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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR THE DETERMINATION OF LINAGLIPTIN AND METFORMIN HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple, economic, sensitive, precise and accurate UV spectrophotometric method was developed and validated for quantification of Linagliptin (LNG) and Metformin hydrochloride (MET) in bulk and in tablet dosage form. Adequate drug solubility and maximum assay sensitivity was found in water at 273nm and 233nm respectively. Calibration graph constructed at 273nm and 233nm was linear in concentration range of 2-10µg/ml with correlation coefficient of 0.998 and 0.999 respectively. The method was validated as per ICH guidelines in terms of linearity (within 2- 10µg/ml), accuracy (% recovery), precision, specificity and robustness

INTRODUCTION:

Linagliptin (LNG) [1] is chemically, 8- [(3R) - 3 -aminopiperidin-1-yl] -7- (but-2-yn-1-yl) -3-methyl-1-[(4-methylquinazolin-2-yl) methyl]-3, 7-dihydro-1H-purine-2, 6-dione is an DPP-4 inhibitor developed by Boehringer Ingelheim for treatment of type-II diabetes. It is novel hypoglycemic drug that belongs to dipeptidyl-peptidase-4 inhibitor class. Metformin hydrochloride (MET) [2] is a biguanide class of oral antidiabetic drug or blood glucose lowering agent, chemically known as 3-(diaminomethylidene)-1, 1-dimethylguanidine. Metformin itself produces the antidiabetic effect in aqueous. It is the first line drug for the treatment of type II diabetes or non- insulin dependent diabetes mellitus (NIDDM). It lowers blood glucose concentrations in without causing overt hypoglycemia. Therefore, it was thought of interest to develop simple, accurate, fast and cost effective method for the analysis of LNG and MET in its tablet formulation. This paper describes development and validation of simple, specific, sensitive, accurate and precise Ultraviolet spectroscopic method for the estimation of LNG and MET in bulk and its formulation.

EXPERIMENTAL PROCEDURE:

Chemicals:

All chemicals were of analytical grade unless stated otherwise. LNG and MET were the kind gift samples from Mylan Laboratories Ltd. (Hyderabad, India). Water was deionized and double distilled. Marketed combination tablet formulations containing 2.5mg of LNG

and 500mg of MET were purchased from local drug store.

Instrumentation:

A double beam Shimadzu UV Visible spectrophotometer (Model: 1800), equipped with 1 cm quartz cells, with a fixed slit width (1 nm), wavelength accuracy of ±0.5 nm (with automatic wavelength correction) was used. For scanning, the wavelength range selected was from 400 nm to 200 nm with medium scanning speed.

Preparation of Standard Solutions:

Stock solution of MET and LNG were prepared by dissolving 10 mg drug in 100 ml Distilled water. Several aliquots of standard solutions of LNG and MET (100µg/ml) were diluted to get standard solutions across the range of 2-10µg/ml.

Preparation of Sample Solutions:

Fixed dose combination of LNG and MET is approved for marketing as a (Trajenta D) containing Linagliptin and Metformin Hydrochloride. 20 Trajenta D tablets were weighed and triturated in a mortar pestle and quantity of sample equivalent to 2.5 mg of LNG and 500 mg of MET were dissolved in Distilled water. Final volume was made up to the mark and filtered through Whatman filter paper (No. 41). The absorbance was taken at 273nm and 233 nm against blank. The concentrations of LNG and MET was calculated.

Determination of λ_{max}:

Standard solution containing 10µg/ml each of LNG and MET was scanned using distilled water as blank in the range of 200-400 nm to determine the wavelength of maximum absorption (λ_{max}) of the drugs. MET showed absorbance maxima at 233 nm and LNG showed the absorbance maxima at 273 nm.

Determination of Isoabsorptive Point and wavelength of Maximum Absorbance (λ_{max}):

Solutions of 10µg/ml of both drugs were prepared from working stock solution and scanned in the range of 200 nm to 400 nm against Distilled water as blank. The isoabsorptive point was found to be 244 nm.

Absorption ratio Method:

In absorbance ratio method, ratios of absorption at two selected wavelengths were taken. One is at iso-absorptive point and other at λ_{max} of one of the component. The concentration of two drugs in mixture was calculated by using the following equations:

$$Cx = \frac{Qm - Qy}{Qx - Qy} \times \frac{A1}{ax1}$$

$$Cy = \frac{Qm - Qx}{Qy - Qx} \times \frac{A1}{ay1}$$

Where, ax_1 and ax_2 are the absorptivities of LNG at 273 nm and 251 nm. ay_1 and ay_2 are absorptivities of MET at 224 and 251 nm, respectively. A1 and A2 are the absorbances of mixture at 224 and 251 nm, respectively. Cx and Cy are the concentrations of LNG and MET, respectively in sample solution.

Method Validation: The method validation was performed based on ICH guidelines [3].

Specificity: Specificity of the method was determined by comparing the spectrum of standards of MET and LNG with that of marketed product.

Linearity: The linearity of an analytical method is its ability to elicit that test results are proportional to the concentration of analyte in samples within a given range. This was determined by means of calibration graph using increasing amounts of standard solutions (2-10µg/ml). Calibration curves were constructed and the proposed method was evaluated by its correlation coefficient and intercept value calculated in the corresponding statistical study.

Accuracy: To check the accuracy of the developed method and to study

interference of formulation additives, analytical recovery experiments were carried out by using standard addition method. Tablet samples solution of each drug was added to reference standard at 3 different concentrations level (80%, 100% and 120%). at each level, samples were prepared in triplicate and the mean percentage recoveries and %RSD values were calculated.

Precision: Repeatability: A mixture containing 10µg/ml each of LNG and MET was prepared and analyzed. Precision is studied in terms of intraday and inter-day precision. Three concentrations of LNG and MET was selected in a mixture and analyzed.

Limit of detection (LOD) and Limit of quantification (LOQ): Limit of detection (LOD) and Limit of quantification (LOQ) for the assay were calculated using the following equations:

$LOD = 3.3 \times S_o/b$ and $LOQ = 10 \times S_o/b$

Where S_o and b are the standard deviation and the slope of the calibration line respectively.

Ruggedness: Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slots by different analysts using similar operational conditions.

RESULTS:

Linearity: Linear detector response for the peak-area ratios of the linagliptin and metformin to internal standard was observed in concentration range between 2-10µg/ml and 2-10µg/ml. calibration curves were constructed by plotting the peak areas versus concentration and the regression equations were calculated. The results obtained are represented in Table 1, and these results indicate that the current method is linear for linagliptin and metformin in the range specified above with a correlation coefficient 0.9989 and0.9991 respectively.

Precision: The intraday and interday precision was evaluated with six sample replicate injections and the %RSD was within 2%. The results of the method validation study for precision are presented in table 1.

Accuracy: Accuracy measures the percentage deviation of nominal concentration as compared to the observed concentration. The accuracy values of the low medium and high quality control samples of LNG and MET were measured (n=3) and mean of the concentration was within the acceptable limits. The results of the method validation study in accuracy are presented in table 2.

Limit of detection (LOD) and Limit of quantification (LOQ): LOD and LOQ are experimentally verified at appropriate concentrations. The LOD and LOQ of linagliptin were calculated to be 0.367 and 1.33µg/ml and LOD and LOQ of metformin were calculated to be 1.113 and 2.037µg/ml respectively. % Recovery for LNG and MET was found within the range of 99.61% and 100.70%. The % RSD value for both Linagliptin and Metformin Hydrochloride was found to be less than 2%. The results did not show any statistical difference between operators suggesting that methods developed were rugged. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical formulations containing both these drugs.

DISCUSSION:

This study represents the development and validation of simple U.V method for the determination of LNG and MET in pharmaceutical formulations. The method is less time consuming and the sensitivity of the method is comparatively higher.

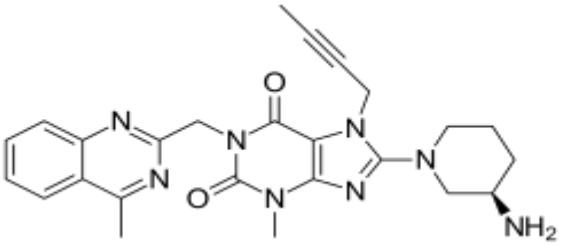

Fig.1: Structure of Linagliptin

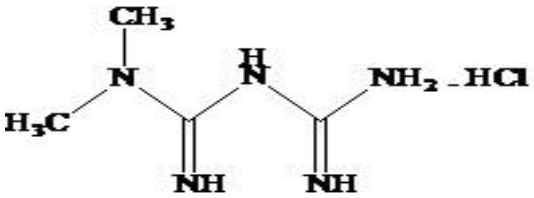

Fig.2: Structure of Metformin

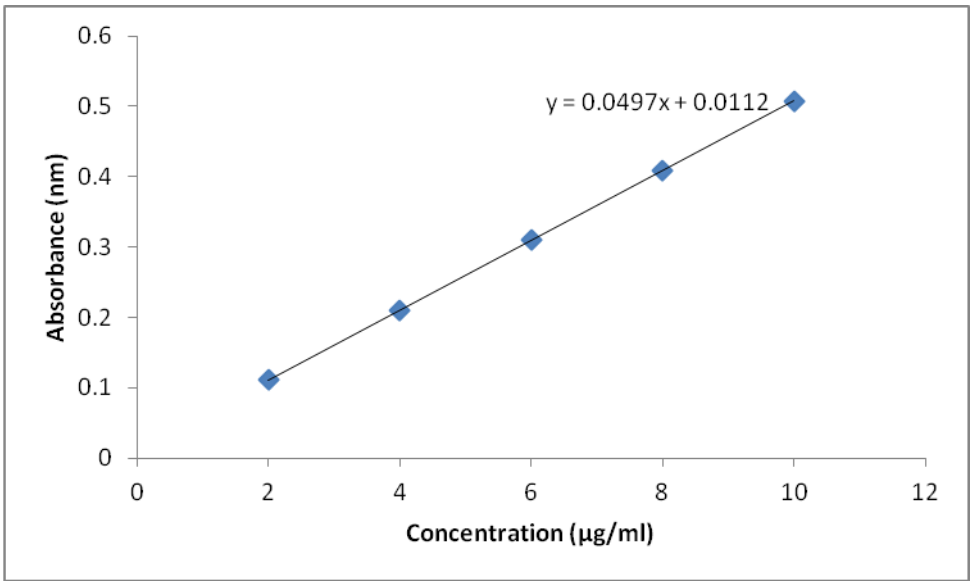

Fig.3: Calibration curve of Linagliptin

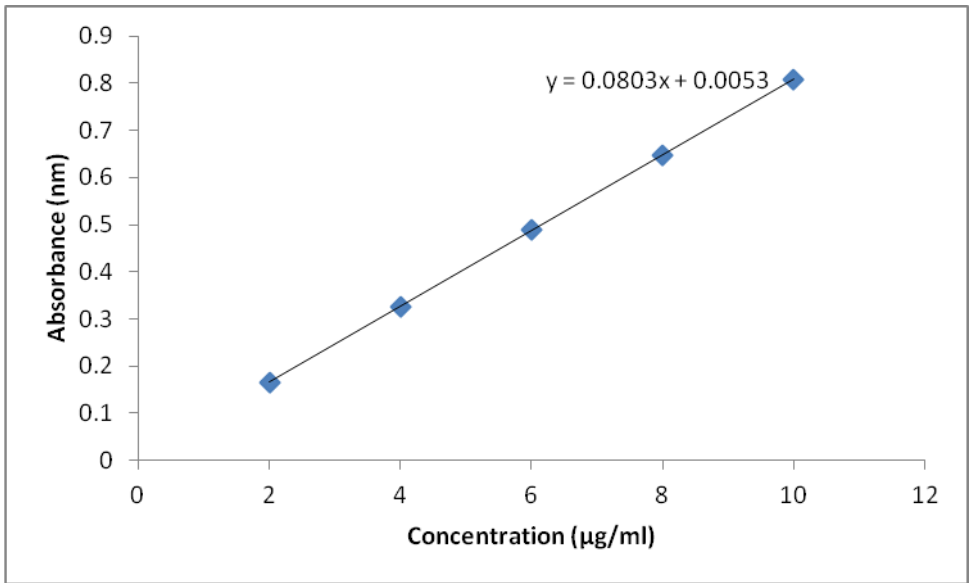

Fig.4: Calibration curve of Metformin Hydrochloride

Table 1: Method validation parameters:

Parameters	Linagliptin	Metformin
Absorption maxima(nm)	273	233
Beer law limit(µg/ml)	2-10	2-10
Correlation coefficient(r^2)	0.9989	0.9991
Regression equation(Y^*)	Y=0.0497x+0.0112	Y=0.0802x+0.0053
Slope(m)	0.0497	0.0802
Intercept	0.0112	0.0053
Precision(%RSD)	99%	99.8%
% Purity	99.61%	100.70%
Limit of detection(LOD)(µg/ml)	0.367	1.33
Limit of quantification(LOQ) (µg/ml)	1.113	2.037

Table 2: Accuracy

Brand name	Concentration Level(µg/ml)	Amount Recovered	%Recovery
Trajenta D	2	5.1	99.44
Trajenta D	6	5.9	99.50
Trajenta D	10	6.4	99.09

Table 3: Assay

Brand	Drug	%Amount found ±SD	%RSD
Trajenta D	Linagliptin	101.74±0.25	0.24
	Metformin	100.11±0.86	0.85

Other UV methods [4, 5, 6] and Sensitive HPLC methods [7] are also reported for the determination of LNG and MET but the cost of this method is less compared to other methods. The concentrations of LNG and MET by absorbance ratio method was found to be 0.11ug/ml and 4.98ug/ml respectively.

CONCLUSION:

The validation study shows that the developed UV method is linear, accurate, rapid, precise, robust and inexpensive with acceptable correlation co-efficient, RSD (%) and standard deviations which make it versatile and valuable for determination of LNG and MET in bulk or pharmaceutical dosage forms. The method was successfully used for determination of drugs in their pharmaceutical formulation.

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