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ANALYSIS OF LINAGLIPTIN IN TABLET DOSAGE FORM BY UV SPECTROSCOPY METHOD, ITS DERIVATIVES AND DIFFERENCE SPECTRA

R.K. Sangeetha* and T. Subashri

Department of Pharmaceutical analysis, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore – 641044, India.

Corresponding Author Dr. R.K. Sangeetha

Department of Pharmaceutical analysis, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore – 641044, India.

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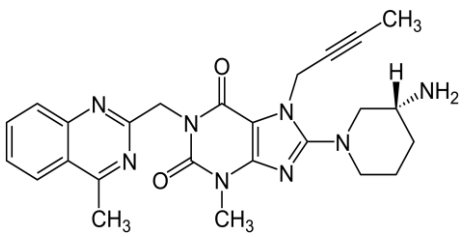
ABSTRACT

A simple and rapid method of analysis of linagliptin has been established by validated UV spectroscopy method, difference spectroscopy and first order & second order derivative spectra after the identification of drug with its IR spectra interpretation. The UV spectroscopic method was validated according to ICH guidelines for its Linearity, Intraday precision, Interday precision, Accuracy, Limit of Detection and Limit of Quantification. The solvent selected was methanol and the maximum absorbance was found at 228 nm. The linearity range was found between 5-40 mcg/ml with a correlation coefficient of 0.9985 and the slope of the curve was found to be 0.0406. The precision values were found to be below 1 showing its repeatability and reproducibility of the method. The accuracy of method was found by the analysis of formulation and recovery studies at 100% level with % RSD value of 0.5472. The difference spectroscopy method was performed with 0.1 M HCl and 0.1 M NaOH. The difference absorbance was used to calculate the assay value and was found to be 95%. The first order spectra and the second order spectra was obtained from the zero order spectrum of standard drug linear range of 10-40 mcg/ml at the wavelength of 238 nm and 228 nm respectively were selected for the calibration graph. The correlation coefficient was found to be 0.9982 and 1.0 for first order and second order spectra. The method adopted for the analysis of linagliptin are economical, reliable, simple and accurate. This metho can be used for further studies of linagliptin.

KEYWORDS: The method adopted for the analysis of linagliptin are linagliptin.

The drug linagliptin is chemically 8-[(3R-3-aminopiperidin-1-yl]-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-2,3,6,7-tetrahydro-1H-purine-2,6-dione with a molecular weight of 472.54 available as flim coated tablet of potent dosage of 5 mg. The structure of linagliptin is as follows



LINAGLIPTIN STRUCTURE

The drug is used for the treatment of Diabetes approved by USFDA in 2011 as a competitive and reversible DPP-4 enzyme inhibitor, that slows the breakdown of insulinotropic hormone glucagon- like peptide for better glycemic control in diabetes patient. GLP and glucose dependent insulinotropic polypeptide are incretin hormones that increase in the production and release of insulin from pancreatic beta cells and decrease the release of glucagon from pancreatic alpha cells. this result in a overall decrease in glucose production in the liver and increase of insulin in a glucose-dependent manner.

INSTRUMENTS AND MATERIALS USED

Jasco V-630 Spectrophotometer are the instrument used for the analysis of the sample.

Shimadzu BL-2204 is the weighing balance used for weighing the samples. The water was collected from Milli - Q Millipore Instrument The organic solvents of LR grade like methanol, DMSO, toluene, petroleum ether was procured from sd Fine Chem limited, Mumbai. The chemical like HCl and NaOH were procured from sd fine Chem Limited Mumbai.

VALIDATION OF UV SPECTROSCOPY

Preparation of stock solution

10 mg of pure drug linagliptin was exactly weighed into 10 ml volumetric flask. The drug was dissolved using minimum quantity of methanol by shaking well and the volume was made upto 10 ml with methanol.

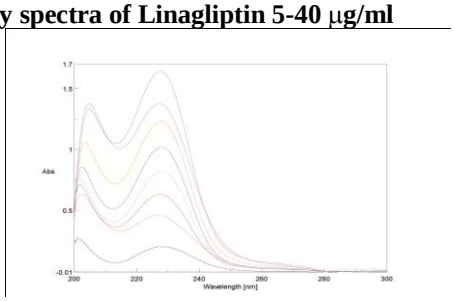
Preparation of serial solution

From the two stock solutions further dilutions were done with the respective solvents and prepared solutions in the concentration range of 5-40µg with methanol.

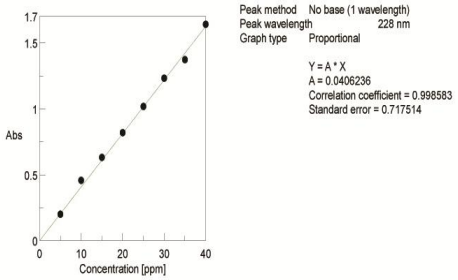
Methanol -Linagliptin Linearity

s.no	Concentration	Absorbance
1.	5	0.1990
2.	10	0.4555
3.	15	0.6299
4.	20	0.8175
5.	25	1.0167
6.	30	1.2307
7.	35	1.3697
8.	40	1.6389

Overlay spectra of Linagliptin 5-40 µg/ml



Calibration graph – Linagliptin 5-40 µg/ml



Range

The solution was found to obey the beer lambert law in the concentration range of 5-40µg/ml in methanol.

Precision

Intraday precision was studied by measuring the absorbance for the standard drug solution of 15µg/ml repeatedly for five consecutive reading.

Interday precision was studied by measuring the absorbance the standard drug solution repeatedly on three different days for the solution of 15µg/ml. The % RSD was calculated and tabulated.

Intraday precision

Precision	Absorbance	%RSD
Day-1	0.6346 0.6344 0.6299 0.6343 0.6243	0.7078

Interday precision

precision	Absorbance	%RSD
Day-1	0.6346 0.6344 0.6299 0.6343 0.6243	0.7078
Day-2	0.6083 0.6087 0.6011 0.6080 0.6072	0.5202
Day-3	0.6075 0.6073 0.6069 0.6065 0.6071	0.0620

Analysis of formulations

Twenty film coated tablets of linagliptin (Trajenta 5 mg) with the label claim of 5mg were weighed accurately and the average weight was calculated. The tablets were powdered in a mortar and mixed thoroughly. The tablet powder equivalent to 10 mg of linagliptin was weighed and transferred into 10 ml volumetric flask. The drug was extracted with methanol and the concentration of the 10µg/ml was prepared. The spectra was recorded and compared with the calibration graph. The absorbance of the 10µg/ml tablet solution was compared with the same concentration in the linearity graph and the amount present was calculated.

Drug	Amount labelled	Amount found	% label claim
Linagliptin	5mg	5.25	105%

Accuracy

The accuracy studies are performed by the addition of standard drug at 100% level of pure drug added to tablet powder. The tablet powder of 10 mg equivalent was extracted in the similar procedure as mentioned in the analysis of formulation. The pure drug of linagliptin at 100% (15 mcg standard drug) level was added and the dilutions were made to give final concentration of 30 µg/ml. The absorbance was measured and % recovery was calculated.

Drug	Concentration of the drug µg/ml	Absorbance	Amount found after addition (mg)	Amount found before addition (mg)	% Recovery
Linagliptin	30	1.3352	0.0325	0.0158	108.49

The value is a mean of five determinations.

Limit Of Detection

The limit of detection was calculated by using statistical formula as expressed below using the S.D of solution of the standard drug and the slope of the calibration graph. LOD was found to be 0.3649µg/ml.

Limit of Quantification

The limit of quantification was found statistically using the SD of solutions of the standard drug and the slope of the calibration graph was expressed by the formula. LOQ was found to be 1.1059µg/ml.

DIFFERENCE SPECTROSCOPY

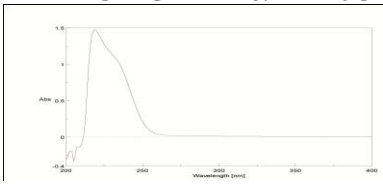
The difference spectrophotometric assay is a the measured value of the different absorbance (ΔA) between 2 equimolar solution of the analyte in different chemical form which exhibit different spectra characterstics. this method is used for the analysis of sample containing absorbing interference and the most common technique employed is the adjustment of pH by the means of aqueous solution of acid or alkalis.

Linagliptin consist of ionisable function group and can undergo ionization based on the pH of the solution.it consist of aminopiperidine ring and aromatic ketone group which the electron transfer takes place and shift in the wavelength from 205nm(λ_1) to 212nm, for HCL and for NaOH solution from 219nm (λ_2)to 235nm.The pH of the 0.1 M HCL solution was found to be 2.59 and for 0.1 M NaOH is found to be 11.05.

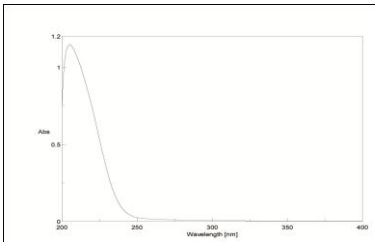
The difference spectra with the alkaline solution the sample cavity and the acid solution in the reference cavity showed, bathochromic shift towards longer wavelength and hypsochromic shift with increased absorbance at 205nm. The absorbance of 0.1 M HCl solution and for 0.1 M NaOH is found to be 1.1440 and 1.4279 respectively.

The isobestic point at which both the acid solution and alkaline have equal absorptivities is at 221 nm. So the measured value of (ΔA) is the quantitative difference spectrophotometric assay measured to the baseline is calculated using the equation $\Delta A = A_{alk} - A_{acid}$. The molar absorptivity was calculated using the formula $\Delta A^{1\%}_{1cm} = A / bc$ and found to be 329.

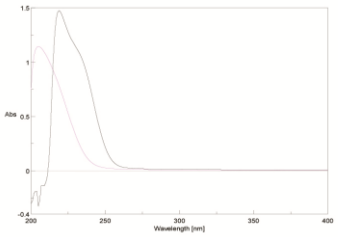
LINAGLIPTIN SPECTRA – 0.1 M NaOH



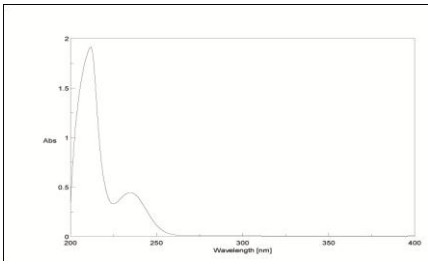
LINAGLIPTIN SPECTRA - 0.1 M HCl



OVERLAY SPECTRA OF LINAGLIPTIN – 0.1 M HCl AND O.1 M NaOH



DIFFERENCE SPECTRA OF LINAGLIPTIN

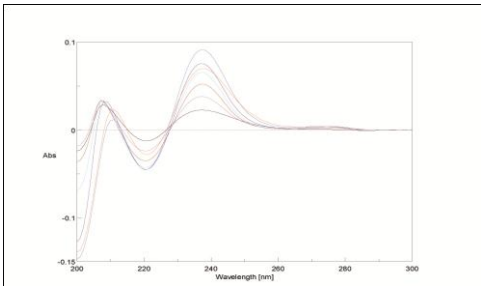


DERIVATIVE SPECTROSCOPY

In derivative spectroscopy, the normal absorption spectrum is referred as the fundamentals, zero order or D^O spectrum. The maximum absorbance of linagliptin was founded at 228 nm. The conversion of normal spectrum was generated automatically using the double beam recording spectrophotometer which is a plot of wavelength against dA/dλ.

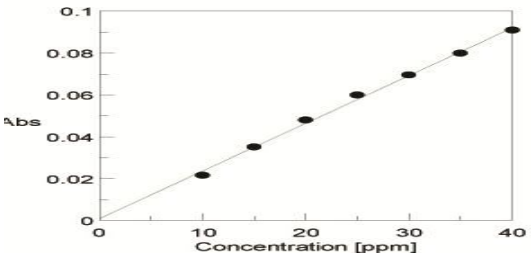
The λ max at λ_3 is a wavelength of the zeroth slope and cross over point is first derivative spectra. the λ_2 and λ_4 of maximum negative and positive slope was found at 209nm and 238nm. The calibration graph of the linearity curve of the concentration range, 10 -40µg/ml was founded to be obeying the beer lamberts law with increase sensitivity with correlation coefficient of 0.9982 as given below. The first order and second derivative spectra was generated for the linearity range of 10-40 mcg/ml and the corr. elation coefficient of 0.9982 and 1.0 as given below.

OVER LAY SPECTRA OF LINAGLIPTIN - 1 ST ORDER DERIVATIVE



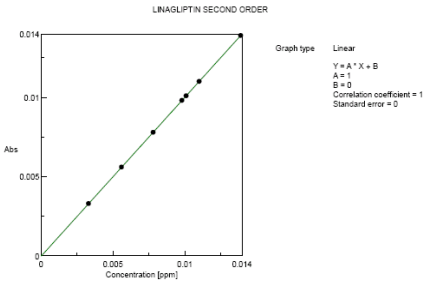
CALIBRATION GRAPH AT 238 nm

Y = A * X + B
A = 0.00228
B = 0.0008
Correlation coefficient = 0.998216
Standard error = 0.707759



LINEARITY RANGE OF LINAGLIPTIN – FIRST DERIVATIVE

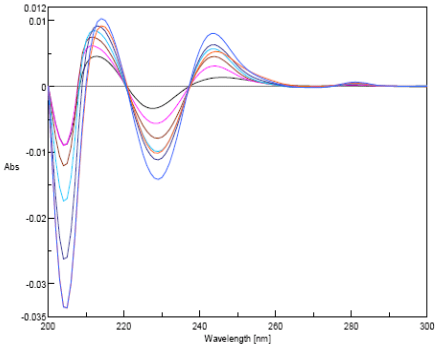
s.no	Concentration	Absorbance
1.	10	0.0215
2.	15	0.0351
3.	20	0.0479
4.	25	0.0599
5.	30	0.0695
6.	35	0.0798
7.	40	0.0909



CALIBRATION GRAPH AT 228 nm – SECOND DERIVATIVE SPECTRA

S.no	Concentration	Absorbance
1.	10	0.0033
2.	15	0.0056
3.	20	0.0078
4.	25	0.0098
5.	30	0.0110
6.	35	0.0101
7.	40	0.0139

OVER LAY SPECTRA OF LINAGLIPTIN - SECOND ORDER DERIVATIVE



DISCUSSIONS AND CONCLUSION

The selected drug linagliptin is a novel hypoglycemic drug that belongs to dipeptidyl-peptidase-4 inhibitor class (DPP-4). It is used in treatment of type II diabetis the function to stimulate glucose dependent insulin release and reduce glucagon level.

A thorough literature survey shows there are few month reported for the analysis of linagliptin by RP-HPLC method and UV-spectroscopic method with 50:50 ratio of methanol and water.

The present study was focused to establish a method by validated UV-spectroscopy with its first order and second order derivative spectra and difference spectroscopy.

The drug was found to be soluble and in methanol, water, 0.1M NaOH, 0.1M HCL, toluene, tetrahydrofuran and DMSO (dimethyl sulphaoxide) and insoluble in petroleum ether and chloroform.

The UV-spectroscopic method was developed with methanol as solvent and the λ_{max} was found to be 228nm. The linearity was found between the range of 5-40 µg with correlation coefficient of 0.9985.

The intraday precision was performed with the standard solution on the same day and the %RSD was found to be0.7078. The interday precision of standard drug was performed on 3 consequetive day the %RSD was found to be 0.7078, 0.5202, 0.0620 respectively.

The LOD and LOQ were calculated statistically using the standard deviation of standard solution and the slope of the curve from the calibration graph with the formula of 3.3 σ/ S and 10σ / S respectively and was found to be 0.3649 µg/ml and 1.1059 µg/ml.

The difference spectroscopy method was perfomed with 0.1M NaOH of pH 11.05 and 0.1M HCL 0f pH 2.59. the spectra of drug solution of 10µg/ml was recorded separately in 0.1m NaOH and 0.1m HCL and λ_{max} was found to be 219nm and 205nm respectively. The difference spectra was recorded with alkaline solution

the sample cavity and acid solution in reference cavity. The shift in the wavelength was noted with the changing pH and the ΔA was calculated. the molar absorptivity and amount of drug in the assay sample was calculated based on ΔA and found to be 95.82%.

The derivative spectroscopy of 1st order with the zero crossing at λ_{max}228nm was measured at λ_4 at 238 nm and the second order spectra was measured at 228 nm. Both derivatives were converted automatically using the software in double beam spectrophotometer. The linearity range of normal spectrum in the range of calibration graph between 10-40 µg/ml was converted to first derivative and second order spectra. The Correlation Coefficient for the absorbance at 238nm and 228 nm were found to be 0.9982 and 1.0 respectively.

Hence, the UV spectroscopic method was developed and validated according to the ICH guideline for its linearity, range, precision, LOD, LOQ which can be applied for the routine analysis of linagliptin in bulk drug and tablet dosage form. The difference spectroscopy and the derivative spectroscopy can be used for the routine analysis of linagliptin in bulk and tablet dosage form.

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