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Development and Validation of Spectrophotometric Method for Metformin and Sitagliptin by Absorbance Ratio Method

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ABSTRACT:

A simple, accurate and precise spectroscopic method was developed for simultaneous stimation of Metformin and Sitagliptin in marketed formulation using Q-Absorbance Ratio Method. In this spectroscopic method, 237 nm (λ max of Metformin) and 253.26 nm (iso absorptive point for oth drugs) were selected for measurement of absorptivity. Both the drugs show linearity in a oncentration range of 5-25 µg/ml for Metformin and 0.5-2.5 µg/ml for Sitagliptin at 237 nm and 253.26 nm respectively . Accuracy, precision and recovery studies were done by QC samples overing lower, medium and high concentrations of the linearity range. The relative tandard deviation for accuracy, precision studies were found to be within the acceptance range (<2%). ecovery of Metformin and Sitagliptin were found to be 99.73-101.16 % and 99.44-101.56 % espectively confirming the accuracy of the proposed method. The proposed method is ecommended for routine analysis since they are rapid, simple, accurate and sensitive.

KEY WORDS: Metformin ; Sitagliptin ;Simultaneous estimation; Q-Absorbance Ratio Method.

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INTRODUCTION:

Metformin, biguanide antihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM). [1] It is for treatment of non-insulin-dependent diabetes mellitus, lowering rather than body weight, not causing hypoglaycemia and of entailing a reduction of triglycerides and LDL cholesterol level, advantageous effect on the prognosis of diabetes. IUPAC name of Metformin is 1-carbamimidamido-N,N-dimethylmethanimidamide (Figure 1).

Sitagliptin[2] is a new oral hypoglycemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. This enzyme-inhibiting drug is to be used either alone or in combination with metformin or a thiazolidinedione for control of type 2 diabetes mellitus.

Cheically Sitagliptin is, (2R)-4-OXO-4-[3-(trifluoromethyl) - 5,6 dihydro [1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine (Figure 2).

Metformin is official in IP 2014[3], USP 2012[4] and BP 2009[5] and estimated potentiometrically. Sitagliptin is not official in any

Pharmacopoiea. Combination of these both drug is used in the treatment of non –insulin-dependent diabetes mellitus.

Some spectrophotometric methods[6-9] and chromatographic methods[10-19] have been reported for the estimation of Metformin in alone and in combination with other drugs. For estimation of Sitagliptin alone or in combination with other drugs some spectrophotmetric[20] and Chromatographic methods[21-22] have been reported.The review of literature regarding quantitative analysis of Metformin and Sitagliptin revealed that there are few analytical methods[23-28] have been reported for simultaneous estimation of these two drugs. The focus of the present study was to develop and validate a rapid, stable, specific, and economic spectroscopic method for the estimation of Metformin and Sitagliptin in marketed formulation.

MATERIALS AND METHODOLOGY

Metformin and Sitagliptin were obtained as gift samples from Vapi Care Pharma Pvt Ltd., Vapi and Sun Pharma , Dadra and Nagar Haveli, Silvassa ,respectively. Marketed formulation JANUMET was procured as Gift sample from local market.

A double beam UV/Visible spectrophotometer (Simadzu-1800, Software –UV Probe, Version 2.42) with spectral width of 2 nm, 1 cm quartz cells was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software.

Digital balance (Mettler toledo) was used for weighing the samples.

Class 'A' volumetric glassware were used (Borosillicate)

Preparation of Standard solution

Preparation of stock solution of Metformin

Accurately weighed quantity of Metformin 100 mg was transferred to 100 ml volumetric flask, dissolved and diluted up to mark with methanol to give a stock solution having strength of 1000 µg/ml. Then this solution further diluted to obtain concentration 100 µg/ml.

Preparation of stock solution of Sitagliptin

Accurately weighed quantity of Sitagliptin 100 mg was transferred to 100 ml volumetric flask, dissolved and diluted up to mark with methanol to give a stock solution having strength of 1000 µg/ml. Then this solution further diluted to obtain concentration 100 µg/ml.

Preparation of standard mixture solution

Stock solution of Metformin and Sitagliptin were properly diluted to obtain the concentration 15 µg/ml for Metformin and 1.5 µg/ml for Sitagliptin .

Calibration curves for Metformin and Sitagliptin

The solutions for Metformin were prepared by pipetting out 5,10,15,20 and 25ml of the working standard solution of Metformin (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with methanol. The absorbance of the solutions was measured at 237.14 nm against methanol as a reagent blank. The solutions for Sitagliptin were prepared by pipetting out 0.5,1,1.5,2 and 2.5 ml of the working standard solution of Sitagliptin (10 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with methanol. The absorbance of the solutions was measured at 253.26 nm against methanol as a reagent blank. Plot the graph of absorbance versus respective concentration of Metformin and Sitagliptin. Linearity range of Metformin and Sitagliptin was found with correlation co-efficient. The overlain spectra for Metformin and Sitagliptin is shown in Figure 3.

Method

Absorbance ratio method (Q-analysis method):

The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that for a substance, which obeys Beer's law at all wavelength, the ratio of absorbance at any two wavelengths is constant value independent of concentration or path length e.g. two dilutions of the same substance give the same absorbance ratio A1/A2. In the USP, this ratio is referred to as Q value. In the quantitative assay of two components in mixture by the absorbance ratio method, absorbance is measured at

two wavelengths, one being the λmax of one of the components (λ2) and the other being a wavelength of equal absorptivity of the two components (λ1), i.e., an iso- absorptive point. A series of standard solutions of Metformin and Sitagliptin in the concentration range of 5-25 µg/ml and 0.5-2.5 µg/ml respectively were prepared in methanol and the absorbance of these solutions were measured at 253.26 nm (iso- absorptive point) and 237.14 nm (λmax of Metformin) (Fig. 3). Calibration curves were plotted to verify the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs.

The concentration of two drugs in mixture was calculated by using the following equations:

$$Cx = (Qm - Qy / Qx - Qy) \times (A1 / ax1)$$

$$Cy = (Qm - Qx / Qy - Qx) \times A1 / ay1)$$

Where,

ax1 = A (1%, 1cm) of Metformin at 237.14 nm

ay1 = A (1%, 1cm) of Sitagliptin at 237.14 nm

ax2 = A (1%, 1cm) of Metformin at 235.26 nm

ay2 = A (1%, 1cm) of Sitagliptin at 253.26 nm

A1 and A2 are the absorbances of mixture at 237.14 nm and 253.26 nm. Cx and Cy are the concentrations of Metformin and Sitagliptin in gm/100 ml respectively in sample solution.

Qm= A2 / A1, Qx = ax2 / ax1 and Qy= ay2 /ay1

Assay of tablets by method :

Tablet Janumet 50/500 contains 500 mg of Metformin and 50 mg of Sitagliptin equivalent. It is marketed by Merck sharp & Dhohme Ltd. Fixed dose combination of Metformin and Sitagliptin marketing in India. Twenty tablets were weighed and triturated in a mortar pestle and the tablet powder equivalent to 100 mg of Metformin and 10 mg of Sitagliptin was transferred to a 100 ml volumetric flask, dissolved and diluted up to mark with methanol. The solution was filtered through Whatman filter paper no. 42 and first few drops of filtrate were discarded. 1 ml of this solution was diluted to 10 ml with methanol and 0.4 ml of this solution was further diluted to 10 ml with methanol. Absorbance of the resulting solution was measured at 237.14 nm and 253.26 nm against methanol. The concentration of Metformin and Sitagliptin can be obtained by using following equations,

$$Cx = (Qm - Qy / Qx - Qy) \times (A1 / ax1)$$

$$Cy = (Qm - Qx / Qy - Qx) \times A1 / ay1)$$

Method validation

The UV spectrophotometric method was validated as per ICH guidelines for method validation. The performance parameters like linearity, precision and accuracy were evaluated.

Linearity:

Linearity was studied by diluting standard stock solution of Metformin 5-25 µg/ml and Sitagliptin 0.5-2.5 µg/ml concentrations (n=3). Calibration curves with concentration verses absorbance were plotted at their respective wavelengths and the obtained data was subjected to regression analysis using the least square method. The standard curves for Metformin and Sitagliptin are shown in Fig. 6, 7, 8 and 9 and data is presented in Table 1.

Precision:

Repeatability: For repeatability 5 ml of working standard solution of Metformin (100 µg/ml) was transferred to 10 ml volumetric flask. 05 ml of working standard solution of Sitagliptin (10 µg/ml) was transferred to another 10 ml volumetric flask. The volume was adjusted up to mark with methanol in both the flask to get 5 µg/ml solution of Metformin and 0.5 µg/ml solution of Sitagliptin . The absorbance of solutions were measured six times and % RSD was calculated.

Intermediate precision: Intermediate precision is studied in terms of intraday and inter-day precision. Three concentrations of Metformin and Sitagliptin were selected in a mixture and analyzed by method (n=3). For intraday, the analysis was carried out at different intervals on the same day and for inter day, the analysis was carried on different days.

Accuracy:

To check the accuracy of the developed methods and to study interference of formulation additives, analytical recovery experiments were carried out by using standard addition method. Reference standard solution of each drug was added to tablet samples at three different concentrations level (80,100 and 120%). At each level, samples were prepared in triplicate and the mean percentage recoveries and % RSD value were calculated. Table 2. shows the result for accuracy of the method.

RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient, precise and accurate way for simultaneous analysis of Metformin and Sitagliptin in its bulk and pharmaceutical dosage form. Absorbance maxima of Metformin at 237.14 nm and isoabsorptive point 253.26 nm were selected for the analysis. Regression analysis shows linearity over the concentration range of 5-25 µg/ml for Metformin and 0.5-2.5 µg/ml for Sitagliptin with respective correlation coefficients of 0.9980 and 0.9970 for Metformin and 0.9970 and 0.9950 for Sitagliptin at 237.14 and 253.26 respectively. The % RSD for repeatability (n=6), intraday and interday (n=3) precision was found to be less than 2% indicating the precision of method. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery. % recovery for Metformin and Sitagliptin were found within the range of 99% and 101%. Values of standard deviation and coefficient of variation was satisfactorily low indicating the accuracy of this method. The assay for Metformin and Sitagliptin was found to be 100.50± 0.78 and 101.58± 0.011 respectively (Table 3). The % RSD value for both Metformin and Sitagliptin were found to be less than 2%. In this study simultaneous estimation of Metformin and Sitagliptin were carried out by absorbance ratio method satisfactorily.

CONCLUSION

Based on the results obtained, it is found that the developed UV-spectrophotometric technique is quite simple, accurate, precise, reproducible, sensitive and economical. These method can become effective analytical tool for routine quality control of Metformin and Sitagliptin bulk drug combination and its combined pharmaceutical dosage form without any prior separation of components.

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Table 1 : Regression analysis of calibration curves and summary of validation parameters.

Sr.No.	Parameter	Metformin		Sitagliptin	
		237.14	253.26	237.14	253.26
1.	Linearity range (µg/ml)	5 - 25	5 - 25	0.5 – 2.5	0.5 – 2.5
2.	Slope	0.036	0.001	0.032	0.01
3.	Intercept	0.212	0.004	0.037	0.003
4.	Limit of Detection (µg/ml)	0.0405	0.0165	0.1727	0.1896
5.	Limit of Quatificatiion (µg/ml)	0.1230	0.1460	0.4233	0.4544

Table 2: Recovery study data for Metformin and Sitaglptin (n=3)

Drug	Pre-analyzed conc. (µg/ml)	Drug added (µg/ml)	Total Conc. of drug (µg/ml)	Conc. Recovered (µg/ml)	% Recovery
Metformin	15	0	15	14.98	-
		12	27	26.99	100.08 %
		15	30	29.94	99.73 %
		18	33	33.19	101.16 %
Sitagliptin	1.5	0	1.5	1.49	-
		1.2	2.7	2.70	100.83 %
		1.5	3.0	3.02	101.56 %
		1.8	3.3	3.28	99.44 %

Table 3 : Analysis of marketed formulation (n=3)

Tablet batch no.	Actual Conc. (mg/tablet)		Mean conc. Obtained ± S.D. (mg/tablet)		% Conc. of Label claim ± S.D.	
	Metformin	Sitaglptin	Metformin	Sitaglptin	Metformin	Sitaglptin
JANUMET (Metformin 500mg + Sitagliptin 50mg)	500	50	499.98	50.69	100.5 ± 0.780	101.58± 0.011

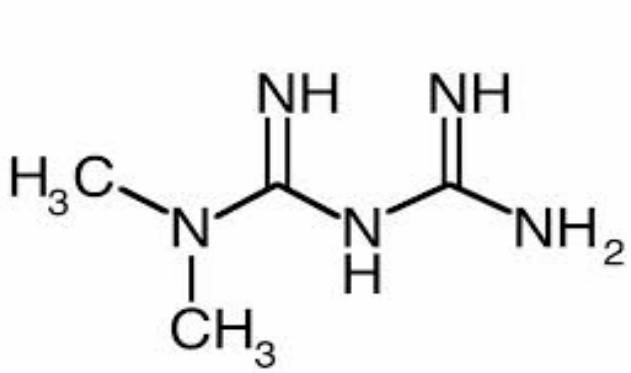

Figure:1 Structure of Metformin

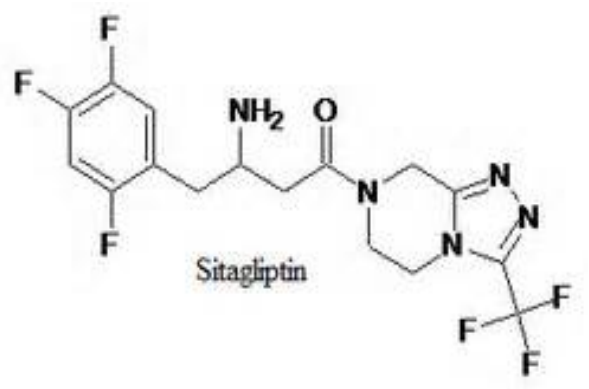

Figure 2: Structure of Sitagliptin

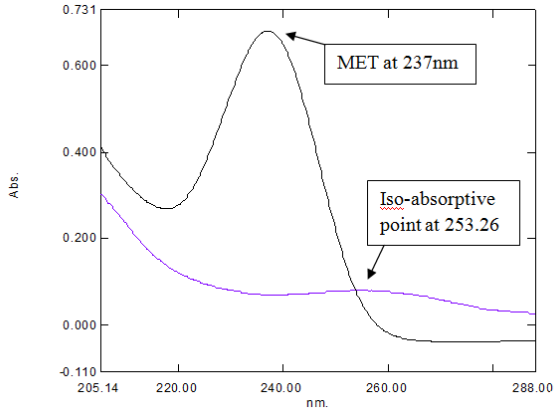

Figure 3. Overlain zero order spectra of Metformin and Sitagliptin (10:1) ratios, respectively

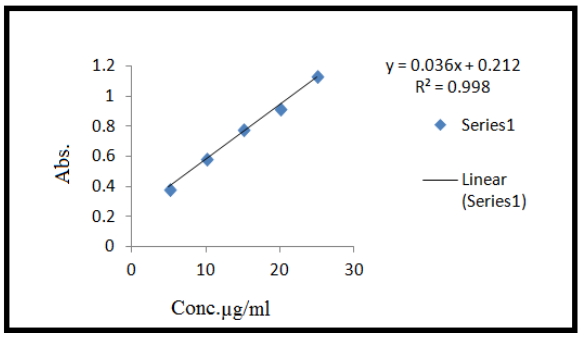

Fig . 4. Calibration curve for Metformin at 237.14 nm in methanol.

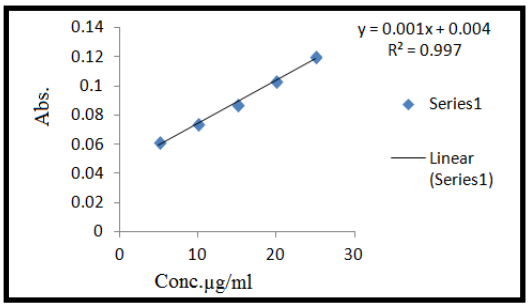

Fig.5. Calibration curve for Metformin at 253.26 nm in methanol

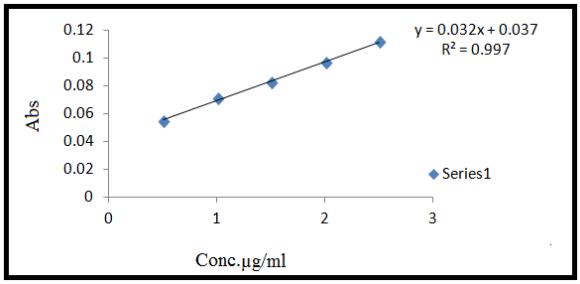

Fig.6. Calibration curve for Sitagliptin at 237.14 nm in methanol

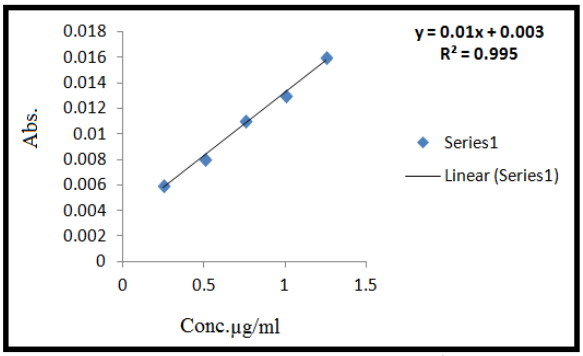

Fig.7. Calibration curve for Sitagliptin at 253.26 nm in me