Original Article

Simultaneous Method Development And Validation Of Metformin And Saxagliptin In Bulk And Combined Dosage Form Using Rp-Hplc

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DOI: 10.47750/pnr.2022.13.S10.802

Abstract

The current study describes the stability indicating RP-HPLC method for metformin in combined dosage form with saxagliptin. The HPLC analysis was done on a Hypersil ODS C_{18}, with phosphate buffer (pH 3 adjusted with 0.5ml TEA and dil. orthophosphoric acid) and methanol [35:65, v/v] at a flow rate of 1ml/min in a column (250mm x 4.6mm, 5µm particle size) and detector wavelength is 210 nm. Metformin and Saxagliptin were shown to have retention times of 2.846 and 3.995 minutes, respectively. Metformin and Saxagliptin were found to have linearity ranges of 125–750µg/mL and 1.25–7.5µg/mL, respectively, with correlation coefficients (R^2) greater than 0.999. The precision and accuracy of the test and recovery study results were statistically assessed in each case. The suggested approach was determined to be specific, accurate, and exact and could be used for the simultaneous quantitative measurement of metformin and saxagliptin, according to the validation results carried out in accordance with ICH recommendations.

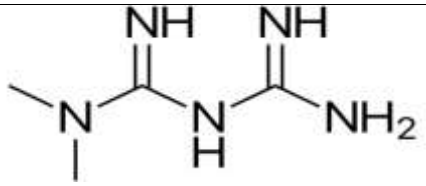
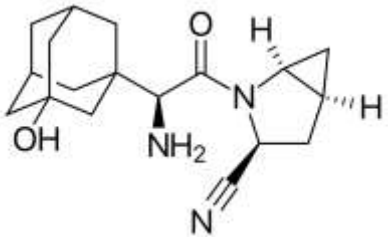
KEY WORDS: Metformin and Saxagliptin, ICH recommendations for reverse-phase high-performance liquid chromatography.

INTRODUCTION

Saxagliptin and metformin are used to treat patients with type 2 diabetes who have high blood sugar (glucose) levels in addition to diet and exercise[1]. Saxagliptin stimulates the pancreas to produce more insulin, which lowers blood sugar levels. When the blood sugar level is too high, it also tells the liver to cease making sugar. Metformin affects how well your body utilizes sugar by reducing the release of sugar from the liver's stored supply, reducing stomach absorption of sugar, and decreasing hepatic sugar release. Diabetic type II patients or diabetes that is insulin-dependent cannot benefit from this medication. According to a study of the literature, relatively few techniques for measuring Saxagliptin and metformin both alone and in combination with other drugs have been published. However, very fewer research has been done to quantify saxagliptin and metformin using HPLC [2-10], HPTLC [11, 12], and UV Spectrophotometric[14-16] methods, either alone or in conjunction with other drugs. As a result, it is necessary to make an effort to create a quick and accurate HPLC technique for the measurement of metformin and saxagliptin. In order to give a suitable level of sensitivity and selectivity in a timely manner for chromatographic run, the HPLC technique was effectively applied. Table 1 lists the names and chemical structures of Saxagliptin and metformin according to IUPAC.

Table 1. IUPAC names and structures of Metformin and Saxagliptin

Official Name	IUPAC Name	Structure

Metformin	N, N-Dimethylimidodicarbonimidic diamide	
Saxagliptin	(1S, 3S, 5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile	

MATERIALS AND METHODS

Instruments used

On a Empower 2 software and a 2998 Photo Diode Array (PDA) detector for the Waters Alliance HPLC system for processing and data collection, an RP-HPLC technique was carried out. As the stationary phase, an ODS C_{18} column (250mm x 4.6mm, 5µm) was employed. The investigation makes use of Whatman filter paper No. 41, an analytical balance (Lab India), and an ultrasonic bath sonicator.

Reagents used

Metformin and Saxagliptin were obtained from Hetero Pharma Ltd, Hyd, India, and Clintech Hyderabad, respectively. From Mumbai, India's Merck Specialties Private Limited, methanol of HPLC quality was procured. HPLC grade orthophosphoric acid, HPLC grade water, and sodium dihydrogen phosphate were procured from SD fine chemicals, India. Kombiglyze XR 5mg/500mg tablets of AstraZeneca were procured from a local chemist.

Preparation of mobile phase

Accurately weighed and transferred 1.36gr of Sodium di-hydrogen phosphate in 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then add 0.5ml TEA and pH adjusted to 3 with dil. Orthophosphoric acid solution and mixed with HPLC grade Methanol in the proportion of 35:65, v/v and it was filtered through 0.45 µm membrane filter and degassed by ultra-sonication.

Preparation of Metformin and Saxagliptin mixed standard drug stock solutions

Weighed accurately then added to a clean 100 ml volumetric flask, add 500 mg of metformin and 5 mg of saxagliptin. The drugs were sonicated after they had completely dissolved to produce a concentration of 5000 µg/mL of metformin and 50 µg/mL of saxagliptin. Pipetting 1 mL of the aforementioned solution, aliquoted, is put to a 10 mL volumetric flask before being diluted with the mobile phase to reach a concentration of 500 µg/mL of metformin and 5µg/mL of saxagliptin.

Preparation of sample solution

Each KOMBIGLYZE XR tablet includes 500 mg of Saxagliptin and 5 mg of Metformin. Twenty KOMBIGLYZE XR pills were weighed and then crushed in a mortar and pestle to produce a powder. To achieve a focus of 5000 µg/mL of Metformin and 50 µg/mL of Saxagliptin, a precisely weighed amount of powder containing 5 mg of Saxagliptin and 500 mg in a 100 ml volumetric flask that was dry and clean was filled with Metformin. Following the addition of the mobile phase, it was thoroughly dissolved using sonication before being filtered through a 0.45mm nylon membrane filter. Pipetting an aliquot of 1 mL from the aforementioned solution it was then diluted to a

concentration in a 10 µL volumetric flask using the mobile phase. 500 µg/mL of metformin and 5 µg/mL, was the first step in the process of Saxagliptin.

RESULTS AND DISCUSSION:

Assay Methodology:

From the aforementioned standard and sample solutions, 10µ liters of 500 µg/mL metformin and 5 µg/mL saxagliptin were injected into an HPLC system. The % assay was computed using the method below, and the findings are displayed in Table 2 and Figures 1 and 2.

Table 2: Reports of Assay studies

Drug	KOMBIGLYZE XR (mg)	The amount found (mg)	Label claim %
Metformin	500	499.3	99.86
Saxagliptin	5	4.98	99.6

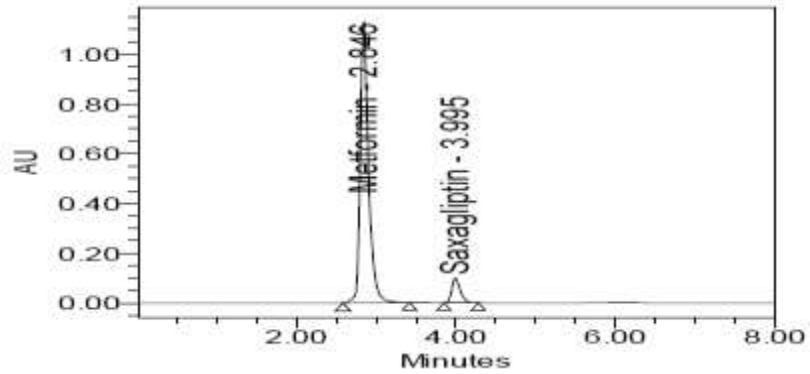


Figure 1: Chromatogram for standard Metformin and Saxagliptin

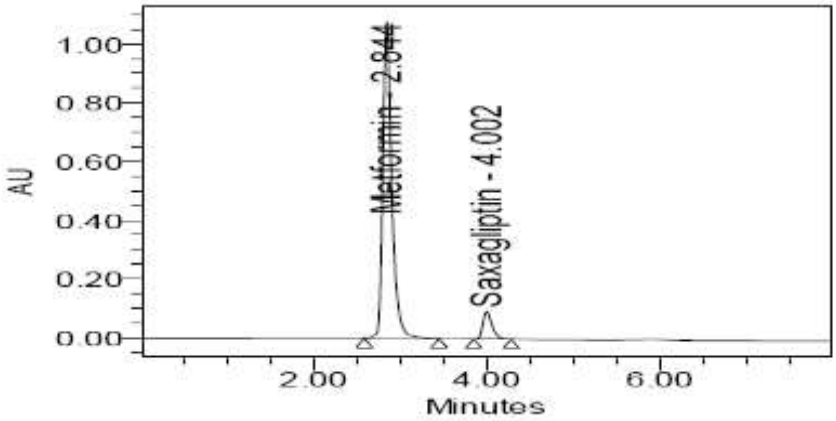


Figure 2: Chromatogram for sample Metformin and Saxagliptin

Analytical method validation[17]:

System Suitability:

It is important for assuring the performance quality of chromatographic system. So, previously prepared solutions were analyzed with respect to chromatographic conditions for system suitability studies as revealed in Table 3.

Table 3: Results of System suitability studies

Parameter	Metformin	Saxagliptin	Acceptance Limits

Theoretical plate number	3994	7159	NLT:2000
Retention time	2.846min	3.995min	-
Tailing factor	1.37	1.36	NMT:1.5
Resolution		6.13	NLT:2
LOD (µg/mL)	0.32	0.21	
LOQ (µg/mL)	0.98	0.65	

Specificity:

Excipients and other additives' impact often found in the combination dosage form of Metformin and Saxagliptin in the determination under optimal circumstances was evaluated and found to be non-interfering. The HPLC system was injected with a placebo solution to assess the sensitivity of the RP-HPLC procedure is shown in fig 3.

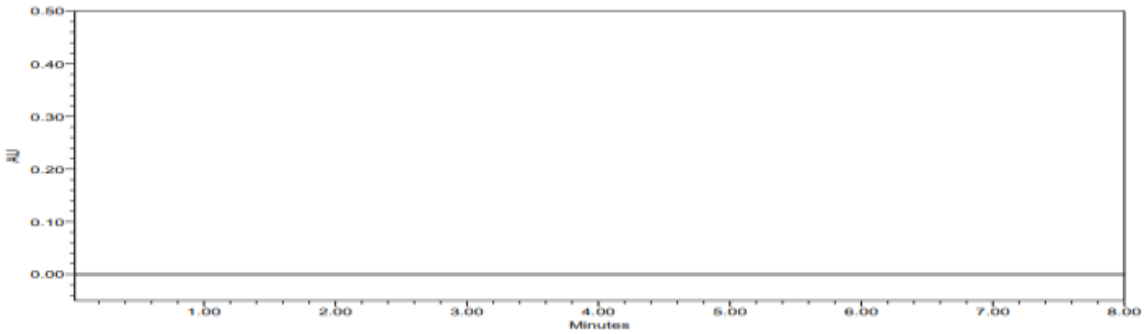


Figure 3: Chromatogram for blank

Linearity:

For linearity, Metformin concentrations of 125, 250, 375, 500, 625, and 750 µg/mL and Saxagliptin concentrations of 1.25, 2.5, 3.75, 5.0, 6.25, and 7.5µg/mL were produced. All of the aforesaid Before testing, solutions were filtered using a 0.45mm nylon membrane filter. being injected three times into the HPLC apparatus. The slope, intercept, and correlation coefficient were all analyzed using least square regression. Figure shows a calibration curve developed The regression equation was calculated by graphing peak area vs. concentration (µg/mL), as illustrated in figures 4 and 5, and the findings are displayed in Table 4.

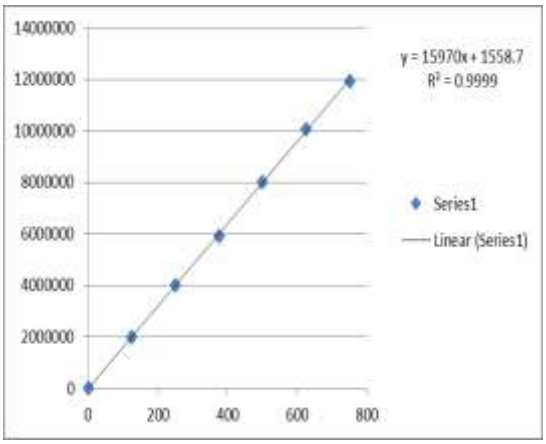


Figure 4: Linearity of Metformin

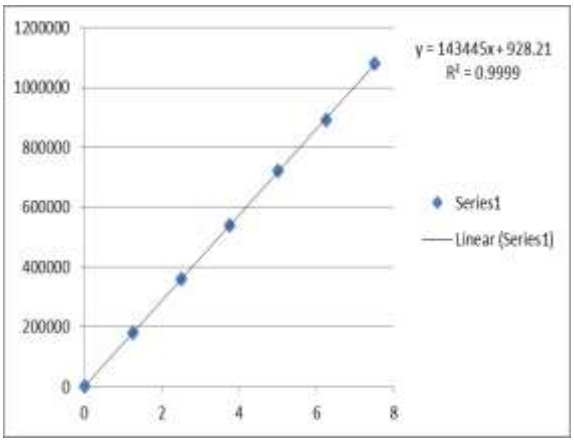


Figure 5: Linearity of Saxagliptin

Table 4: Linearity of Metformin and Saxagliptin

Concentration of Metformin (µg/mL)	Peak Area	Concentration of Saxagliptin (µg/mL)	Peak Area
125	2002848	1.25	180317

250	4014411	2.5	360457
375	5917816	3.75	540208
500	8011191	5	720427
625	10055559	6.25	890314
750	11931410	7.5	1080202

Accuracy:

By calculating the recoveries of Metformin and Saxagliptin using the customary addition approach, the Accuracy of the proposed a method was assessed. Saxagliptin and Metformin were added to the pre-analyzed sample solution at concentrations of 50%, 100%, and 150% to perform recovery studies. Tables 5 and 6 provide information on KOMBIGLYZE XR tablet powder and the precision of the proposed strategy

. **Table 5: Results of accuracy studies of Metformin**

Concentration Level in %	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery	% Mean Recovery
50%	250	250.4	100.16	99.57
	250	248	99.21	
	250	248.4	99.37	
100%	500	498.6	99.73	99.90
	500	498.8	99.76	
	500	501.05	100.21	
150%	750	747	99.6	99.80
	750	750	100.01	
	750	748.4	99.79	

Table 6: Results of accuracy studies of Saxagliptin

Concentration Level in %	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery	% Mean Recovery
50%	2.5	2.509	100.36	99.96
	2.5	2.488	99.52	
	2.5	2.5	100.00	
100%	5	5.004	100.08	99.78
	5	4.98	99.60	
	5	4.983	99.66	
150%	7.5	7.48	99.73	99.78
	7.5	7.44	99.20	
	7.5	7.53	100.40	

Precision:

A homogenous sample preparation of metformin and saxagliptin in a solution was injected into the HPLC system six times to ensure that the analytical method is operating properly. Results of the Metformin and Saxagliptin method accuracy are shown in Tables 7 and 8. In order to determine the analytical method's intermediate precision (also known as Ruggedness), six injections of 500 µg/mL of metformin and 5µg/mL of saxagliptin into the HPLC system were used to perform precision on within-laboratory variations, such as different days and different analysts. Tables 9 and 10 include the findings of the intermediate precision or roughness of Saxagliptin and Metformin.

Table 7: Method precision of Metformin

Injection No.	Name of the drug	Area
1	Metformin	8286937
2	Metformin	8269334
3	Metformin	8253883
4	Metformin	8269023
5	Metformin	8214425
6	Metformin	8221271
AVG		8252478.833
SD		28876.2
% RSD		0.3

Table 8: Method precision of Saxagliptin

Injection No.	Name of the drug	Peak Area
1	Saxagliptin	723754
2	Saxagliptin	724779
3	Saxagliptin	725253
4	Saxagliptin	724887
5	Saxagliptin	729087
6	Saxagliptin	731371
AVG		726522
SD		3002.5
% RSD		0.4

Table 9: Ruggedness of Metformin

Injection No.	Name of the drug	Peak Area
1	Metformin	8008297
2	Metformin	8102945
3	Metformin	8008503
4	Metformin	8085368
5	Metformin	8059329
6	Metformin	8128062
AVG		8065417.333
SD		49537.4
% RSD		0.6

Table 10: Ruggedness of Saxagliptin

Injection No.	Name of the drug	Peak Area
1	Saxagliptin	720637
2	Saxagliptin	730901
3	Saxagliptin	715439
4	Saxagliptin	725787
5	Saxaglipti	721034

6	Saxagliptin	730006
AVG		723967.33
SD		6004.67
% RSD		0.83

Limit of detection and Limit of quantitation

The minimum concentration of an analyte at which a reaction may be produced to produce a detectable quantity. The limit of quantitation is the lowest concentration of an analyte that produces a quantifiable reaction. Table 3 displays the outcomes of the LOD and LOQ estimates for the drugs metformin and saxagliptin.
The following formula is used to determine LOD and LOQ.

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$$\text{Limit of Detection (LOD)} = 3.3\times \frac{\text{Standard deviation of the response}}{\text{Slope of the calibration curve}}$$

$$\text{Limit of Quantitation (LOQ)} = 10\times \frac{\text{Standard deviation of the response}}{\text{Slope of the calibration curve}}$$

Robustness

Testing the method's robustness involved purposefully altering the wavelength and flow rate. The system suitability parameters show no significant fluctuations, and Tables 11 and 12 give the robustness results.

Table 11: Robustness data of Metformin

Variations in method parameters	**Retention Time (mins)**	**Average peak area**	**System suitability parameters**	
			Theoretical Plates	**Asymmetry**
Wavelength (211nm)	2.8	8100411	4636	1.44
Wavelength (209nm)	2.8	8086744	4441	1.39
0.9 mL/min Flow rate	2.8	8069949	3529	1.45
1.1 mL/min Flow rate	2.8	8067238	4321	1.4

Table 12: Robustness data of Saxagliptin

Variations in method parameters	**Retention Time (mins)**	**Average peak area**	**System suitability parameters**	
			Theoretical Plates	**Asymmetry**
Wavelength (211nm)	3.9	723517	7230	1.36
Wavelength (209nm)	3.9	723679	7454	1.35
0.9 mL/min Flow rate	3.9	725847	5412	1.40
1.1 mL/min Flow rate	3.9	723589	6524	1.49

Summary and conclusion

RP-HPLC is a contemporary method for the simultaneous analysis of quantification of Saxagliptin and Metformin in its developed and proven to use pharmaceutical and bulk dosage forms, in compliance with ICH recommendations. Metformin and saxagliptin were obtained using ODS C_{18} (250 x 4.6 mm ,5µm particle size) and a mobile phase containing a mixture of HPLC grade methanol in the ratio of 35:65, v/v, and Phophate buffer (pH adjusted to 3 with dil. Orthophosphoric acid and 0.5ml of TEA). The retention time for the metformin and saxagliptine were found to be, respectively, 2.8 and 3.9 minutes with a resolution of 6.3. The percentage recoveries for Saxagliptin and Metformin, respectively, ranged from 99.78% to 99.96% and from 99.57% to 99.90%. For both drugs, linearity was demonstrated in the 125–750 µg/mL range for metformin and 1.2–7.50 µg/mL range for saxagliptin. Metformin and saxagliptin were found to have method precision RSD% values of 0.3% and 0.4%, respectively, whereas the values for intermediate precision were discovered to be 0.6% and 0.8%, respectively. Saxagliptine's LOD level was discovered to be 0.21 µg/mL, while metformin's LOD level was found to be 0.32 µg/mL. Meanwhile, the drugs' respective LOQ values were determined to be 0.98 µg/mL and 0.65µg/mL. These studies show that the recommended approach was accurate and exact for determining Saxagliptin and Metformin simultaneously in pharmaceutical combination dose forms and bulk. The method developed is simple, precise, and accurate. Therefore, both in bulk and in pharmaceutical dose forms, Metformin and Saxagliptin may be routinely analyzed using the RP-HPLC technique.

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