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

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ABSTRACT

A RP-HPLC method was developed and validated for the simultaneous estimation of MetforminHydrochloride (MET) and Saxaagliptin (SGL) in pure and pharmaceutical dosage form. Chromatography was carried on PhenominexC18(250 mm × 4.6 mm, 5 µm) column with mobile phase comprising of phosphate buffer and acetonitrile in the ratio(60:40) v/v. The flow rate was adjusted to 0.7 ml/min with UV detection at 242 nm. The retention times of MET and SGL were found to be 1.7 min, 2.9 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 100-600µg/ml, 1-6 µg/ml for MET, SGL respectively. The proposed RP-HPLCmethod is sensitive, precise and accurate so it was successfully applied for the reliable quantification of drugs in the commercial dosage form.

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

INTRODUCTION

Metformin hydrochloride (MET), an oral anti-diabetic 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.

Saxagliptin is a new oral hypoglycemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs.

IUPAC name for Saxagliptin is (1S, 3S, 5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo hexane-3-carbonitrile. Saxagliptin is part of a class of diabetes medications called dipeptidyl peptidase-4 (DPP-4) inhibitors. DPP-4 is an enzyme that breaks down incretion hormones. As a DPP-4 inhibitor, Saxagliptin slows down the breakdown of incretion hormones, increasing the level of these hormones in the body. Type 2 diabetes mellitus is a common chronic disease that causes significant morbidity and mortality worldwide. The primary goal of treatment is to target glycemic control by maintaining the glycosylated

hemoglobin (HbA1c) level near 6% to 7% without predisposing patients to hypoglycemia. Currently available anti-diabetic agents work by different mechanisms to lower blood glucose levels. The usual adult dose is 2.5 to 5 mg once daily regardless of meals. A daily dose of 2.5 mg is recommended for patients with moderate to severe renal impairment or those who are taking potent CYP 3A4 inhibitors. In randomized clinical trials, Saxagliptin alone lowered HbA1c levels by about 0.5%; with
better efficacy seen when combined with other agents.

MATERIALS AND METHODS

Apparatus and chemicals

Waters HPLC system connected with UV dual λ absorbance Detector 2487 and Empower-2 Software was used. MET and SGL pure drugs were kindly supplied as a gift sample by Natco Pharma Ltd., Hyderabad, Andhra Pradesh, India. Methanol was of HPLC grade, collected from E.Merck, Mumbai. Potassium dihydrogenortho 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 SGL tablets available in the market in composition of Metformin HCL (500 mg),Saxagliptin (5 mg). The tablets were checked and stored properly.

Reagents and Solutions

Preparation of mobile phase

Preparation of 0.1M Phosphate buffer (pH 5.3)

Accurately weighed portion of 2.722g of potassium dihydrogen orthophosphate was dissolved in 200 ml of HPLCwater. Mobile phase consisting of phosphate buffer(p^H5.3),acetonitrile in the ratio of 60:40 respectively. The mobile phase was filtered through 0.22µ membrane filter and sonicated for 15 min.
Mobile phase is used as diluent.

Preparation of standard solution

Accurately weighed 100mg of Metformin and it is dissolved in 100ml of mobile phase as diluent ,that gave 1000µg/ml. from that gave at take serial dilutions 1-6,that gave concentrations 100,200,300,400,500 and 600µg/ml.

Accurately weighed 5mg of saxagliptin , dissolve in 5ml of mobile phase as diluent that gave1000µg/ml, from hat take 1ml and diluted to 10ml with mobile phase as diluent it gives 100µg/ml. from that take 1ml and diluted to 10ml,it gave 10µg/ml, from this take the serial dilutions 1-6ml,that take the concentrations 1,2,3,4,5 and 6 µg/ml.

Preparation of test solution

20 tablets of combined formulation of Metformin and Saxagliptin were weighed, average weight was calculated and triturated in a mortar with pestle from that, powder equivalent to 500 mg of Metformin and 5 mg of Saxagliptin was weighed and dissolved in diluent and test concentration was prepared by further dilution with same.

Chromatographic Conditions

The mobile phase, a mixture of 0.1M phosphste buffer pH 5.3 and acetonitrile (60:40 v/v) pumped at a flow rate of 0.7 ml/min through the column (Phenomex C18, 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 242 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 SGL 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 containing 100% test concentration of MET and SGL. Various chromatographic parameters such as retention time, peak area, tailing factor, theoretical plates of the column 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 SGL 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 SGL of different concentrations level (100,200,300,400,500,600 and 1,2,3,4,5,6 µg/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 SGL were added to preanalyzed 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 × SD/SLOPE

LOQ = 10 × SD/SLOPE

Robustness of Method

The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by changing parameters like flow rate of mobile phase and detection wavelength

- Change in the detection wavelength by ± 2nm (238nm and 242nm)
- Change in flow rate by ± 0.2 ml/min (0.6 ml/min and 0.9 ml/min)

After each change, sample solution was injected and % assay with system suitability parameters were checked.

RESULTS AND DISCUSSION

The developed method has been validated as per ICH guidelines. Every 20 µL of the working standard solution of SGL in the concentration range of 1 to 6 µg/mL, and that for MET in the concentration range of 100 to 600 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 SGL and MET were obtained by plotting the peak area versus concentrations of SGL and MET. System suitability and precision study are shown in Table No.1. Precision study of the developed method is in Table No.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 SGL

S.No	Parameters	SGL	MET
1	Linearity range	1-6(µg/ml)	100-600(µg/ml)
2	correlation	0.999	0.999
3	slope	829998	251867
4	Intercept	33734	66985
5	Retention time	2.9	1.7
6	USP plate count	3452	3568
7	Tailing factor	1.5	1.0
8	Limit of detection(LOD)	0.0042(µg/ml)	0.0148(µg/ml)
9	Limit of quantification(LOQ)	0.212(µg/ml)	0.662(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
SGL	0.8656	0.7324	0.6322	0.8213
MET	1.1563	1.7669	0.1935	0.8241

Accuracy

Table:No.3: Recovery Studies

Sample	Spiked Amount (mg)	Recovered Amount (mg)	% Recovered	% Average Recovery
SGL	3	3.02	99.54	100.6
	4	4.03	100.03	
	5	5.01	100.006	
MET	200	200.76	100.7	99.9
	300	300.03	100.27	
	400	400.01	101.07	

Table:No.4:Robustness

Drug	Parameters count	Changes	RT(min)	USP Tailing	USP Plate
SGL	Flow rate (ml/min)	0.6	3.0	1.5	7241
		0.9	2.8	1.0	5835
MET	Flow rate (ml/min)	0.6	1.6	1.0	5167
		0.9	1.9	1.5	7334

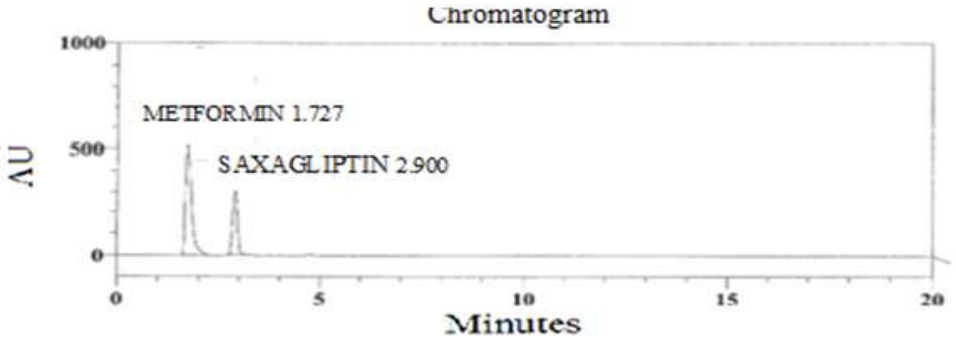

Figure No.1: Typical Chromatogram of standard MET and SGL

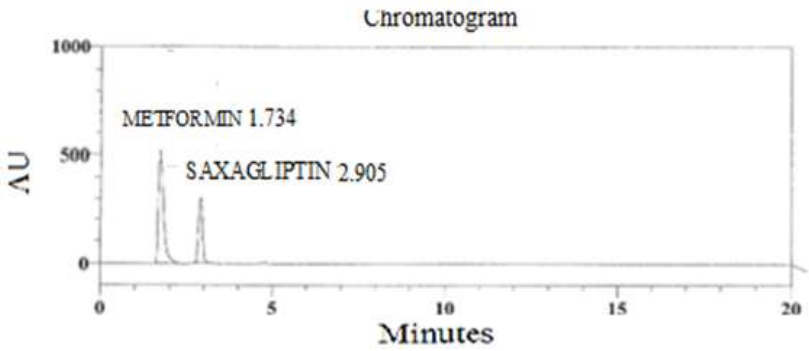

Figure No.2: Typical chromatogram of MET and SGL in marketed formulation

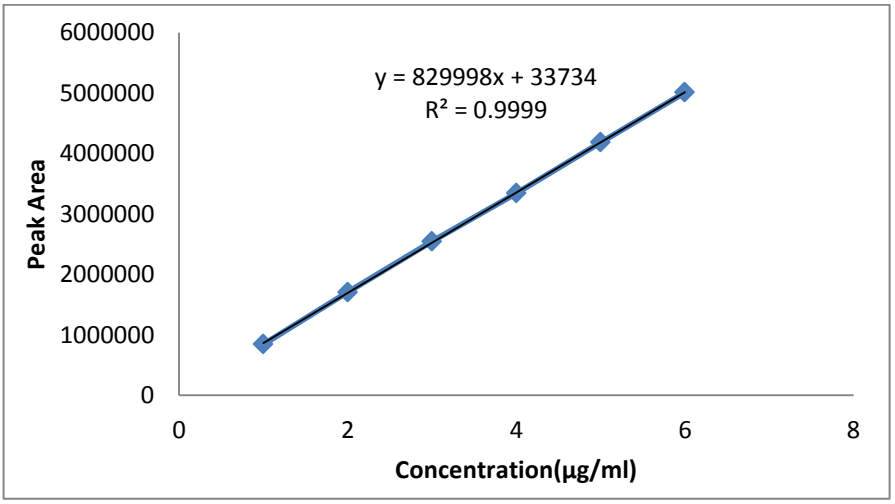

Figure No.4: Linearity of SGL

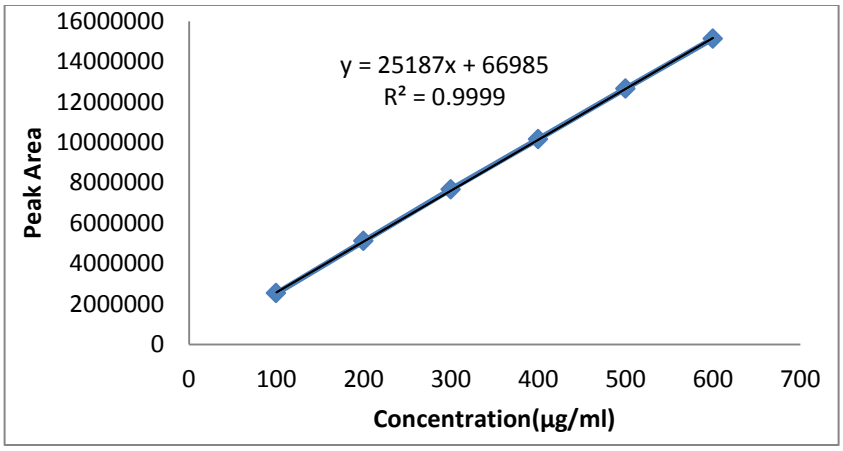

Figure No.3: Linearity of MET

CONCLUSION

The new RP-HPLC method developed and validated for simultaneous estimation of MET and SGL 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 SGL 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 SGL and MET simultaneously, from its pharmaceutical formulations and pure drug.

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