

A VALIDATED RP – HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN AND SAXAGLIPTIN IN TABLETS

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ABSTRACT

This is a simple, economic, sensitive RP-HPLC method for the simultaneous estimation of metformin and saxagliptin in tablets. The method was carried out on C18 column (5 μm, 25 cm x 4.6 mm, i.d) using phosphate buffer (pH 5.0), acetonitrile and methanol in the ratio 75:15: 10 respectively as a mobile phase at a flow rate of 1.5mL/min. The wavelength for metformin and saxagliptin at 225 nm was found to be appropriate. The retention time of metformin and saxagliptin was found to be 5.65 and 6.20 min, respectively. The developed method is found to be rapid and sensitive which can be used for estimation of combination of metformin and saxagliptin in pharmaceutical dosage forms.

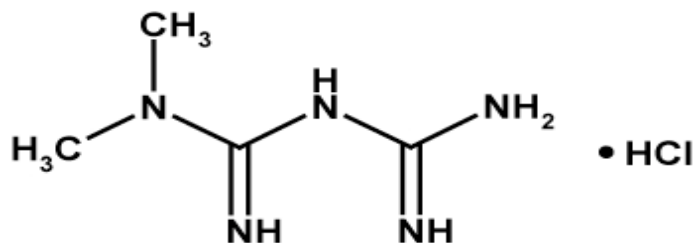
Keywords: Metformin, Saxagliptin, RP-HPLC, wavelength.

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INTRODUCTION

Metformin hydrochloride is a white to off-white crystalline compound with a molecular formula of C₄H₁₁N₅•HCl and a molecular weight of 165.63. Metformin hydrochloride is freely soluble in water and is practically insoluble in acetone, ether and chloroform.

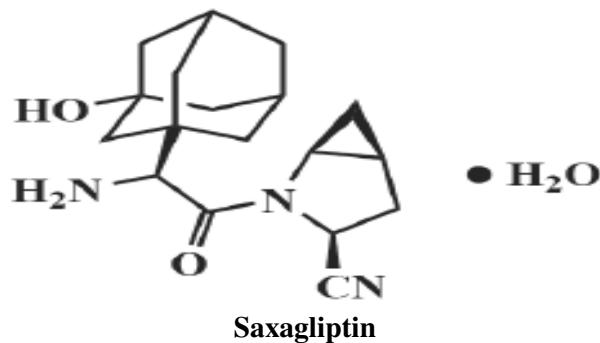
Metformin is widely used in the treatment of hyperglycaemia in individuals with type 2 diabetes. The structural formula is-



Metformin Hydrochloride

Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state¹.

Saxagliptin monohydrate is described chemically as (1*S*,3*S*,5*S*)-2-[(2*S*)-2-Amino-2-(3-hydroxytricyclo[3.3.1.1]dec-1-yl)acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile, monohydrate^{3,7}. The structural formula is:



Saxagliptin monohydrate is a white to light yellow or light brown, non-hygroscopic, crystalline powder. It is sparingly soluble in water at $24^{\circ}\text{C} \pm 3^{\circ}\text{C}$, slightly soluble in ethyl acetate, and soluble in methanol, ethanol, isopropyl alcohol, acetonitrile, acetone, and polyethylene glycol 400 (PEG 400). Saxagliptin is a new oral hypoglycaemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs², licensed for the treatment of type II diabetes in combination with either metformin, a sulphonylurea or a thiazolidinedione.

Saxagliptin once daily added to metformin therapy was generally well tolerated and led to statistically significant improvements in glycaemic indexes versus placebo added to metformin in patients with type 2 diabetes inadequately controlled with metformin alone⁴.

EXPERIMENTAL

Acetonitrile and methanol used were of HPLC grade and obtained from Merck Chemicals. All other chemicals used were of AR grade and obtained from SD Fine Chemicals, Mumbai. Reference standards of metformin and saxagliptin were obtained from Bizten Impex, India.

Instrumentation

Quantitative HPLC was performed on an isocratic HPLC of SHIMADZU prominence consisting of LC – 20AT liquid pump, manual with $20\mu\text{L}$ sample injection loop and SPD20A UV-visible absorbance detector. The output – signal was monitored and integrated by Shimadzu spin chrome software.

Chromatographic conditions

The process was carried out on C18 column ($5\mu\text{m}$, $25\text{ cm} \times 4.6\text{ mm}$, *i.d.*) using the mobile phase consisting of phosphate buffer (pH 5.0), acetonitrile and methanol in the ratio (70:15: 10 v/v) respectively at a flow rate of 1.5 mL/minutes . Wavelength was adjusted to 225 nm . The mobile phase was filtered through $0.2\mu\text{ m}$ membrane filter and sonicated for 15 min.

Preparation of solutions

Standard solution of the pure drug was prepared by dissolving 500 mg of metformin hydrochloride and 5 mg of saxagliptin hydrochloride in a 100 mL volumetric flask using 25 mL of water. Then the volume made up to the mark with the water. Appropriate volume from this solution was further diluted to get appropriate concentration levels according to the requirement. Ten tablets were weighed the average weight was determined and these were powdered. Sample solution was then prepared by dissolving the powdered tablets equivalent to 500 mg of metformin and 5 mg of saxagliptin in a 100 mL of volumetric flask. Then the drugs were dissolved by using 25 mL water and the volume was made up to the mark with water. 5 mL of this solution was further diluted to 25 mL with the same solvent. $50\mu\text{L}$ of solution was injected into HPLC system to obtain chromatogram for standard drug solution and sample solution. Concentrations of metformin and saxagliptin in the formulation were calculated by comparing AUC of the sample with that of the standard.

Assay method

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time of metformin and saxagliptin was found to be 4.657 and 6.200 min respectively. This procedure was repeated for the sample solution obtained from the formulation (Table-1) and recovery studies (Table-2).

Table-1: System suitability parameters

	Parameters	RT	AUC	No. of Theoretical Plates	Tailing factor
Metformin Hydrochloride	Mean	7.340	780503	760.11	1.36
	S.D	0.033	535.448	221.45	0.12
	% R.S.D.	0.44	0.686	29.13	8.82
Saxagliptin	Mean	2.36	141431	698.94	1.46
	S.D.	0.019	763.58	194.13	0.12
	% R.S.D.	0.80	0.53	27.77	8.2

Table-2: Data of Validation

S.No.	Validation Parameters	Metformin	Saxagliptin
1.	Specificity	Should not interfere with the placebo	
2.	Linearity (r ²)	0.999	0.998
3.	Precision (%RSD)	0.8	2.18
4.	Accuracy (% found)	100.032	100.09
5.	Robustness (%RSD) Same Mobile Phase Different Mobile Phase	0.18 0.39	0.40 1.5

Method validation

System suitability

Three replicates of reference standard of Metformin and saxagliptin were injected. Peak report and column performance report were recorded for all chromatogram.

Precision

Sample solutions were checked for repeatability and intermediate precision

Repeatability

Sample solution were prepared as per test method and injected six times. Intermediate Precision: Sample solution was prepared as per test method and study was conducted by two analysts as per test method.

Accuracy

Drug assay was performed in triplicate as per test method in each volumetric flask for each spike level to get the concentration of drugs equivalent to 50%, 75%, 100%, 125% and 150% of the labelled amount as per the test method. The average % recovery was calculated.

Ruggedness

Mobile phase variability study was conducted on different mobile phase. Three samples were prepared and each was analysed as per test method.

RESULTS AND DISCUSSION

The typical chromatogram obtained from the formulation is presented in Figure 1. The retention time for metformin and saxagliptin was found to be 4.657 and 6.200 minutes respectively. Peaks were well resolved with resolution of 6.986 between the two drugs and were symmetrical in shape with asymmetry factor less than 2.00.

System suitability

The % RSD for the retention times and peak area responses of principal peak from five replicate injections were found to be less than 2%. Table-1.

Precision

The mean of the individual assays of Metformin was 101.76% and saxagliptin was 99.23% which lied in the limits (98%-102%). The % RSD was found to be not more than 2%.

Accuracy

The Mean % recovery of Metformin was 100.9 % and saxagliptin was 100.03% which lied between 98%-102%.

Ruggedness

The Mean % Assay of Metformin was 100.22 % and Saxagliptin was 100.13% which lied between 98%-102%. The % RSD was not more than 2%. The method validation Parameters are given in Table- 2.

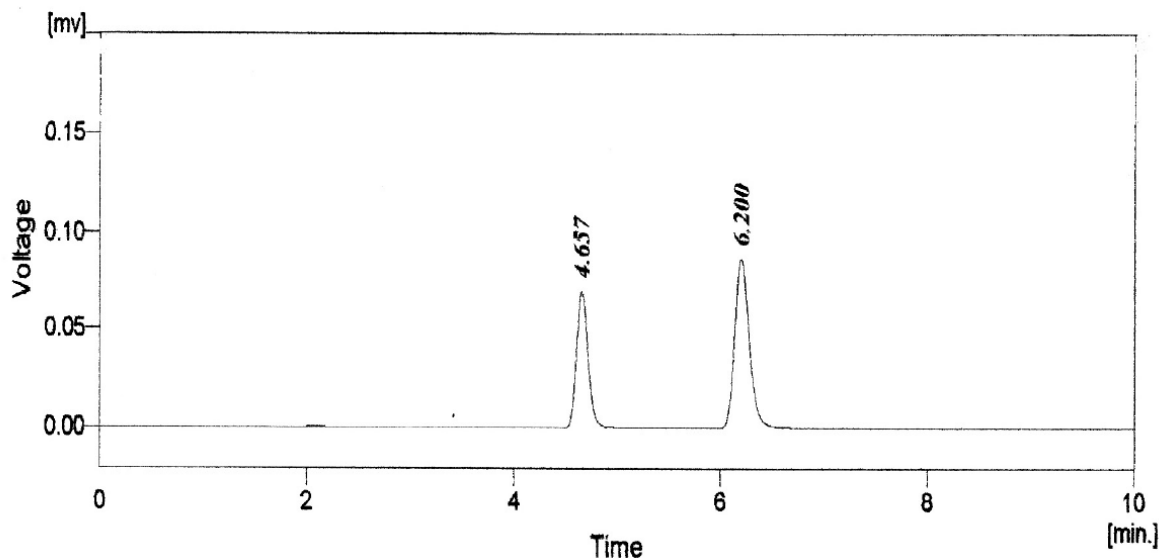


Fig.-1: Chromatogram showing the peaks for metformine and saxagliptin

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of metformin and saxagliptin from tablets. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims. Hence, it can be easily and conveniently adopted for routine analysis of metformin and saxagliptin in tablets.

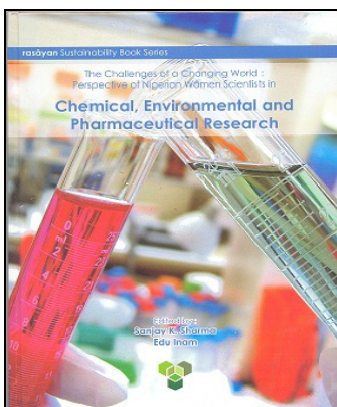
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