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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND SITAGLIPTIN PHOSPHATE IN TABLET DOSAGE FORM

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ABSTRACT

An accurate, precise, sensitive and economical UV spectrophotometric method was developed for the estimation of Metformin HCl and Sitagliptin in tablet dosage form by simultaneous equation method. Method development was done at wavelengths of 232 nm and 225nm, which are the λ_{max} values of Metformin and Sitagliptin respectively. Both the drugs obey Beer's law over the concentration range of 2-16µg /ml. Validation of the proposed methods was carried out for its linearity, accuracy, precision, LOD and LOQ according to ICH guidelines. The proposed method can be successfully applied in routine work for determination of Sitagliptin and Metformin in combined dosage form.

KEYWORDS: Metformin HCl, Sitagliptin, UV Spectroscopic, Simultaneous Equation.

INTRODUCTION

Metformin HCl chemically N,N-dimethylimidodicarbonimidic diamide hydrochloride is an antihyperglycemic agent which improves the glucose tolerance in patients with type-2

diabetes. It decreases the hepatic glucose production, decreases intestinal absorption of glucose and improves insulin sensitivity by increasing peripheral glucose uptake and utilization.

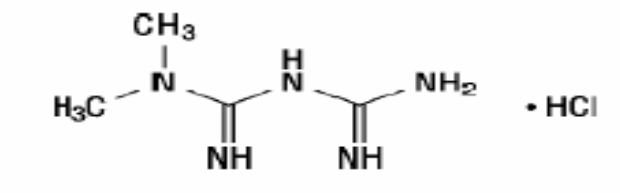

Fig No.1: Chemical Structure of Metformin HCl

Sitagliptin chemically 7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)buthyl]-5,6,7,8tetrahydro-3-(trifluoromethyl)-1,2,4-triazolo[4,3-apyrazinephosphate] is an antidiabetic agent. Sitagliptin is an orally active inhibitor of the dipeptidyl peptidase-4(DPP-4) enzyme. These enzyme breaks down the incretins GLP-1 and GIP, gastrointestinal harmones released in responses to a meal. By preventing GLP-1and GIP inactivation, Sitagliptin is able to increase the secretion of insulin and suppress the release of glucagon by alpha cells of the pancreas.

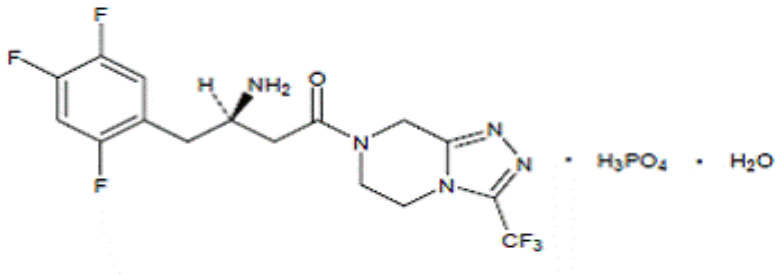

Fig No.2: Chemical Structure of Sitagliptin

Literature survey reveals that there are few UV spectrophotometric[1-6] and RP HPLC[7-8] methods for the estimation of Metformin HCl and Sitagliptin. Methods have been reported for the determination of Metformin HCl and Sitagliptin in pharmaceutical dosage forms, biological fluids Individually or in combination with other drugs. The present study was involved in a research effort aimed at developing and validating simple, specific, accurate, and economical method for the simultaneous estimation of these drugs in pharmaceutical dosage forms.

MATERIALS AND METHODS

Instruments used

SHIMADZU double beam UV/Visible Spectrophotometer model UV 1800s was employed with a spectral band width of 1nm and a wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1cm matched quartz cells). SHIMADZU Electronic balance model AX 200 and Ultra Sonicator (Fast clean) model 2k811056 were also used during the analysis.

Materials

Analytically pure sample of Metformin and Sitagliptin were obtained as gift sample from KP Labs (Hyderabad). Tablets of brand Janumet (Mfg by Merck, Label Claim-500mg Metformin HCL, 50mg Sitagliptin) were purchased from local pharmacy. Ethanol (SD Fine Chemicals) was used as solvent.

PREPARATION OF STANDARD STOCK SOLUTION

Metformin and Sitagliptin of 50mg were individually weighed and transferred to 25ml volumetric flask and dissolved in ethanol. Then the volume was made upto 25ml with ethanol. Further dilutions were made to get appropriate concentrations.

SELECTION OF ANALYTICAL WAVELENGTH

λmax of both the drugs were determined by scanning the two solutions individually in the range of 200-400nm. Metformin showed maximum absorbance at **232**nm and Sitagliptin at **225**nm (Fig No.3).

PREPARATION OF SAMPLE SOLUTION

Twenty tablets were taken, weighed and powdered. The powder equivalent to 50mg of Metformin was transferred into clean dry 25ml volumetric flask and 20mg of sitagliptin (API) was added to it (standard addition method) and dissolved in ethanol. Then the volume was made upto 25ml with ethanol. Further dilutions were made to get appropriate concentration.

SIMULTANEOUS EQUATION METHOD

Simultaneous equation method is used to determine both the drugs simultaneously. The absorptivities of X at λ1 and λ2 are a_{x1}, a_{x2} respectively. The absorptivities of Y at λ1 and λ2

are a_{y1} and a_{y2} respectively. The absorbance of diluted samples at λ1 and λ2 are A_1 and A_2 respectively.

$C_x = A_2a_{y1} - A_1a_{y2} / a_{x2}a_{y1} - a_{x1}a_{y2}$

$C_y = A_1 a_{x2} - A_2 a_{x1} / a_{x2}a_{y1} - a_{x1}a_{y2}$

METHOD VALIDATION

LINEARITY

Metformin HCl and Sitagliptin standard solutions were prepared in the concentration range of 2-16µ g/ml from the standard stock solution. Absorbance of the solutions were determined at respective λ_{max} values and the calibration graphs were plotted (Fig No. 4, 5).

PRECISION

The intraday precision was done by measuring the absorbance of the standard solution for six times on the same day. The interday precision was done by measuring the absorbance of the standard solution for six times on different days. The %RSD values were calculated. The results (Table.1) are proving that the developed method is precise.

ACCURACY

Recovery studies were done at 3 different levels of 80%, 100% and 120%. The sample solutions were spiked with known concentration of the standard solutions and the mixtures were analysed by the proposed method to calculate the % recovery.

SENSITIVITY

LOD and LOQ decide about the sensitivity of the method. LOD is the lowest detectable concentration of the analyte by the method while LOQ is the minimum quantifiable concentration. LOD and LOQ were calculated by standard calibration curves.

RESULTS AND DISCUSSION

Estimation of Metformin HCL and Sitagliptin was achieved by simultaneous equation method. Both the drugs were showing the linearity in the range of 2-16 µg/ml (Fig 4&5). The slope, intercept and correlation coefficient values were found to be 0.991, 0.1889 and 0.9976 for Metformin HCL and 0.0739,0.0906 and 0.9957 for Sitagliptin. Intraday and Inter day precision studies were performed. Low % RSD values were obtained which indicate that the developed method is precise (Table 1, 2). In this method, accuracy was determined by calculation of percentage recovery. The recovery values were found to be within the limit of

98-102% (Table 3). LOD and LOQ were found to be 0.021µg/ml and 0.064µg/ml for Metformin and 0.01 µg/ml and 0.05 µg/ml for Sitagliptin respectively.

Analysis of Marketed Formulation

The developed and validated method was applied for the assay of marketed formulation. The results are shown in Fig No 6, Table 4.

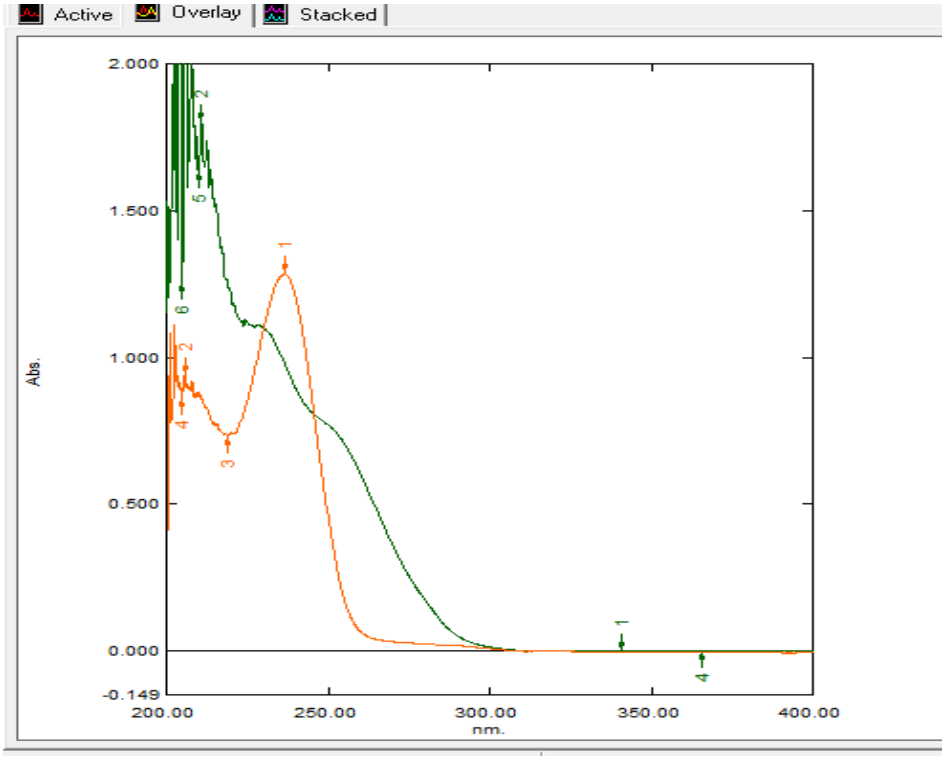

Fig No. 3: Absorbance spectra of Metformin and Sitagliptin

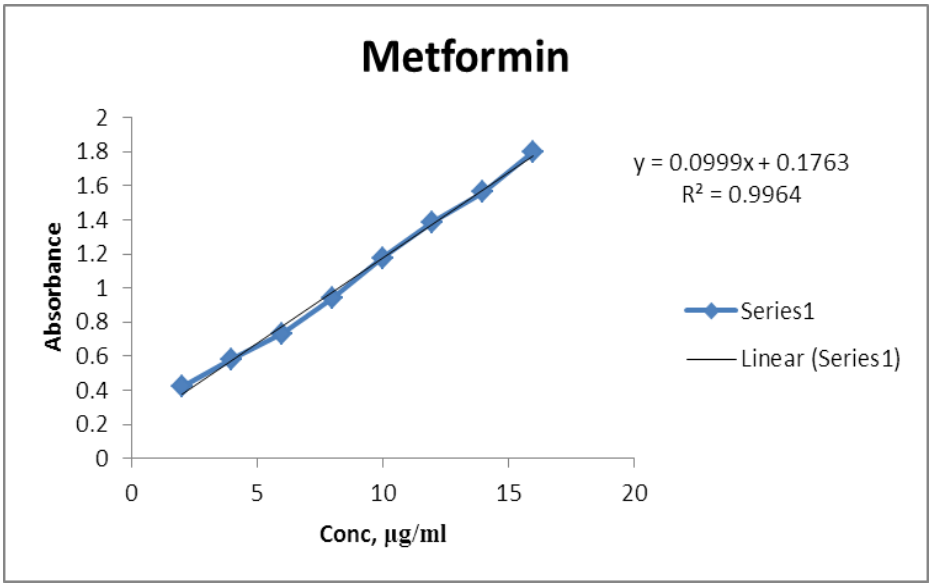

Fig No.4: Calibration curve of Metformin

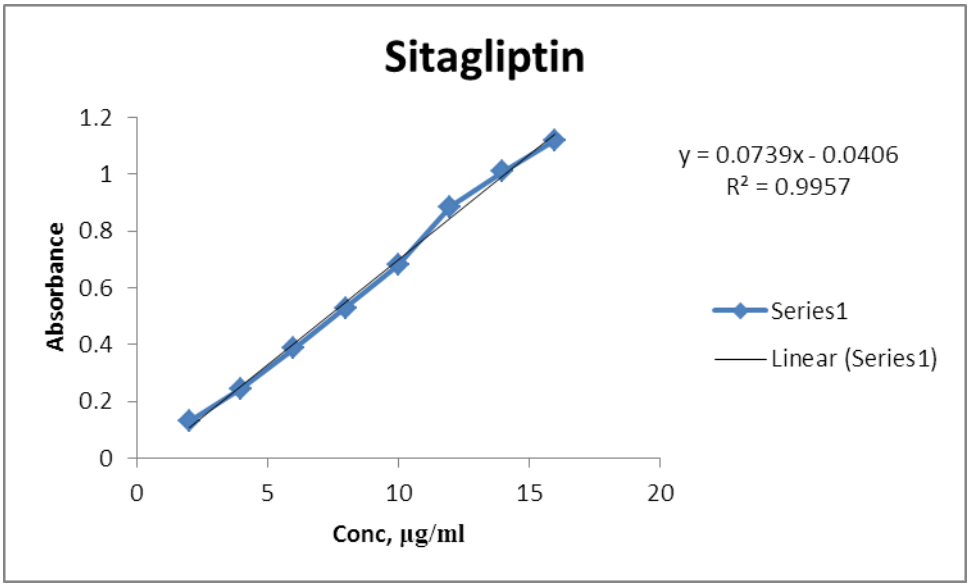

Fig No. 5: Calibration curve of Sitagliptin

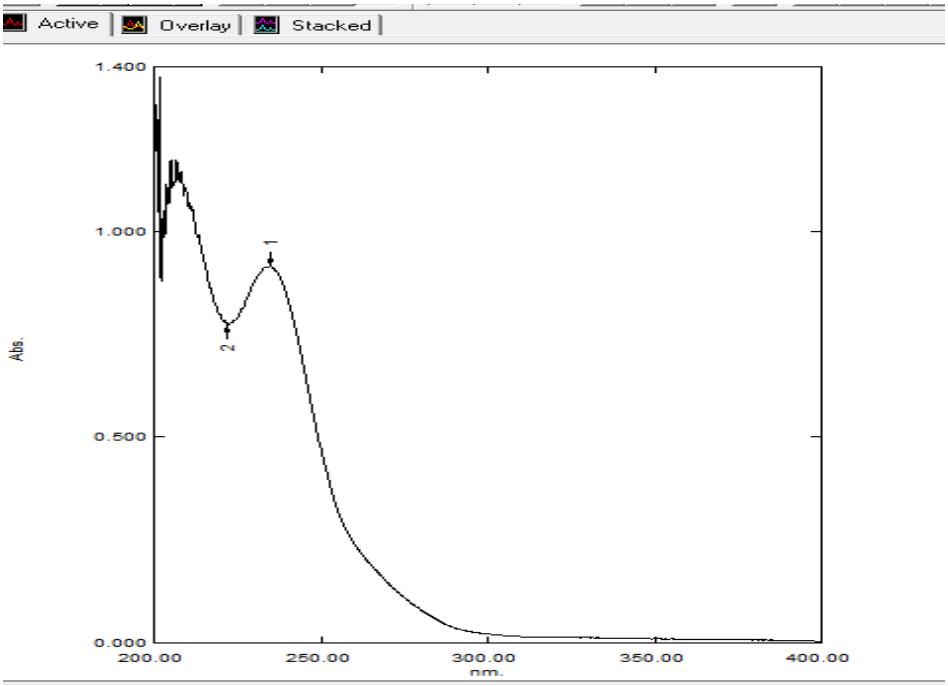

Fig No. 6: Analysis of marketed formulation

Table 1: Intraday precision

S. No	Concentration µg/ml	Absorbance		%RSD	
		232 nm	225 nm	232 nm	225 nm
1	Metformin 6 + Sitagliptin 6	1.225	1.004	0.0855	0.5184
2		1.225	1.006		
3		1.227	1.017		
4		1.224	1.013		
5		1.226	1.015		
6		1.226	1.014		

Table 2: Interday precision

S.No	Concentration µg/ml	Absorbance		%RSD	
		232 nm	225 nm	232 nm	225 nm
1	Metformin 6 + Sitagliptin 6	1.224	1.006	0.420	0.773
2		1.226	1.015		
3		1.227	1.018		
4		1.229	1.024		
5		1.233	1.025		
6		1.238	1.027		

Table 3: Accuracy Studies

S No.	Concentration (µg/ml)	%	Amount added	Total Amount recovered	% Recovery
1	Metformin 6	80	4.8	10.84	99.44
2		100	6	11.92	99.33
3		120	7.2	12.98	98.33
4	Sitagliptin 6	80	4.8	10.86	99.633
5		100	6	11.95	98.76
6		120	7.2	13.29	99.17

Table 4: Analysis of Marketed formulation

S No.	Drug	Labeled amount, mg/ tablet	Amount found, mg/ tablet	% Label claim
1	Metformin	500	500.4	100.08
2	Sitagliptin	50	49.6	99.2

CONCLUSION

The evaluation of obtained values suggests that the proposed UV Spectrophotometry method provide simple, precise, rapid and accurate analytical method for simultaneous determination of Metformin and Sitagliptin in tablet dosage form. Correlating the obtained results with the standard values, the method is found to be valid and hence the method can be easily and conveniently adopted for routine estimation Metformin and Sitagliptin in tablet dosage form.

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