International Journal of Advanced Research in Engineering Technology & Science
ISSN: 2349-2819
Email: editor@ijarets.org Volume-10 Issue-1 January-2023 www.ijarets.org

METFORMIN AND SAXAGLIPTIN SIMULTANEOUS ESTIMATION IN TABLETS USING A VALIDATED RP-HPLC METHOD

Pramod Bhaskar Rao Kharwade,

Research Scholar, School of Pharmacy,

Glocal University Saharanpur (U.P) Glocal University Saharanpur (U.P)

Prof. (Dr.) H. S. Lamba ,

Research Supervisor, School of Pharmacy,

Glocal University Saharanpur (U.P) Glocal University Saharanpur (U.P)

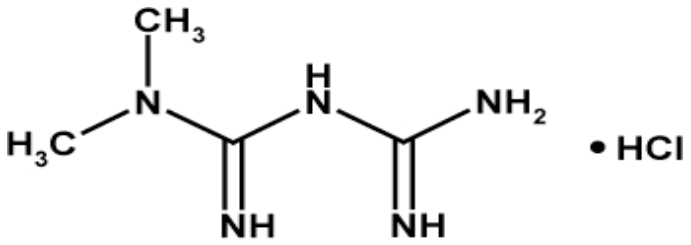
ABSTRACT

This RP-HPLC approach is straightforward, affordable, and sensitive for simultaneously estimating metformin and saxagliptin in tablets. The procedure was performed using a C18 column (5 m, 25 cm x 4.6 mm, i.d.) and a mobile phase consisting of phosphate buffer (pH 5.0), acetonitrile, and methanol in the proportions of 75:15:10, respectively. It was determined that the 225 nm wavelength for metformin and saxagliptin was suitable. Saxagliptin and metformin had retention times of 5.65 and 6.20 minutes, respectively. The developed method, which can be used to estimate the combination of metformin and saxagliptin in pharmaceutical dose forms, is proven to be both quick and sensitive.

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

INTRODUCTION

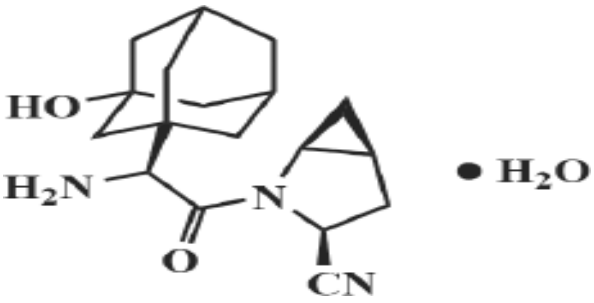
Metformin hydrochloride is a white to off-white crystalline compound with a molecular formula of C4H11N5•HCl and a



Metformin Hydrochloride

molecular weight of 165.63. Metformin hydrochloride is freely soluble in water andis 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 inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state[1]. 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-l-yl)acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile, monohydrate[3,7] .The structural formula is:



Saxagliptin

Saxagliptin monohydrate is a white to light yellow or light brown, non-hygroscopic, crystalline powder. Itis sparingly soluble in water at 24°C ± 3°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 drugs[2], 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 alone[4].

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 standardsof metformin and saxagliptin were obtained from Bizten Impex, India.

Instrumentation

Quantitative HPLC was performed on a isocratic HPLC of SHIMADZU prominenceconsisting of LC – 20AT liquid pump, manual with 20µL sample injection loop and SPD20A UV-visible absorbance detector. The output – signal was monitored and integrated byShimadzu spin chrome software.

Chromatographic conditions

The process was carried out on C18 column (5µm, 25 cm x 4.6 mm, *i.d*) using the mobilephase consistingof

phosphate buffer (pH 5.0), acetonitrile and methanol in the ratio(70:15: 10 v/v) respectively at a flow rate of 1.5mL/minutes. Wavelength was adjusted to 225 nm.The mobile phase was filtered through 0.2 µ membrane filter and sonicated for 15 min.

Preparation of solutions

Standard solution of the pure drug was prepared by dissolving 500 mg of metforminhydrochlorideand 5 mg of saxagliptinhydrochloride 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 thissolution was further diluted to get appropriate concentration levels according to therequirement.Ten tablets were weighed the average weight was determined and these werepowdered. Sample solution was then prepared by dissolving the powdered tablets equivalentto 500 mg of metformine and 5 mg of saxagliptin in a 100 mL of volumetric flask. Then thedrugs were dissolved by using 25 mL water and the volume was made up to the mark withwater. 5 mL of this solution was further diluted to 25 mL with the same solvent.50µL of solution wasinjected into HPLC system to obtain chromatogram for standarddrug solution and sample solution. Concentrations of metformin and saxagliptin in theformulation were calculated by comparing AUC of thesample with that of the standard.

Assay method

With the optimized chromatographic conditions, a steady baseline was recorded, the mixedstandardsolution was injected and the chromatogram was recorded. The retention time ofmetformin and saxagliptin was found to be 4.657 and 6.200 min respectively. This procedurewas repeated for the sample solution obtained from the formulation (Table-1) and recoverystudies (Table-2).

Table-1: System suitability parameters

	Parameters	RT	AUC	No. of TheoriticalPlates	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 theplacebo	
2.	Linearity (r2)	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 validationSystem suitability

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

Precision

Sample solutions where checked for repeatability and intermediate precision

Repeatability

Sample solution were prepared as per test method and injected six times. Intermediate Precision: Samplesolution 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 preparedand each was analysed as per test method.

RESULTS AND DISCUSSION

The typical chromatogram obtained from the formulation is presented in Figure 1. Theretention time for metformin and saxagliptin was found to be 4.657 and 6.200 minutesrespectively. Peaks were wellresolved with resolution of 6.986 between the two drugs andwere 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 replicateinjections 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 inthe limits (98%-102%). The % RSD was found to be not more than 2%.

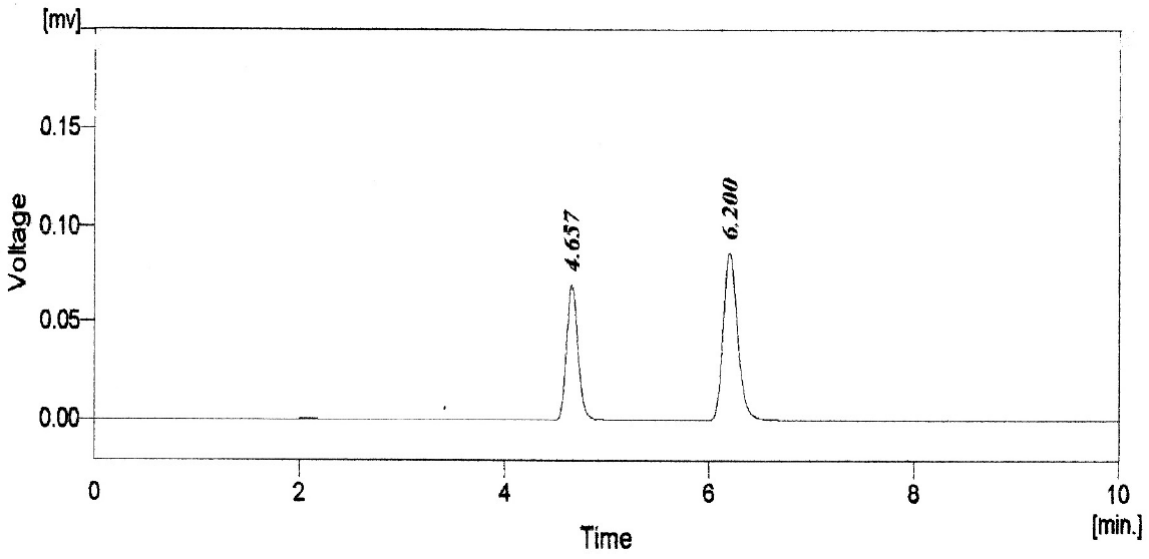
Accuracy

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.

REFERENCE

1. Marc Foretz, Sophie Hébrard, Jocelyne Leclerc, ElhamZarrinpashneh, Maud Soty, Gilles Mithieux, Kei Sakamoto, FabrizioAndreelli and Benoit Viollet, *J Clin Invest*; **120(7),** 2360,(2010).
2. Deanna S. Kania, Jasmine D. Gonzalvo, and Zachary A.Weber., *Clinical Therapeutics*,**33**, 1006,(2011).
3. N. Sultana , M.S. Arayne , N. Shafi et al. , *J. ChromatogrSci* , **49(10),** 776, (2011).
4. André J. Scheen, *Pharmacotherapy*, **13(1)** 139-146 (2012).
5. L.R. Snyder, J.J. Kirkland, and L.J. Glajch, Basics of Separation; Practical HPLC MethodDevelopment, John Wiley and Sons, Inc, New York, 2nd edn., ,5-17, (1997)
6. Validation of analytical procedures; text and methodology guidelines Q2(R1), ICH, 6-13, 2005