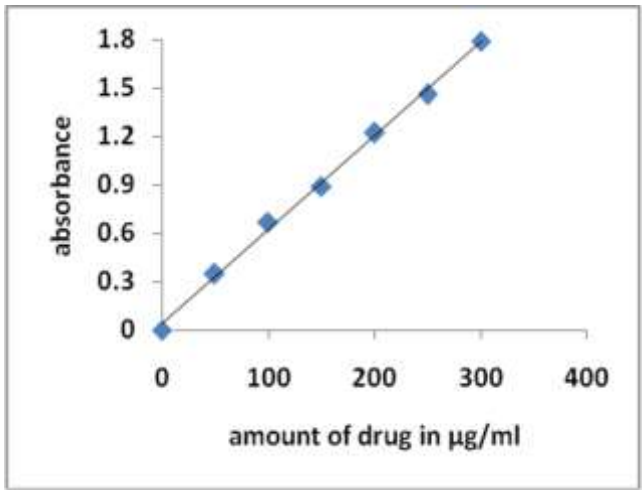


Figure 3: Calibration curve –Verification of Beer-Lambert's Law

It is obviously clear from the data presented in Table 2 and from this calibration straight line as shown in Figure 3 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 suitably employed for the spectrophotometric quantitative determination

of the drug Megagliptin in the range 10 µg/ml to 300 µg/ml. The molar absorptivitiy and the Sandell Sensitivity of the method are found to be 2.346 x 10^4 lit. $mole^{-1}.cm^{-1}$ and 0.01818 µg ml^{-1} cm^2 respectively

Stoichiometric Composition of the Charge Transfer complex : Job's Continuous Variation Method:

The composition of the CT complex between the drug Megagliptin and the reagent DDQ is established by the Job's "continuous variation method". In this method, equi molar concentration (5 x 10^{-4} M) of both the drug and DDQ are varied continuously keeping the total volume of the mixed solution as constant at 5 ml. In each case, the absorbance is measured at 385 nm against a suitable blank. The data obtained is presented in Table 3 and Figure 4

Table 3: Job's method of continuous variation

S.No.	Volume of Drug (5 x 10^{-4} M) V_1 in ml	Volume of DDQ (5 x 10^{-4} M) V_2 in ml	Total volume in ml	Volume fraction (X) of the drug (V_1/V_1+V_2)	Absorbance
1	4.5	0.5	5.0	0.1	0.34
2	4.0	1.0	5.0	0.2	0.46
3	3.5	1.5	5.0	0.3	0.71
4	3.0	2.0	5.0	0.4	0.89
5	2.5	2.5	5.0	0.5	1.20
6	2.0	3.0	5.0	0.6	0.84
7	1.5	3.5	5.0	0.7	0.72

8	1.0	4.0	5.0	0.8	0.63
9	0.5	4.5	5.0	0.9	0.39

The data in Table- 3 are plotted in the form of a graph between volume fraction ((V_1/V_1+V_2) on the x- axis and the absorbance values on the y-axis. The graph obtained is shown in Figure 4

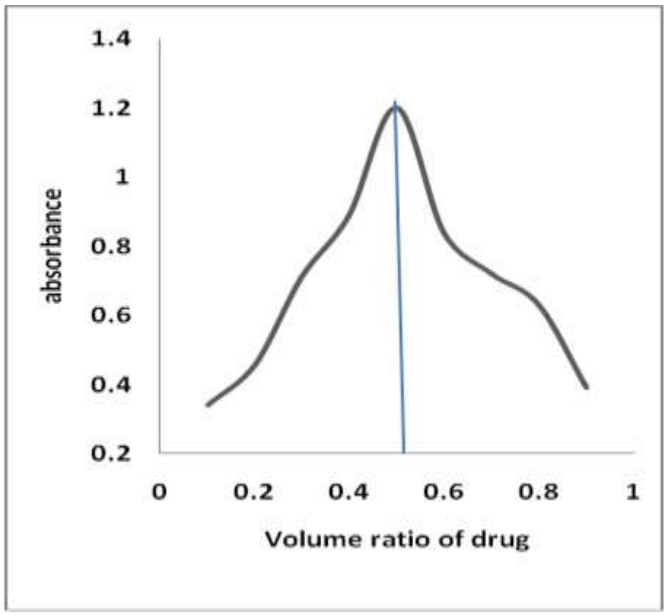
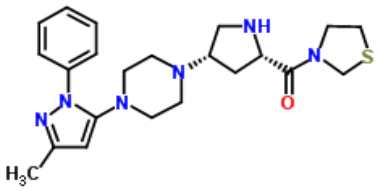
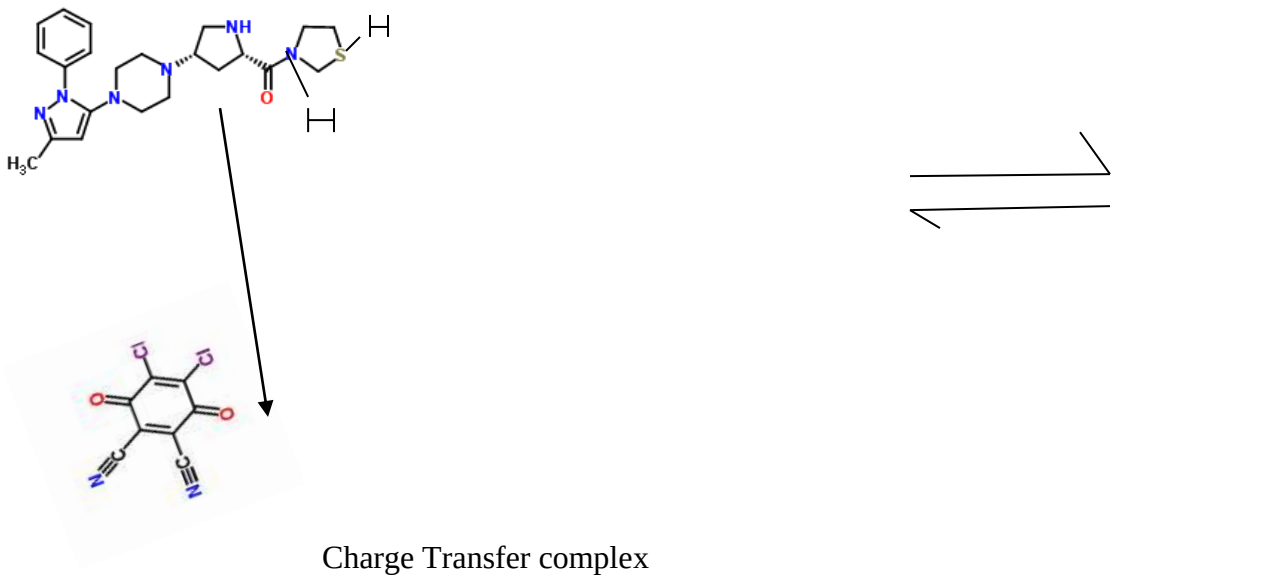


Figure 4: Job's Continuous Variation Method

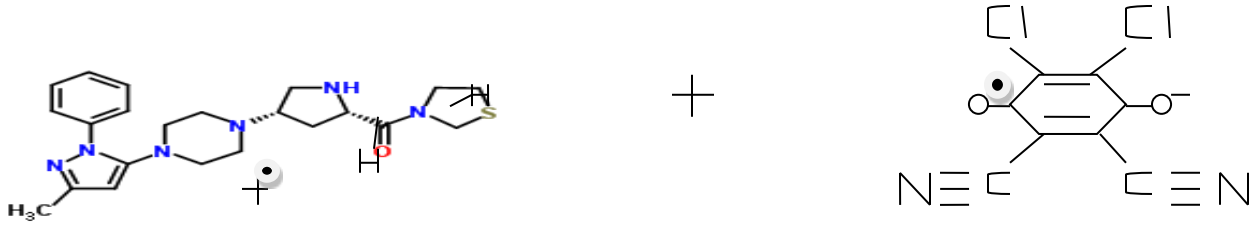
The data in Table 3 are plotted in the form of a graph between volume fraction (V_1/V_1+V_2) on the x- axis and the absorbance values on the y-axis. The graph obtained is shown in Figure 4

From the Figure 4, it is found that one mole of the drug is reacting with one mole of DDQ thereby establishing the stoichiometry of the CT complex as 1:1 (Drug: DDQ). The probable reaction sequence may be represented as shown in Figure 5.





Charge Transfer complex



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cation anion

Figure 5 : Formation of 1: 1 CT Complex

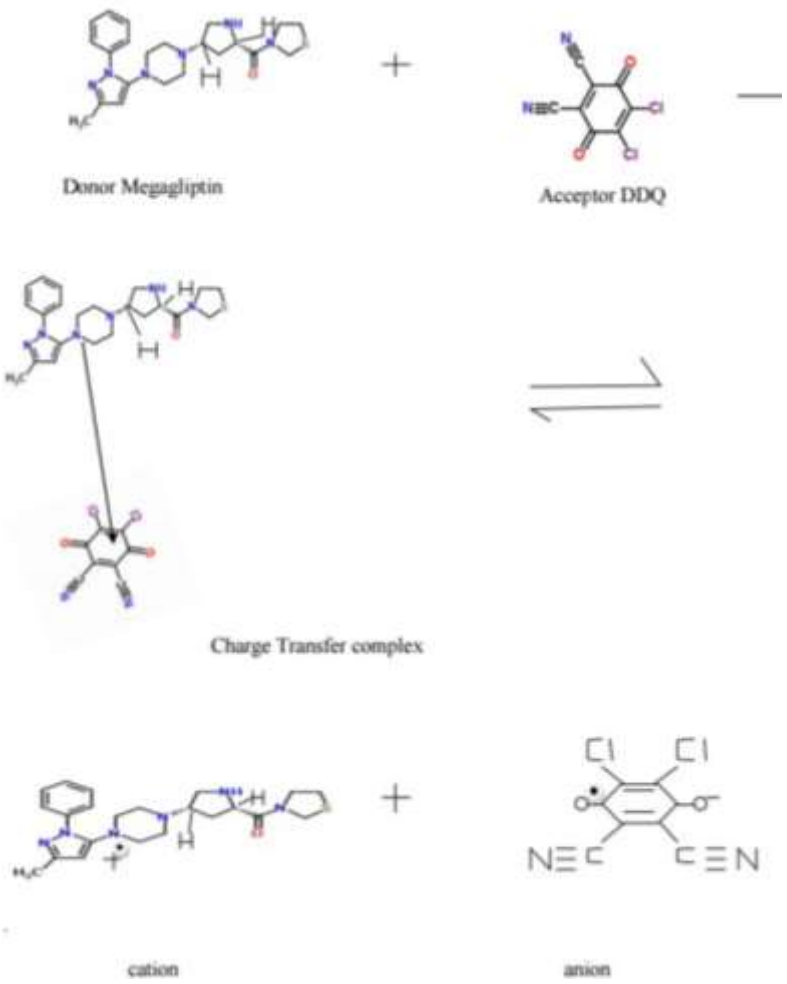

Figure 5 : Formation of 1: 1 CT Complex

Assay of Megagliptin drug In pharmaceutical formulations :

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

20 tablets of Megagliptin are weighed and finely powdered. An accurately weighed portion of the powdered sample equivalent to 50 mg of Megagliptin 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 the filtrate. The final volume is made up to 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 shown in Table 4.

Table 4: Assay of Megagliptin in Tablets

Sample	Labelled amount in mg	Amount found by present method ±SD*	Percentage of Label claim	*t_{cal}
Tablet I	20	20.02±0.19	100.1	0.2412
Tablet II	20	19.96±0.40	99.8	0.2216

* Average of 5 determinations based on label claim

CONCLUSION:

The calibration curve is linear up to 300 µg/ml indicating the suitability of the proposed method for the spectrophotometric determination of Megagliptin in the range of 10 µg/ml to 300 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 of estimation of Megagliptin in pharmaceutical formulations.

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