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DEVELOPMENT OF UV SPECTROSCOPIC METHOD FOR ESTIMATION AND VALIDATION OF SAXAGLIPTIN AND METFORMIN HYDROCHLORIDE BY ABSORBANCE RATIO METHOD

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ABSTRACT

A simple, rapid, accurate, precise and improved analytical method for estimation of Saxagliptin (SXG) and Metformin (MET) in active pharmaceutical ingredient was established. The method was validated in terms of linearity, accuracy, precision, specificity, limit of detection and limit of Quantitation. The optimum conditions for the analysis of the drug were established. The maximum wavelength (λ_{max}) of Saxagliptin and Metformin were found to be 212nm and 233nm respectively. The percentage recovery of Saxagliptin and Metformin were 100.1 and 99.98 respectively. Beer's law was obeyed in the concentration range 5-25µg/ml for Saxagliptin and 2-10µg/ml for Metformin.

INTRODUCTION:

Saxagliptin [1] is also an oral anti diabetic drug and it belongs to new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. Saxagliptin exerts its actions in patients with type 2 diabetes by slowing the inactivation of incretin hormones, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Chemical name is (1S, 3S, 5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo hexane-3-carbonitrile. It is used for the treatment of type II diabetes in combination with Metformin, a sulphonyl urea. Metformin [2] is an oral antidiabetic drug and is chemically, 1, 1-dimethyl biguanide hydrochloride. This drug is included in first line therapy to treat patients with type 2 diabetes mellitus. The main action of Metformin is to decrease fasting plasma glucose levels and it is achieved by suppressing excessive hepatic glucose production and improving glucose clearance. It is the principal component in combination therapies intended for diabetes and is frequently used in high doses of about 500 to 850 mg.

EXPERIMENTAL WORK:

Chemicals:

SXG and MET were the kind gift samples from Mylan Laboratories Ltd. (Hyderabad, India). Marketed combination tablet (Kombiglyze) formulations containing 5mg of SXG and 500mg of MET were purchased from local drug store.

Instrumentation:

Shimadzu double beam UV-Visible spectrophotometer with spectral width of 1nm, wavelength accuracy of ± 0.1 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software.

Selection of detection wavelength:

Solutions of drug were scanned over the range of 200-400 nm. It was observed that the drugs showed considerable absorbance at 212 nm for SXG and 233nm for MET and were selected as the wavelength for detection.

Preparation of standard solutions:

SXG and MET were weighed (100 mg each) and transferred to two separate 100 ml volumetric flasks and dissolved in 50 ml of distilled water and make up the volume up to the mark with distilled water to obtain the final concentrations of solution containing 1000 µg/ml of SXG and MET.

Preparation of working standard:

Aliquots from the standard stock solutions of SXG and MET were appropriately diluted with distilled water to obtain working standard of SXG and MET.

Preparation of Sample Solutions:

Fixed dose combination of SXG and MET is approved for marketing as a (Kombiglyze) tablet containing Saxagliptin and Metformin Hydrochloride. 20 Kombiglyze tablets were weighed and triturated in a mortar pestle and quantity of sample equivalent to 5 mg of SXG and 500 mg of MET were dissolved in Distilled water. Final volume was made up to the mark and filtered through Whatmann filter paper (No. 41). The absorbance was taken at 212 and 233 nm against blank. The concentrations of SXG and MET was calculated.

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 iso-absorptive point was found to be 244 nm.

Absorbance 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 SXG at 212 nm and 233 nm. ay_1 and ay_2 are absorptivities of MET at 233 and 212 nm, respectively.

A1 and A2 are the absorbances of mixture at 212 and 233 nm, respectively. Cx and Cy are the concentrations of SXG and MET, respectively in sample solution.

METHOD VALIDATION

The method validation was performed based on ICH guidelines [3]. The method was validated for

Accuracy, Precision, Linearity, Detection limit, Quantitation limit

Accuracy:

The accuracy is expressed as percentage recovery obtained from three levels of samples of SXG and MET. The accuracy of the method was established by using samples at low, medium and high concentrations i.e., 2,6and10µg/ml for MET and 5, 15 and25µg/ml for SXG.

Precision: The repeatability studies were carried out by estimating response of SXG (20 µg/ml) and MET (6 µg/ml) six times and results are calculated in terms of relative standard deviation. The results are reported in terms of relative standard deviation (%R.S.D).

Linearity:

Standard calibration samples were prepared by making serial dilutions from the stock solution of SXG and MET (1mg/ml).Calibration curve of concentration versus absorbance was plotted at concentration range of 5-25µg/ml for SXG and 2-10µg/ml for MET.

Limit of Detection (LOD): The Limit of Detection is defined as the lowest amount of SXG and MET in a sample which can be detected but not necessarily quantitated. The Limit of Detection (LOD) may be expressed as: $LOD = 3.3\sigma/S$

Where

σ=Relative standard deviation of the response. S = the slope of the calibration curve (of the analyte).

Limit of Quantitation (LOQ):

The Limit of Quantitation is defined as the lowest amount of SXG and MET in a sample, which can be quantitatively determined with suitable precision and accuracy.

Quantitation Limit (LOQ) may be expressed as:

$$LOQ = 10\sigma/S$$

Where

σ = Relative standard deviation of the response.

S = the slope of the calibration curve (of the analyte).

RESULTS:

Linearity

Linear detector response was observed in concentration range between 2-10µg/ml for MET and 5-30µg/ml for SXG. An aliquot (10µl) of each solution was analyzed. Calibration curves were constructed by plotting the peak areas versus concentration and the regression equations were calculated. The results obtained are listed in the Table 1, and these results indicate that the current method is linear for SXG and MET in the range specified above with a correlation coefficients better than 0.998.

Precision

The repeatability studies were evaluated with six sample replicates and the % RSD was reported. The results of the method validation study for precision are presented in Table 2.

Accuracy: The accuracy of the method was determined by calculating recoveries of SXG and MET by method of standard additions at three different levels 80, 100 and 120 %. Mean percentage recovery was determined. Recovery values were calculated and shown in Table 1.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were experimentally verified at the appropriate concentrations. The LOD was calculated to be 0.798µg/ml for SXG and 0.50µg/ml for MET. The LOQ was calculated to be 2.41µg/ml for SXG and 1.52µg/ml for MET. The results of the method validation study for Limit of Detection (LOD) and Limit of Quantitation (LOQ) are presented in Table 2.

DISCUSSION:

This study represents the development and validation of simple U.V method for the determination of SXG and MET in pharmaceutical formulations. The cost of the method is reduced. The method is less time consuming and the sensitivity of the method is comparatively higher. Other U.V methods (4, 5, 6) and sensitive HPLC methods (7, 8) are also reported for the determination of SXG and MET but the cost of this method is less compared to other methods. The concentrations of SXG and MET by absorbance ratio method was found to be 2.15ug/ml and 9.08ug/ml respectively.

The U.V method developed and validated for the determination of Saxagliptin and Metformin in pharmaceutical formulations, assured the satisfactory precision and accuracy. The method was found to be simple, accurate and precise as per ICH guidelines. The method was successfully used for determination of drugs in their pharmaceutical formulation.

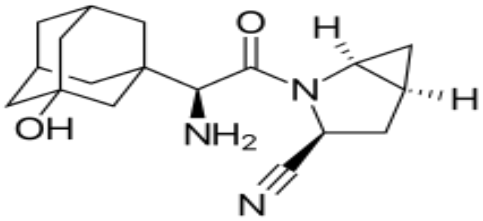

Fig. 1: Structure of Saxagliptin

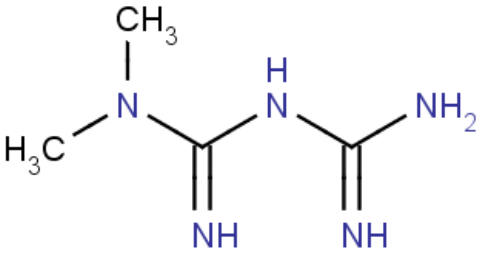

Fig. 2: Structure of Metformin

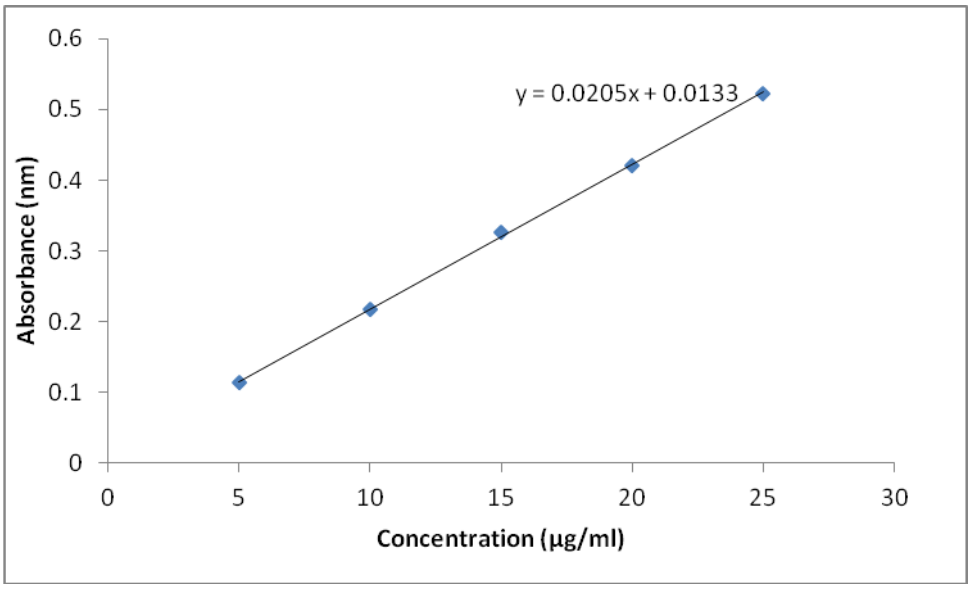

Fig. 3: Calibration curve of Saxagliptin

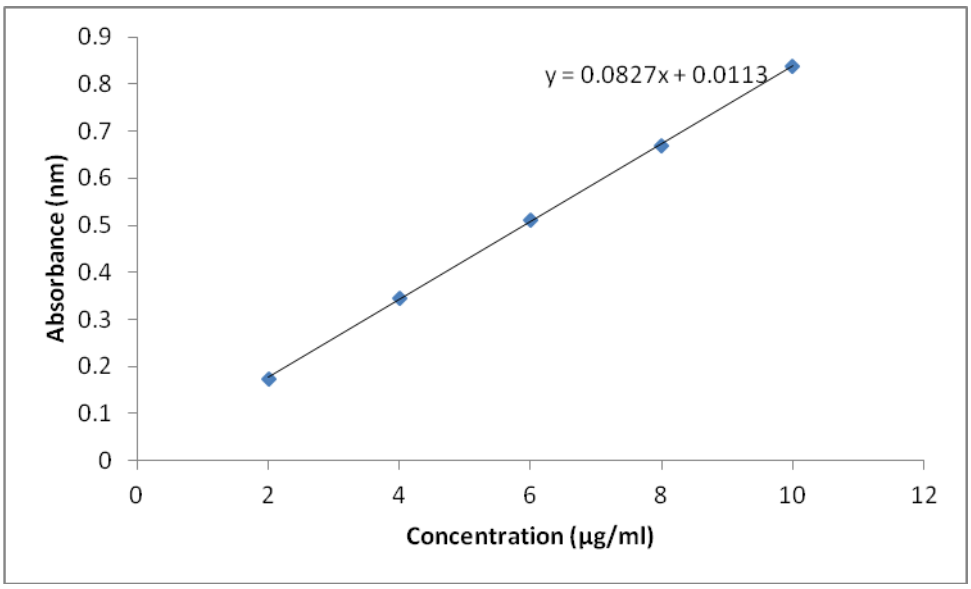

Fig. 4: Calibration curve of Metformin

Table 1: Recovery Studies:

Theoretical amount Added (%)	Amount of drug sample used Metformin	Obtained (µg) Metformin (n=3)	%Recovery	Amount of drug sample used Saxagliptin	Obtained (µg) Saxagliptin (n=3)	%Recovery
80	4mg	3.21	100.12	20mg	19.05	99.92
100	4mg	3.99	99.95	20mg	19.08	100.16
120	4mg	4.795	99.86	20mg	20.13	99.93
			Mean % Recovery 99.97			Mean % Recovery 100.01

Table 2: Method Validation Parameters:

Validation Parameters	Metformin	Saxagliptin
Linearity Range (µg/ml)	2-10 µg/ml	5-25 µg/ml
Beer's law limits (µg/ml)	2-10	0.2-1.0
λ max (nm)	233nm	212nm
Regression equation (Y*)	Y=0.0205x+0.0133	Y = 0.0827x+0.0113
Slope (b)	0.0205	0.0827
Correlation coefficient (r^2)	0.999	0.998
Percentage purity	99%	98%
Percentage recovery	100.1%	99.98%
Precision (RSD)	3.97	4.46
Limit Of Detection (LOD) (µg/ml)	0.50	0.798
Limit Of Quantitation (LOQ)	1.52	2.41

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