

SIMULTANEOUS ESTIMATION OF SAXAGLIPTIN HYDROCHLORIDE AND METFORMIN HYDROCHLORIDE IN ACTIVE PHARMACEUTICAL INGREDIENT BY RP-HPLC

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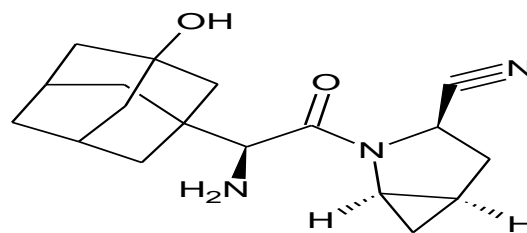
ABSTRACT

A new simple, accurate, precise and reproducible RP-HPLC method has been developed for the simultaneous estimation of Saxagliptin and Metformin in bulk drug form using C₁₈ column (Phenomenex, 250 x 4.6 mm, 5 μm) in isocratic mode. The mobile phase consisted of 0.02M Potassium dihydrogen phosphate (KH₂PO₄), Acetonitrile, Methanol in the ratio of 50:25:25 (v/v/v) at pH 4.3. The detection wavelength was carried out at 240 nm. The method was linear over the concentration range for Saxagliptin 10-50 μg/ml and for Metformin 5-25 μg/ml. The recoveries of Saxagliptin and Metformin were found to be 100.48 and 101.1% respectively. The validation of method was carried out utilizing ICH-guidelines. The described HPLC method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form.

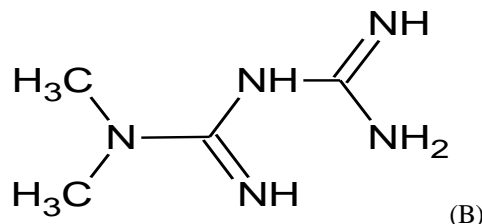
Key Words: - Saxagliptine Metformine, Simultaneous estimation, RP-HPLC

INTRODUCTION

Saxagliptin (SXG) is chemically (1S, 3S, 5S)-2-[(2S)-2-Amino-2-(3 hydroxytricyclo [3.3.1.1^{3,7}] dec-1-yl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile previously identified as BMS-477118. This is new oral hypoglycemic agent of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. The empirical formula is C₁₈H₂₅N₃O₂ · H₂O and the molecular weight is 333.43¹⁻⁵. The structural formula is:



(A)



(B)

Fig 1 Structure of Saxigliptin (A) and Metformin (B)

Saxagliptin recently approved for the treatment of type-2 diabetes mellitus. It has been used in conjunction with exercise and diet to improve glycaemic control in patients with type 2 diabetes and is to be used with metformin, a sulphonylurea or pioglitazone when blood sugar levels are not adequately controlled by one of these agents alone⁶⁻¹². Literature survey reveals that the drug can be estimated only by LC-MS/MS¹³, Spectrophotometric method¹⁴ have been reported.

Metformin Hydrochloride(MTF) ($C_4H_{11}N_5.HCl$) is 1, 1-dimethylbiguanidine monohydrochloride (Fig 1), is an anti-diabetic drug from the biguanide class of oral hypoglycaemic agents, given orally in the treatment of non –insulin-dependent diabetes mellitus¹⁵. Major action of metformin HCl in increasing glucose transport across the cell membrane in skeletal muscle¹⁶⁻¹⁷. Several analytical methods based on UV¹⁸⁻²⁰, Spectrofluorimetry²¹, RP-HPLC²²⁻³⁰, HPTLC³¹ and LC-MS/MS³² was reported for the determination of metformin. Although literature survey reveals that various methods were reported for Saxagliptin and Metformin both for single estimation and in combination with others drugs, but no estimation method has been reported for the analysis of these drugs in combination.

EXPERIMENTAL

Apparatus

The liquid chromatographic system consists of shimadzu 20 AT with UV-VIS detector, binary pump and rheodyne injector valve with 20 μ l fixed loop. The analytes were monitored at 240 nm. Chromatographic analysis was performed on Phenomenex C_{18} column having 250 mm \times 4.6 mm i.d. and 5 μ m particle size. Chromatogram were automatically obtained by spinchrome system software.

Reagents and Materials

All chemicals and reagents were used of AR grade. Authentic of SXG and MTF were obtained as gift samples from Astra Zeneca Pharma India Ltd.

Selection of detection wavelength

Solutions of drug were scanned over the range of 200-400 nm. It was observed that both the drugs showed considerable absorbance at 274 nm for SXG and 231nm for MTF. the Isobestic point of both drugs was found to be 240nm.(Fig. 2)

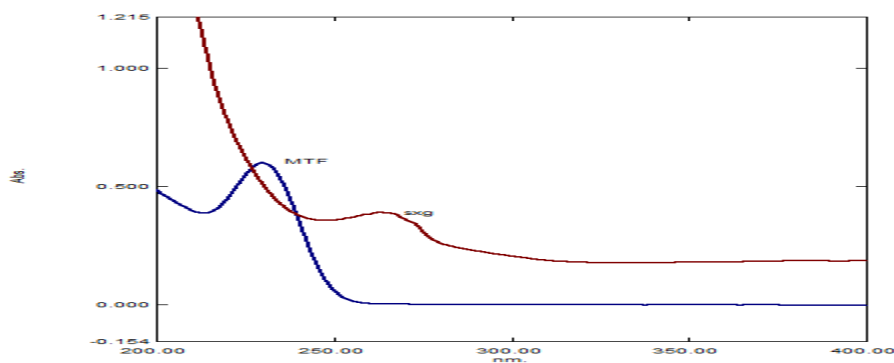


Fig 2 Isobestic point of Saxagliptin and Metformin

Chromatographic Conditions

The Phenomenex C_{18} column (250 x 4.6mm, 5 μ m) equilibrated with mobile phase 0.02M Potassium dihydrogen phosphate (KH_2PO_4), Acetonitrile, Methanol in the ratio of 50:25:25 (v/v/v) at pH 4.3. The flow rate was maintained at 1 ml/min, detection wavelength 240 nm, and the injection volume was 20 μ l and run time was kept 12 min.

Preparation of standard solution

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

Preparation of working standard

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

METHOD DEVELOPMENT

Lots of mobile phase and there different proportions were tried and finally was selected as 0.02M Potassium dihydrogen phosphate (KH_2PO_4), Acetonitrile, Methanol in the ratio of 50:25:25 (v/v/v) at pH 4.3 appropriate mobile phase which gave good resolution and acceptable system suitability parameters. The chromatogram of working standard solution is shown in fig 3.

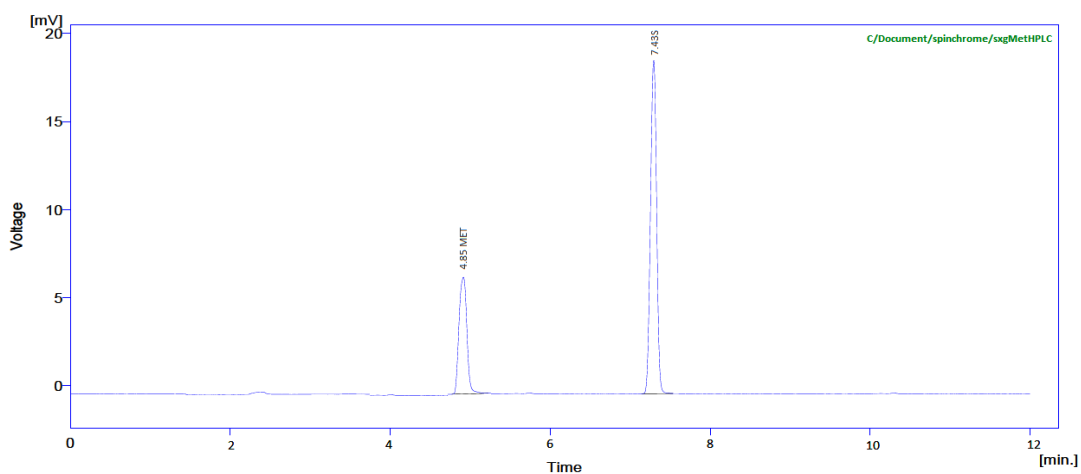


Fig 3 HPLC chromatogram of Saxagliptin and Metformin.

Linearity

Accurately measured volumes of working standard solution of SXG and MTF were transferred into a series of 10 ml volumetric flasks and diluted appropriately with water. 20µl of each solution was injected at same chromatographic conditions. Calibration curves were obtained by plotting the peak area versus concentration of drug. Regression equations were calculated. The method was found linear over a concentration range 10-50µg/mL of SXG and 5-25 µg/mL of MTF. (Fig. 4, 5)

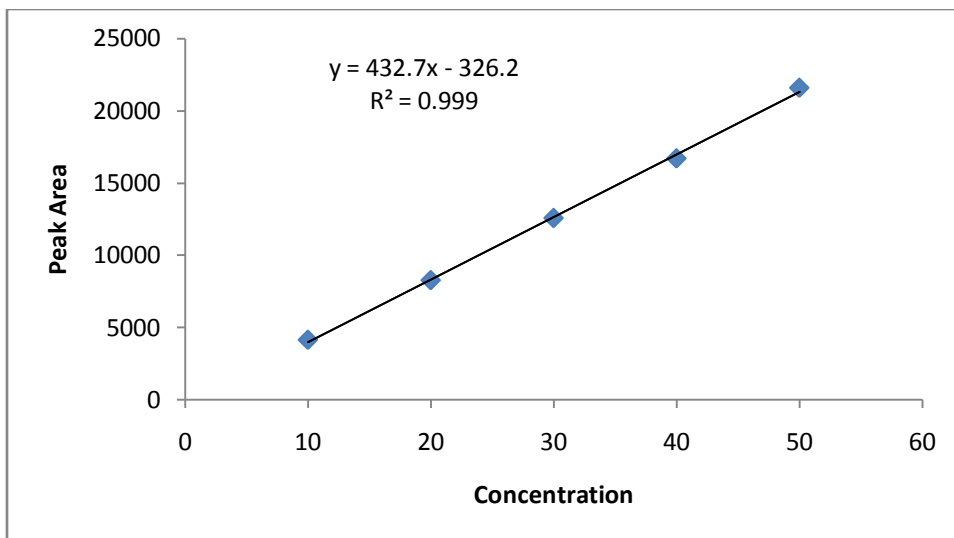


Fig. 4 Calibration curve of Saxagliptine

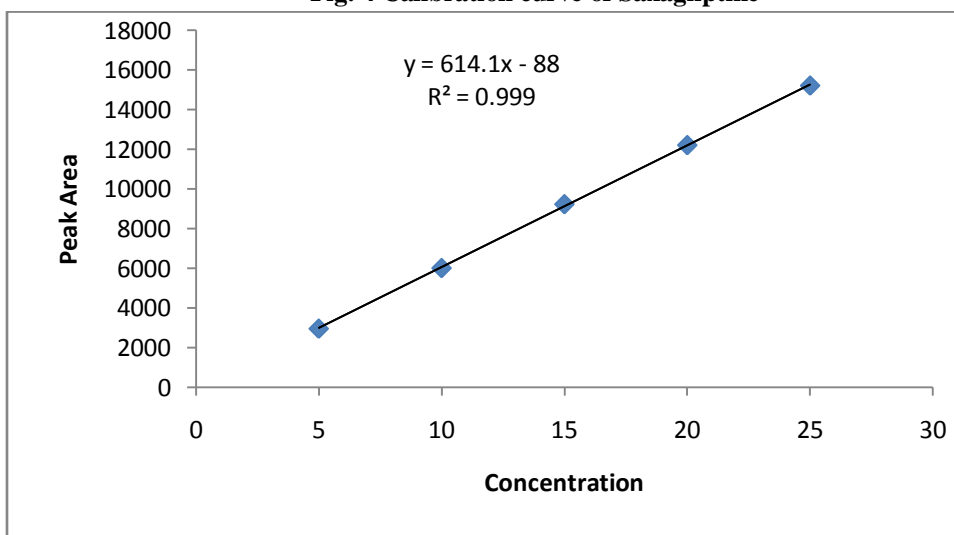


Fig. 5 Calibration curve of Metformin

Precision

The repeatability studies were carried out by estimating response of SXG (20 µg/mL) and MTF (10µg/mL) five times. The intra-day and inter-day precision studies were carried out by estimating the corresponding responses five times on interday and intraday for three different concentrations of SXG (10, 30, 50 µg/ml) and MTF (10, 15, 20 µg/ml). It is expressed as the percentage coefficient of variation (% CV) which is calculated as per the following expression.

$$\% \text{ CV} = (\text{standard deviation} / \text{mean}) \times 100.$$

Accuracy

Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 80%, 100% and 120% to the pre analysed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated which sense to conformation that the proposed method was accurate.

Table 1 Recovery study

S. No.	Saxagliptin			Metformin		
	80	100	120	80	100	120
Recovery Level						
Amount present	20	20	20	10	10	10
Amount added	16	20	24	8	10	12
Amount recovered	36.23	40.12	44.23	18.4	19.87	22.32
%Recovery	100.6	100.3	100.5	102.2	99.35	101.45
Mean % Recovery	100.48			101.08		

Detection Limit and Quantitation Limit

The detection limits were found to be 1.91µg/ml and 0.40µg/ml for SXG and MFT respectively. The quantitation limits were found to be 5.78µg/ml and 1.22µg/ml for Saxagliptin SXG and MFT respectively. These values indicate that the method is sensitive.

Specificity

The method was determined as specific by comparing test results obtained from analyses of sample solution containing placebo ingredients with that of test results those obtained from analyses of standard solution.

RESULTS AND DISCUSSION

The present work done on this combination comprises a simple, precise and accurate method by reverse phase high performance liquid chromatography. An attempt has been made to estimate SXG and MTF by RP-HPLC. Calibration curve depicting the linearity and range for SXG and MTF were determined from mixed standards and were found to be of the order 10-50 µg /ml of SXG and 5-25 µg /ml of MTF. The retention times 4.85 minutes for MTF and 7.43 minutes for SXG. The results obtained from HPLC method were reproducible and encouraging. The values percentage deviation was within limit (>2%) and recovery close to 100% indicating reproducibility and accuracy of method.

CONCLUSION

Proposed study describes method for the estimation of SXG and MTF combination in mixture. The method was validated and found to be simple, sensitive, accurate and precise as per ICH guidelines .The method was successfully used for determination of drugs in their pharmaceutical formulation.

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Table 2: Analytical parameters

S.NO.	PARAMETERS	Saxagliptin	Metformine
1	Limit of linearity (µg/ml)	10-50	5-25
2	Regression equation	$y = 432.7x - 326.2$	$y = 614.1x - 88$
3	Slope	432.7	614.1
4	Intercept	326.2	88
5	Correlation coefficient (r^2)	0.999	0.999
6	Retention time (min)	4.85	7.43
7	Detection limit (µg/ml)	1.91	0.40
8	Quantitation limit (µg/ml)	5.78	1.22
9	Accuracy (%)	100.48	101.01
10	Precision (% CV)		
	Repeatibility	0.30	0.75
	Intra-day precision (n=3)	0.435-0.545	0.442-0.987
	Inter-day precision (n=3)	0.268-0.157	0.442-1.67

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