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Method development and validation for the simultaneous estimation of sitagliptin and metformin in tablet dosage form by RP-HPLC

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ABSTRACT

A RP-HPLC method was developed and validated for the simultaneous estimation of Metformin Hydrochloride (MET) and Sitagliptin (STG) in pure and pharmaceutical dosage form. Chromatography was carried on Phenomex (kromosil-250 mm × 4.6 mm, 5 µm) column with mobile phase comprising of phosphate buffer and acetonitrile in the ratio 75:25 v/v. The flow rate was adjusted to 1.0 ml/min with UV detection at260 nm. The retention times of MET and STG were found to be 1.43 min, 2.3 min respectively. The different analytical parameters such as accuracy, linearity, precision, robustness, limit of detection (LOD), limit of quantification (LOQ) were determined according to the ICH-Q2B guidelines. The detector response was linear in the range of 25-250 µg/ml, 2.5-25 µg/ml for MET, STG respectively. The proposed RP-HPLC method is sensitive, precise and accurate so it was successfully applied for the reliable quantification of drugs in the commercial dosage form.

Keywords: Metformin hydrochloride, Sitagliptin, RP-HPLC and Simultaneous estimation.

INTRODUCTION

Metformin hydrochloride (MET), an oral antidiabetic drug which is the first line of choice for the treatment of type 2 diabetes, particularly in overweight or obese peoples and those with normal kidney function. MET improves hyperglycemia, primarily through its suppressive action on production of hepatic glucose (hepatic gluconeogenesis). MET activates AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats; activation of AMPK is required for metformin's inhibitory effect on the production of glucose by liver cells. MET is known chemically as 3-(diaminomethylidene) - 1, 1- dimethyl guanidine.1 – 4 .Sitagliptin (STG) (*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine is an oral anti-hyperglycemic agent of the new dipeptidylpeptidase-4 (DPP-4) inhibitor class of drug. STG inhibits the inactivation of GLP- 1 and GIP byDPP-4, allowing GLP-1 and GIP to potentiate the secretion of insulin in the beta cells and suppress glucagon release by the alpha cells of the islets of Langerhans in the pancreas. STG has been shown to reduce hyperglycemia in type 2

diabetes mellitus. Novartis has since withdrawn its intent to submit STG to the FDA, as of July 2008. The Food and Drug Administration had demanded additional clinical data before it could approve STG including extra evidence that skin lesions and kidney impairment seen during an early study on animals have not occurred in human trials. While the drug is still not approved for use in the Medicines Agency for use within the EU and is listed on the Australian PBS with certain restrictions. The EMEA has also approved a new oral treatment released by Novartis, called Eucreas, a combination of STG and MET 5,6.Literature survey shows that there are many methods for the quantitative estimation of MET separately and in combination STG with other drugs 7-10. To our knowledge simple and economical analytical method for simultaneous estimation of MET and STG has not been reported so far. So attempt was taken to develop and validate an economic, rapid reverse phase high performance liquid chromatographic method for the quality control of MET and STG in pharmaceutical preparations with lower solvent consumption along with the short analytical run time that leads to an environmental friendly chromatographic procedure and will allow the analysis of a large number of samples in a short period of time. The method was validated as per ICH guidelines and found to be accurate, precise and reproducible.

MATERIALS AND METHODS

Apparatus and chemicals

Waters HPLC system connected with UV dual λabsorbance Detector 2487 and Empower-2 Software was used. MET and STG pure drugs were kindly supplied as a gift sample by Natco pharma limited, Hyderabad, Andhra Pradesh, India. Methanol was of HPLC grade, collected from E.Merck, Mumbai. Potassium di hydrogen ortho phosphate, di-sodium hydrogen orthophosphate were analytical reagent grade supplied by Fischer Scientific Chemicals, India. Water HPLC grade was obtained from a Finar Limited, Ahmedabad, India.

Commercial Formulation

MET and STG tablets available in the market in of Metformin HCL (500 mg), Sitagliptin (50 mg). The tablets were checked and stored properly.

Preparation of solutions

Preparation of mobile phase Preparation of 0.1M Phosphate buffer (pH 6.8) was carried out by dissolving accurately weighed portion of 2.722g of potassium di hydrogen orthophosphate in 200 ml of HPLC water. Separately 700 mg of disodium hydrogen orthophosphate was weighed and dissolved in 20 ml of HPLC water, the pH adjusted to 6.8 using disodium hydrogen orthophosphate, and then the solution was filtered through a 0.22 µg/ml filter membrane and stored in closed container. HPLC grade acetonitrile is filtered and store in a tightly caped container. Preparation of standard solution500 mg of MET and 50 mg of VIL was weighed accurately and dissolved in HPLC water in 50 ml volumetric flask, which gave 10000 µg/ml of MET and 1000 µg/ml of STG. From the above solution 1 ml was diluted to 10ml (1000 µg/ml MET and 100 µg/ml of STG). From this into a series of five 10 ml volumetric flasks 0.25,0.5, 1, 1.5, 2, 2.5 ml were transferred and diluted to10 ml with HPLC water, that gave 2.5, 5, 10, 15, 20,and 25 µg/ml of STG and 25, 50, 100, 150, 200 and250 µg/ml of MET. Preparation of test solution20 tablets of combined formulation of MET and STG were weighed, average weight was calculated and triturated in a mortar with pestle from that, powder equivalent to 500 mg of MET and 50 mg of STG was weighed and dissolved in HPLC water and test concentration was prepared by further dilution with same.

Chromatographic Conditions

The mobile phase, a mixture of 0.1M phosphate buffer pH 6.8 and acetonitrile (75:25 v/v) pumped at a flow rate of 1.0 ml/min through the column (Phenomexkromosil 5µ, 4.6 × 250 mm). The mobile phase was degassed prior to use under vacuum by filtration through a 0.22 µ membrane filter. Both drugs showed good absorbance at 260 nm, which was selected as wavelength for further analysis.

DEVELOPMENT AND VALIDATION OF HPLC METHOD

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of MET and STG in tablet dosage form. The experiment was carried out according to the official specifications of ICH. The method was validated for the parameters like system suitability,

specificity, linearity, precision, accuracy, LOD, LOQ and robustness.

System Suitability

System suitability study of the method was carried out by six replicate analysis of solution containing100% test concentration of MET and STG. The colum Various chromatographic parameters such as retention time, peak area, tailing factor, theoretical plates of and resolution between the peaks were determined and the method was evaluated by analyzing these parameters.

Specificity

Specificity test determines the effect of excipients on the assay result. To determine the specificity of the method, standard solution of MET and STG. Were injected first. Then commercial product, blank and excipients solution were run in the instrument one after another. No any interference at retention time of drugs was observed.

Linearity

Linearity of the method was determined by constructing calibration curves. Standard solutions of MET and STG of different concentrations level (25, 50, 100, 150, 200, 250 and 2.5, 5, 10, 15, 20, 25µg/ml) were used for this purpose. Each measurement was carried out in 6 replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients

Accuracy

Accuracy is the percentage of analyte recovered by assay from a known added amount. To check the degree of accuracy of the method, recovery studies were performed in six times by standard addition method at 50%, 100% and 150%. Known amounts of standard MET and STG were added to pre analyzed samples and were subjected to the proposed HPLC method.

Precision

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (% RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ were calculated for the sensitivity of the method. They were quantified based on the signal to noise ratio. LOD is lowest detectable concentration of the analyte by the method while LOQ is the minimum quantifiable concentration. LOD and LOQ were calculated according to ICH guidelines.

$$LOD = 3.3 \times SD/SLOPE$$

$$LOQ = 10 \times SD/SLOPE$$

Robustness of Method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate and absorbance (nm) on their retention time and tailing factor were studied. The method was found to be unaffected by small changes1±0.1ml change in flow rate and small changes in absorbance as 260±2nm.

RESULTS AND DISCUSSION

The developed method has been validated as per ICH guidelines. Every 20 µL of the working Standard solution of STG in the concentration range of 2.5 to 25 µg/mL, and that for MET in the concentration range of 25 to 250 µg/mL were injected into the chromatographic system. The chromatograms were recorded and the peak area was determined for each concentration of the drug solution. Calibration curves of STG and MET were obtained by plotting the peak area versus concentrations of STG and MET. System suitability and precision study are shown in Table No.1.Precision study of the developed method is in TableNo.2. Standard chromatogram and marketed formulation chromatogram are in Figure No.1 and 2.Accuracy of the method was tested by carrying out recovery studies at different spiked levels. The estimation was carried out as described earlier. At each level, three determinations were performed and results obtained. The amounts recovered and the values of percent recovery were calculated, Limit of detection (LOD) and limit of quantification (LOQ) were calculated. Results of accuracy study are presented in Table No.3. The measured value was obtained by recovery test. Spiked amount of both the drugs were

compared against the recovery amount .All the results indicate that the method is highly accurate. The results of robustness of the present method showed that small changes were made in the flow rate did not produce significant changes in analytical results; we can say that the method is robust. Results of robustness are presented in Table No. 4.

Table No.1: Result of system suitability tests of MET and STG

S.No	Parameters	STG	MET
1	Linearity range	2.5-25 (µg/ml)	25-250(µg/ml)
2	correlation	0.999	0.999
3	slope	13271	36108
4	Intercept	2037.7	2769.7
5	Retention time	2.3	1.43
6	USP plate count	3752	3654
7	Tailing factor	1.2	1.3
8	Limit of detection(LOD)	0.0053(µg/ml)	0.219(µg/ml)
9	Limit of quantification(LOQ)	0.0159(µg/ml)	0.669(µg/ml)

Intermediate Precision

Table: No.2: Intermediate Precision

Repeatability (% RSD) (n=6)	Intermediate precision (% RSD) (n=6)			
	Day 1		Day 2	
	Analyst 1	Analyst 2	Analyst 1	Analyst 2
MET	0.4981	0.8152	0.6697	0.6057
STG	0.4504	0.8257	0.8317	0.7139

Accuracy

Table: No.3: Recovery Studies

Sample	Spiked Amount (mg)	Recovered Amount (mg)	% Recovered	% Average Recovery
STG	5	5.02	100.7	100.06
	10	10.01	100.27	
	15	15.01	101.07	
MET	50	49.76	99.54	99.90
	100	100.03	100.03	
	150	150.01	100.006	

Robustness

Table: No.4: Robustness

Drug	Parameters count	Changes	RT(min)	USP Tailing	USP Plate
STG	Flow rate (ml/min)	0.9	2.36	1.2	3856
		1.1	2.33	1.3	3789
MET	Flow rate (ml/min)	0.9	1.42	1.3	3850
		1.1	1.45	1.2	3758

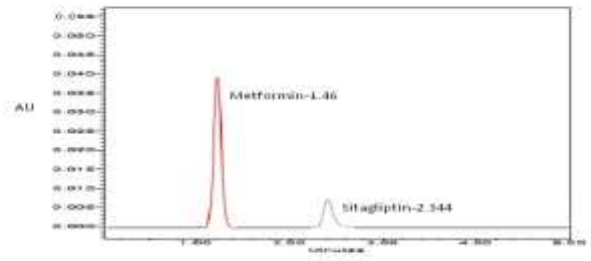

Figure No.1: Typical Chromatogram of standard MET and STG

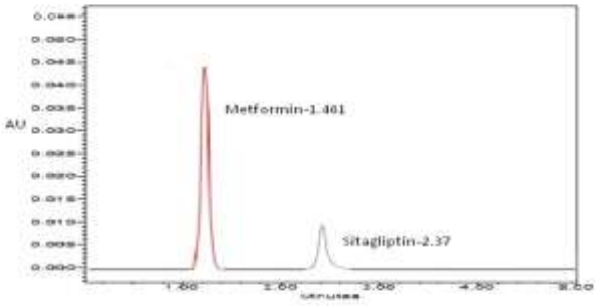

Figure No.2: Typical chromatogram of MET and SGL in marketed formulation

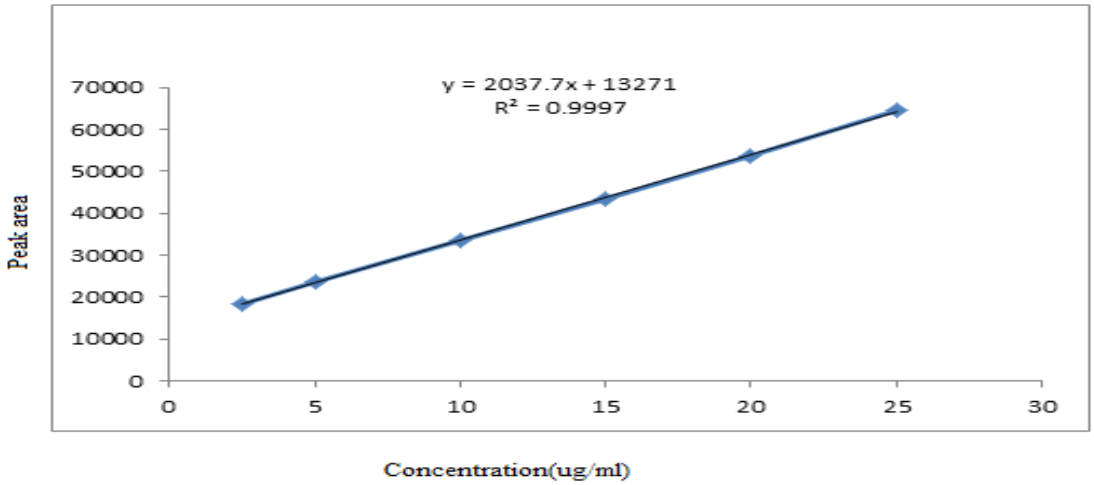

Figure No.3: Linearity of STG

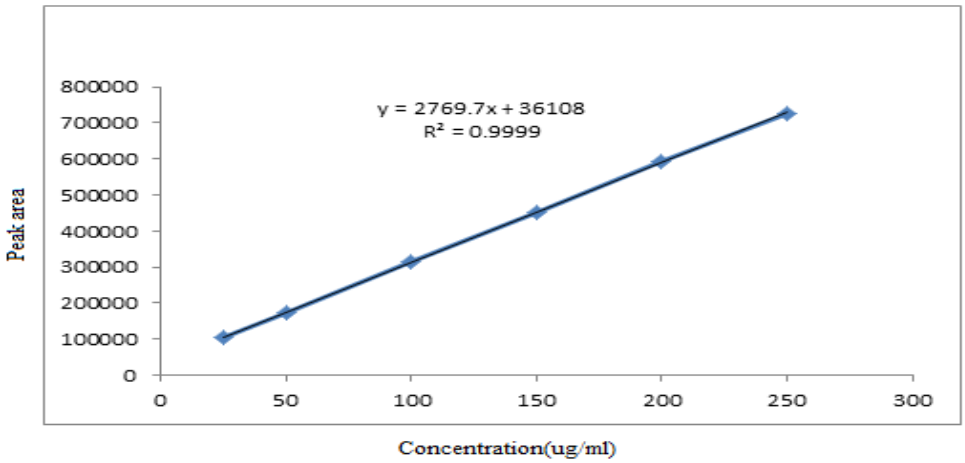

Figure No.4: Linearity of MET

CONCLUSION

The new RPHPLC method developed and validated for simultaneous estimation of MET and STG in pure and in pharmaceutical dosage form and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation. The developed method was validated in terms of accuracy, repeatability, and precision. The assay experiment showed that the contents of STG and MET estimated in the tablet dosage form were free from the interference of excipients. This demonstrated that the developed HPLC method was simple, linear, precise, and accurate, and could be conveniently adopted for the routine quality control analysis of STG and, from its pharmaceutical formulations and pure drug.

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