



Research Article

Analytical Method Development & Validation for Simultaneous Estimation of Saxagliptin & Metformin in Combined Pharmaceutical Dosage Form By RP-HPLC

A. Raja Reddy*, E. Revanth, T. Rama Rao

Department of Pharmaceutical Analysis, CMR College of Pharmacy, Hyderabad, India- 501401.

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ABSTRACT

A rapid and precise reverse-phase high-performance liquid chromatographic method has been developed for the validation of Metformin and Saxagliptin, in their pure form as well as in tablet dosage form. Chromatography was carried out on an X terra C18 (4.6 x 150mm, 5 μ m) column using a mixture of ACN, Methanol, and Water (35:50:15 v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 250nm. The method produces linear responses in the concentration range of 25-125ppm of Metformin and 10-50ppm of Saxagliptin. The method precision for the determination of assay was below 2.0 %RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

INTRODUCTION

Saxagliptin HCl (SAG), (Fig. 1a) is chemically named as (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile hydrochloride [1]. Saxagliptin HCl (SAG) is an oral hypoglycemic (anti-diabetic drug) of the dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. SAG is used as monotherapy or in combination with other medicines for the treatment of type II diabetes. The drug works to competitively inhibit a protein/enzyme, dipeptidyl

peptidase 4 (DPP-4), that results in an increased amount of active incretins; Gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), reduced amount of release of glucagon and increased release of insulin [2], [3].

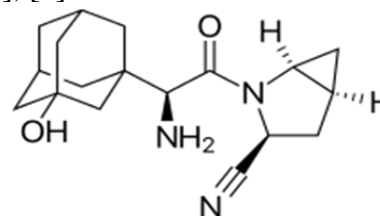



Fig. No: 1 Structure of Saxagliptin

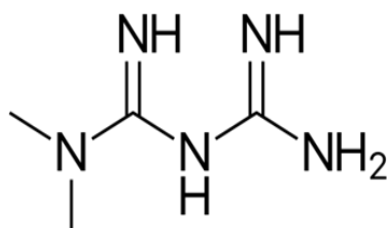
*Corresponding Author: A. Raja Reddy

Address: Department of Pharmaceutical Analysis, CMR College of Pharmacy, Hyderabad, India- 501401.

Email : rajareddyaleti050@gmail.com

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**Fig. No: 2 Structure of Metformin**

Metformin hydrochloride (MET), (Fig. 2b) is chemically designated as 1,1- Dimethyl biguanide hydrochloride [1]. *Metformin hydrochloride (MET)* is a biguanide antidiabetic. It is given orally

MATERIALS AND METHODS

in the treatment of type II diabetes mellitus; a disease characterized by defects in both insulin secretion and insulin sensitivity, and is the drug of first choice in overweight patients [2]. SAG is not an official drug in any pharmacopeia. While MET was determined by British Pharmacopoeia (BP) [4] and United States Pharmacopoeia (USP) [5], both suggest a nonaqueous titration method for the assay of MET using anhydrous formic acid as a solvent and 0.1 M perchloric acid as a titrant. The end point is determined potentiometrically.

Table 1: Drug details

S. No	Drug name	Formulation	Manufacturer	Procurement
1	Metformin	–	–	Procured from sun pharma, provided by Sura labs
2	Saxagliptin	–	–	Procured from sun pharma, provided by Sura labs

Table 2: Instruments and Equipment

S. No	Instruments	Software	Model	Company
1	HPLC	Empower 2	Alliance 2695 separation module. 996 PDA detector.	Waters
2	Weighing Balance	N/A	XEX 200	Sartorius
3	Sonicator	N/A	SE60US	Labman

Preparation of sample stock solution: Weighed 50mg of Emtricitabine, 6.25mg of tenofovir, and 12.5mg of Bictegravir working std 50ml volumetric flask, add 10ml of diluent, sonicate for 10min and make up to the final volume with diluents.(1000µg/Emtricitabine and 125 µg/ml and

Tenofovir and 250µg/ml of Bictegravir) Preparation of sample stock solution: 5 tablets of average equivalent average taken in 100ml of volumetric flask add diluents sonicate filter it (1000µg/ml Emtricitabine and 125 µg/ml Tenofovir and 250µg/ml of Bictegravir.

Table 3: Chemicals and Reagents

S. No	Chemical	Brand names
1	Water for HPLC	LICHROSOLV (MERCK)
2	Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

RESULTS AND DISCUSSION:

Method Development

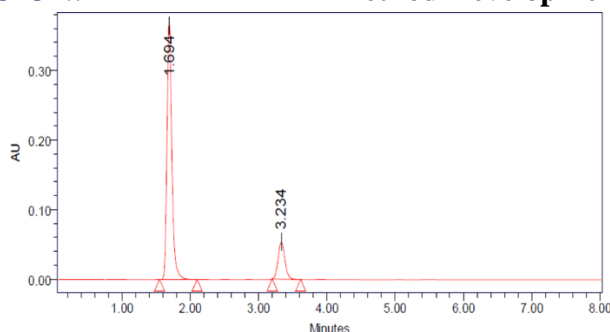


Fig 3: Optimized Chromatogram

Table 5: Observation of Optimized Chromatogram

S.No	Peak Name	Retention Time	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Saxagliptin	1.694	1429524	364752	1.23	6993	10.69
2	Metformin	3.234	300414	53626	1.12	5735	

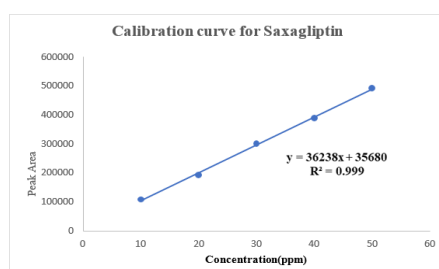


Table 8: Linearity Observation of Saxagliptin

Concentration □ g/ml	Average Peak Area
12.5	504954
25	958753
37.5	1426583
50	1845498
62.5	2272948

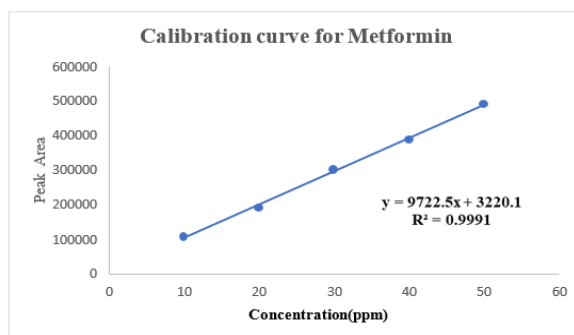


Fig No: 6 Calibration curve for Metformin

Table 9: Linearity Observation of Metformin

Concentration □ g/ml	Average Peak Area
10	107359

20	191497
30	300389
40	388105
50	490352

Optimized chromatographic conditions

Table 6: Shows Optimized chromatographic conditions

Parameter	Optimized Chromatographic Conditions
Mobile phase:	Methanol: Acetonitrile: Water (50:35:15% v/v)
Column :	X-Terra (4.6 ×150mm, 5µm particle size)
Flow rate :	1ml/min
Diluent	Methanol: Acetonitrile: Water (50:35:15% v/v)
Injection Volume	10 µl
Wavelength:	250 nm
Column temp:	35°C
Run mode	Isocratic
Runtime	8minutes

- From the above experiment it was found that Saxagliptin and Metformin can effectively be analyzed by using the RP- HPLC method with Mobile phase at a flow rate of 1 ml/min and detection wavelength of 215nm.

CONCLUSION:

The analytical method was developed by studying different parameters. First of all, the maximum absorbance was found to be at 250nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for the study was X terra C18 because it gave a good peak. 35 ° C temperatures were found to be suitable for the nature of the drug solution. The flow rate was fixed at 1.0ml/min because of the good peak area and satisfactory retention time. The mobile phase is ACN, Methanol, and Water (35:50:15 v/v) was fixed due to a good symmetrical peak. So this mobile phase was used for the proposed study. The run time was selected to be 8 min because the analysis gave a

peak. In the present investigation, a simple, sensitive, precise, and accurate RP-HPLC method was developed for the quantitative estimation of Metformin and Saxagliptin in bulk drug and pharmaceutical dosage forms. This method was simple since diluted samples are directly used without any preliminary chemical derivatization or purification steps. Metformin and Saxagliptin were freely soluble in ACN and methanol. ACN, Methanol, and Water (35:50:15 v/v) were chosen as the mobile phase. The solvent system used in this method was economical. The % RSD values were within 2 and the method was found to be precise. The results expressed in the Tables for the RP-HPLC method were promising. The RP-HPLC method is more sensitive, accurate, and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Metformin and Saxagliptin in bulk drugs and Pharmaceutical dosage forms.

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