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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN AND SAXAGLIFLOZIN IN BOTH BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

A novel, reliable, and reproducible RP-HPLC method has been successfully developed and validated for the simultaneous quantification of Metformin and Saxagliptin in bulk drug and pharmaceutical dosage forms. Separation was achieved on a Thermo C18 column (250 × 4.6 mm, 5 µm) under isocratic conditions, employing a mobile phase of 0.1 M KH_2PO_4 and methanol (65:35, v/v) at a flow rate of 1.0 mL/min. Detection at 256 nm yielded sharp peaks with retention times of 2.737 min for Metformin and 3.384 min for Saxagliptin. The method demonstrated excellent linearity over the concentration range of 50–150 µg/mL for both drugs, with correlation coefficients of 0.999 and 1.000, respectively. Sensitivity studies revealed LOD/LOQ values of 0.747/2.488 µg/mL for Metformin and 0.0268/0.0893 µg/mL for Saxagliptin. Accuracy was confirmed through recovery studies, with mean recoveries of 100% for both analytes. Specificity testing showed no interference from formulation excipients, underscoring the reliability of the method. Validation in line with ICH guidelines for linearity, accuracy, precision, specificity, and robustness confirms the method's suitability for routine quality control of Metformin and Saxagliptin in bulk and tablet dosage forms.

KEYWORDS: Metformin, Saxagliptin, RP-HPLC, Validation, Method development.

INTRODUCTION

Metformin (1, 1-Dimethylbiguanide hydrochloride; CAS No. 1115-70-4) has a molecular formula of C4H11N5 and a molecular weight of 165.62 g/mol (Figure 1). It is a biguanide antihyperglycemic agent and the first-line pharmacotherapy for type II diabetes mellitus. Unlike many other antidiabetic drugs, Metformin does not induce hypoglycemia, instead, it improves insulin sensitivity, reduces hepatic gluconeogenesis, and lowers fasting plasma insulin levels. Additionally, Metformin is associated with modest weight loss, offering therapeutic advantages for overweight or obese patients with type II diabetes.[1-4]

Saxagliptin ((1S, 3S, 5S)-2-[(2S)-2-amino-2-(3-hydroxyadamantan-1-yl) acetyl]-2 azabicyclo [3.1.0] hexane-3-carbonitrile) has a molecular formula of C18H25N3O2 and a molecular weight of 315.41 g/mol (Figure 2). It is water-soluble and belongs to the dipeptidyl peptidase-4 (DPP-4) inhibitor class of antidiabetic drugs. Administered orally, Saxagliptin lowers blood glucose by modulating the activity of incretin hormones. These hormones enhance glucose-dependent insulin secretion from pancreatic β-cells while suppressing hepatic glucose production, thereby contributing to improved glycemic control in type II diabetes.[5]

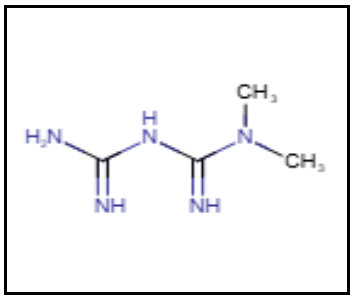

Figure 1: Structure of Metformin.

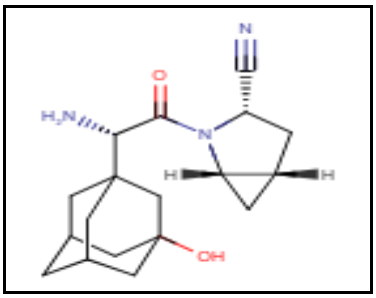

Figure 2: Structure of Saxagliptin.

Although several analytical methods have been reported for the individual estimation of Metformin and Saxagliptin, and a few approaches for their simultaneous estimation are also available, most of these methods are associated with longer retention times and less optimized chromatographic conditions.[6-16]. In contrast, the present study focused on developing and validating a simple, precise, accurate, and economical RP-HPLC method that offers shorter retention times and employs an improved mobile phase composition, thereby enhancing resolution and reducing analysis time. This optimized method, validated in accordance with ICH guidelines, ensures reliable quantification of both drugs in bulk and pharmaceutical dosage forms and represents a practical improvement over previously reported simultaneous estimation methods.

MATERIALS AND METHODS

Equipment

The analysis was performed on a Waters HPLC system (Model 2695) equipped with a photodiode array (PDA) detector and an automated sample injector. Data acquisition and integration were carried out using Empower 2 software. Chromatographic separation was achieved on a Thermo C18 column (250 × 4.6 mm, 5 µm).

Preparation of Mobile Phase: A buffer of 0.1 M KH_2PO_4 was prepared and mixed with methanol in a 65:35 (v/v) ratio, then sonicated for 20 minutes.

Preparation of Standard Solution: 500 mg Metformin and 5 mg Saxagliptin were dissolved in methanol, sonicated, diluted to 100 mL, and further diluted to obtain the working standard.

Preparation of Sample Solution: Tablet powder equivalent to 1176 mg of active ingredients was extracted with methanol, sonicated, diluted to 100 mL, and filtered through a 0.45 µm membrane before HPLC analysis.

Method development trials

Table 1: Method development Trails.

Column	Mobile phase	Flow rate	Observation	Result
WATERS, C18, 25cmx4.6mm, 5µm	OPA: Methanol (50:50)	1.0 ml/Min	Second peak not detected; peak tailing observed	Method rejected
INTERSIL, C8, 25cmx4.6mm, 5µm	KH_2PO_4: Methanol (50:50)	1.0 ml/Min	Two peaks eluted, but poor peak shape	Method rejected
INTERSIL, C8, 25cmx4.6mm, 5µm	OPA: Methanol (50:50)	1.0 ml/Min	Two peaks eluted, but the peak shape is not good	Method rejected
THERMO, C18, 25cmx4.6mm, 5µm	KH_2PO_4: Methanol (60:40)	1.0 ml/Min	Two peaks eluted, but with poor peak shape	Method rejected
THERMO, C18, 25cmx4.6mm, 5µm	KH_2PO_4: Methanol (70:30)	1.0 ml/Min	Two peaks eluted, but the peak shape is not good	Method rejected
THERMO, C18, 25cmx4.6mm, 5µm	KH_2PO_4: Methanol (65:35)	1.0 ml/Min	Two peaks eluted with acceptable peak shape; all system suitability parameters within limits	Method Accepted

Method Validation

As per ICH Q2 (R1) guidelines, the developed RP-HPLC method for the simultaneous estimation of Metformin and Saxagliptin was validated by evaluating the following parameters: specificity, linearity, accuracy, precision, LOD, LOQ, robustness, system suitability, and assay.

Specificity: The method specificity was established by injecting blank, placebo, standard, and sample solutions. Chromatograms were examined to confirm the absence of interfering peaks at the retention times of Metformin and Saxagliptin, ensuring that the analyte responses were not affected by excipients.

Linearity: Linearity was studied by preparing a series of dilutions from the standard stock solutions of Metformin and Saxagliptin. Six concentrations, covering the range of 50% to 150% of the target concentration, were prepared and injected. Calibration curves were constructed by plotting peak areas against concentrations, and correlation coefficients (r^2) were determined.

Accuracy: Accuracy was assessed by recovery studies using the standard addition method. Sample solutions were spiked at three levels 50%, 100%, and 150% of the nominal concentration by adding known amounts of standard stock solutions of Metformin and Saxagliptin. Each level was analyzed in triplicate, and percentage recoveries were calculated.

Precision: Precision was evaluated at two levels: repeatability (intra-day) and intermediate precision (inter-day). Six replicate injections of standard solutions were performed, and % RSD of peak areas and assay values were calculated to demonstrate the reproducibility of the method.

LOD & LOQ: LOD and LOQ were determined based on the standard deviation of response (σ) and slope (S) of the calibration curve, using the formulae LOD = 3.3σ/S and LOQ = 10σ/S. Solutions were prepared at calculated concentrations and analyzed to confirm method sensitivity.

Robustness: Method robustness was verified by introducing deliberate changes in chromatographic conditions, such as variation in flow rate (±0.1 mL/min), and column temperature (±5 °C). Standard solutions were analyzed under these varied conditions, and system suitability parameters were compared with those under optimized conditions.

System Suitability: System suitability was assessed by six replicate injections of standard solutions of Metformin and Saxagliptin. Parameters such as retention time, theoretical plate count, tailing factor, and resolution were evaluated against acceptance criteria.

Assay: The assay of marketed formulations containing Metformin (500 mg) and Saxagliptin (5 mg) was performed by preparing sample solutions equivalent to the labeled claim and injecting them into the HPLC system. The content of each drug was determined by comparing sample peak areas with those of the standard.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

To develop and establish a suitable RP-HPLC method for the estimation of Metformin and Saxagliptin in bulk and tablet dosage forms, preliminary trials were performed using different chromatographic conditions. After several modifications, the optimized conditions were finalized as follows: mobile phase of 0.1 M KH_2PO_4: Methanol (65:35, v/v) at a flow rate of 1.0 mL/min, Thermo C18 column (250 × 4.6 mm, 5 µm), column temperature maintained at 25 °C, injection volume of 10 µL, and detection wavelength at 256 nm. Under these conditions, sharp, well-resolved peaks were obtained with retention times of 2.737 min for Metformin and 3.384 min for Saxagliptin, as shown in the optimized chromatogram (Figure 3). The method was optimized to achieve good resolution, minimal tailing, andacceptable system suitability parameters.

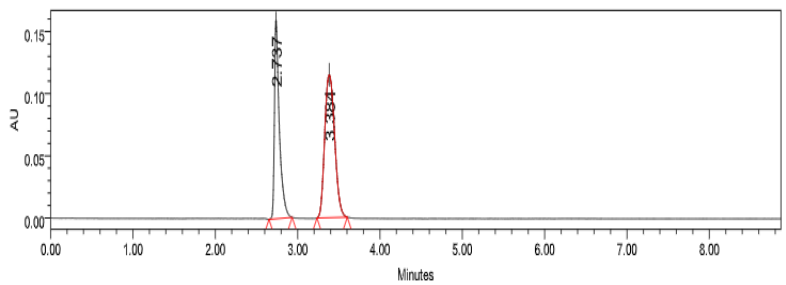

Figure 3: Typical Chromatogram of Optimized Method.

Method Validation

Linearity: Linearity was established for Metformin in the range of 50-150 µg/mL and for Saxagliptin in the same range. Six different concentrations were prepared and injected in duplicate. The calibration plots of peak area versus concentration showed good linearity with correlation coefficients of 0.999 for Metformin and 1.000 for Saxagliptin (Table 2, Figures 4 & 5).

Table 2: Linearity data for Metformin and Saxagliptin.

S. No	Conc (µg/ml)	RT	
		MET	SAX
1.	50	2.798	3.440
2.	75	2.797	3.442
3.	100	2.796	3.440
4.	125	2.795	3.440
5.	150	2.797	3.442
Correlation coefficient (r^2)	MET: 0.999 SAX: 1.00		

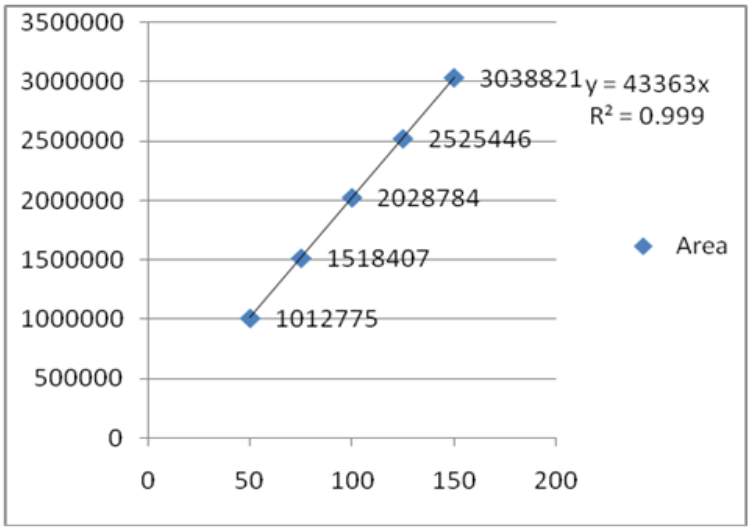

Figure 4: Linearity plot of Metformin.

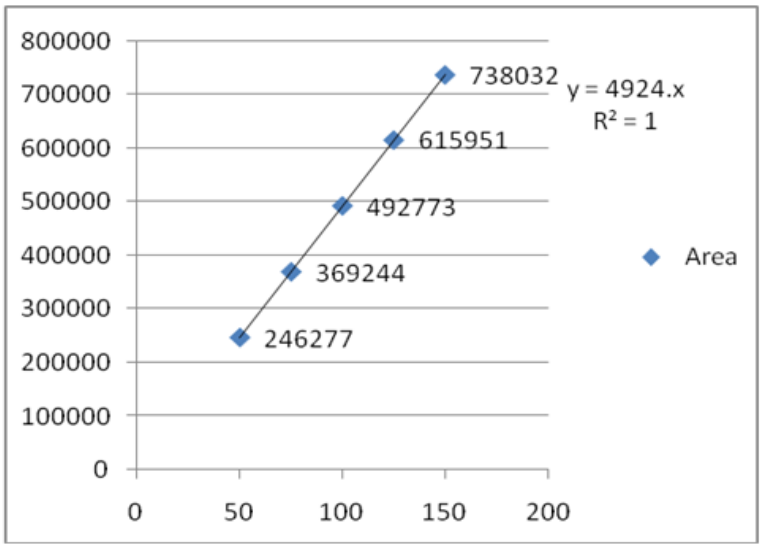

Figure 5: Linearity plot of Saxagliptin.

Specificity: Specificity was confirmed by injecting blank, placebo, standard, and sample solutions. No interfering peaks were observed at the retention times of Metformin (2.737 min) and Saxagliptin (3.384 min), demonstrating that the method is specific (Table 3 & Figures 6,7).

Table 3: Specificity for Metformin and Saxagliptin.

S No	Sample name	Metformin area	Rt	Saxagliptin Area	Rt
1	Standard	2053748	2.787	499528	3.436
2	Sample	2026822	2.793	492099	3.442
3	Blank	-	-	-	-
4	Placebo	-	-	-	-

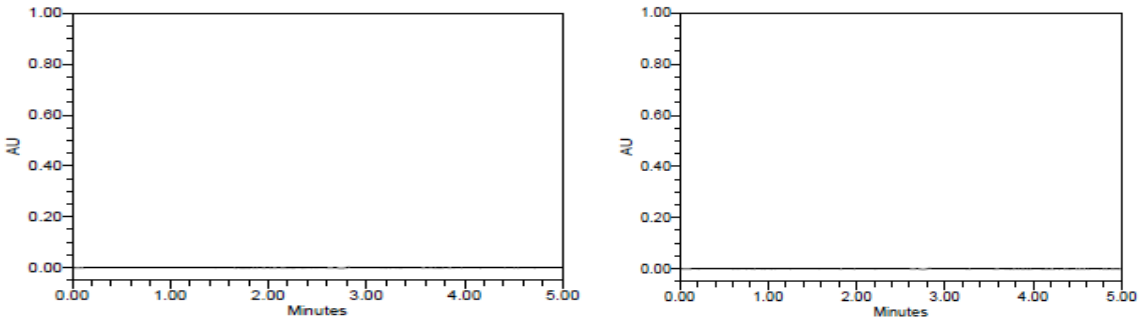

Figure 6: Typical Chromatogram of the blank and Placebo.

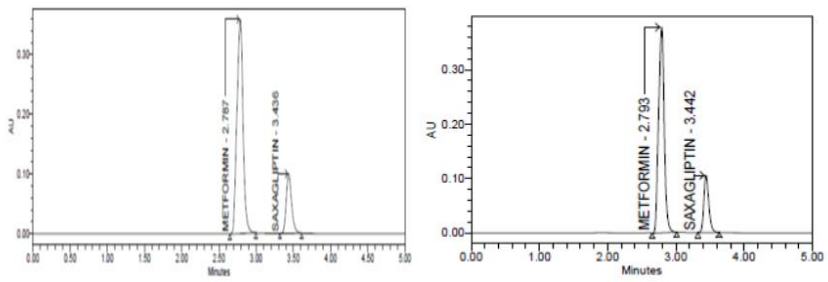

Figure 7: Chromatogram representing the specificity of the standard and the Sample.

Accuracy: Accuracy was determined through recovery studies at three concentration levels (50%, 100%, and 150%). Triplicate injections were given at each level, and the mean percentage recoveries were found to be 100.0% for both Metformin and Saxagliptin, indicating excellent accuracy (Tables 4 & 5, Figure 8).

Table 4: Accuracy (%recovery) results of Metformin.

S.NO	Accuracy level	Sample name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	587.00	250.000	251.08	100	100
		2	587.00	250.000	250.21	100	
		3	587.00	250.000	248.97	100	
2	100%	1	1175.60	500.000	499.75	100	100
		2	1175.60	500.000	498.49	100	
		3	1175.60	500.000	498.57	100	
3	150%	1	1763.40	750.000	748.31	100	100
		2	1763.40	750.000	747.98	100	
		3	1763.40	750.000	748.79	100	

Table 5: Accuracy (%recovery) results of Saxagliptin.

S.NO	Accuracy level	Sample name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	587.80	2.500	2.50	100	100
		2	587.80	2.500	2.50	100	
		3	587.80	2.500	2.50	100	
2	100%	1	1175.60	5.000	4.98	100	100
		2	1175.60	5.000	4.99	100	
		3	1175.60	5.000	4.99	100	
3	150%	1	1763.40	7.500	7.47	100	100
		2	1763.40	7.500	7.47	100	
		3	1763.40	7.500	7.47	100	

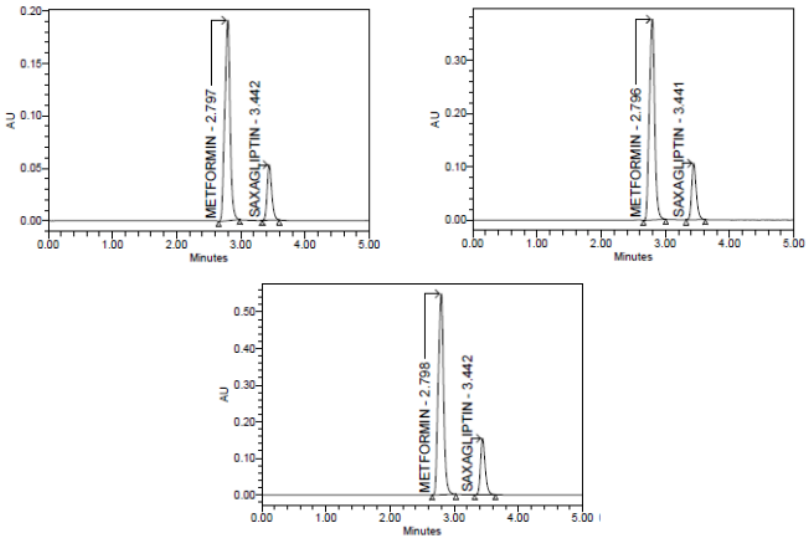

Figure 8: Typical chromatograms for Accuracy of 50 %, 100% & 150%.

Precision: Precision was assessed by repeatability and intermediate precision. Six replicate injections of standard solutions were performed, and the % RSD of peak areas was calculated. The results showed low %RSD values, confirming that the method is precise (Tables 6 & 7, Figure 9).

Table 6: Precision data for Metformin.

S. No.	RT	Area	%Assay
Injection1	2.793	2026822	100
Injection2	2.791	2021083	100
Injection3	2.794	2027836	100
Injection4	2.795	2027706	100
Injection5	2.797	2020325	100
Injection6	2.787	2025153	100

Table 7: Precision data for Saxagliptin.

S.no	RT	Area	%Assay
injection1	3.422	492099	100
injection 2	3.439	492476	100
injection 3	3.440	492067	100
injection 4	3.441	492012	100
injection 5	3.443	492917	100
injection 6	3.433	492309	100

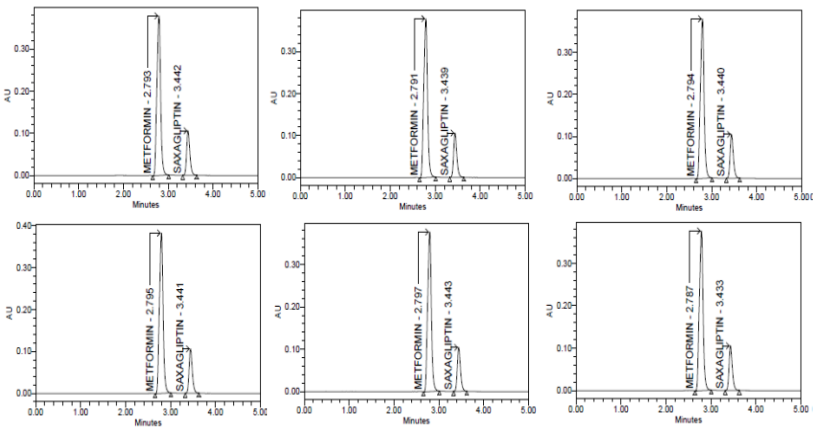

Figure 9: Chromatograms for precision.

LOD and LOQ: The limits of detection and quantification were calculated based on the standard deviation of response and slope of the calibration curve. The LOD was found to be 0.747 µg/mL for Metformin and 0.0268 µg/mL for Saxagliptin, while the LOQ was determined as 2.488 µg/mL and 0.0893 µg/mL, respectively (Figures 10 & 11).

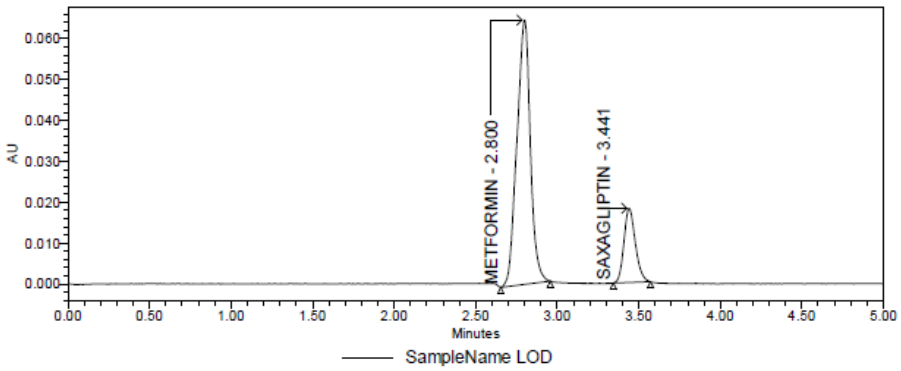

Figure 10: Chromatogram for LOD.

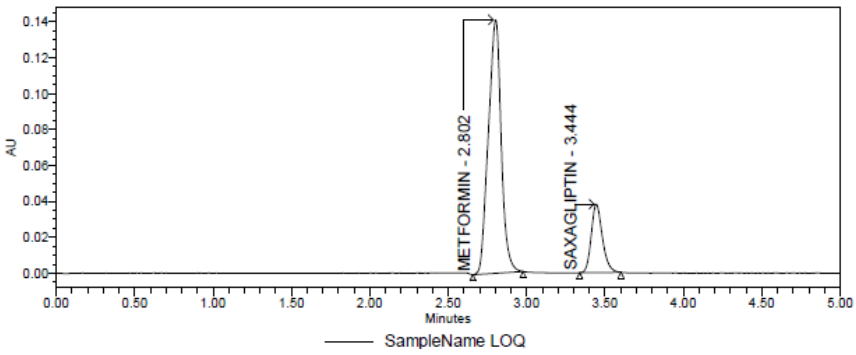

Figure 11: Chromatogram for LOQ.

Robustness: Robustness was studied by making deliberate variations in flow rate (0.8–1.2 mL/min) and column temperature (20–30 °C). No significant changes were observed in retention times, theoretical plates, or tailing factors, proving that the method is robust (Table 8, Figures 12 & 13).

Table 8: Robustness data for Metformin and Saxagliptin.

Parameter	RT	
	MET	SAX
Decreased flow rate(0.8ml/min)	2.321	2.860
Increased flow rate(1.2ml/min)	3.508	4.285
Decreased temperature(20^0c)	2.323	2.864
Increased temperature(30^0c)	3.505	4.276

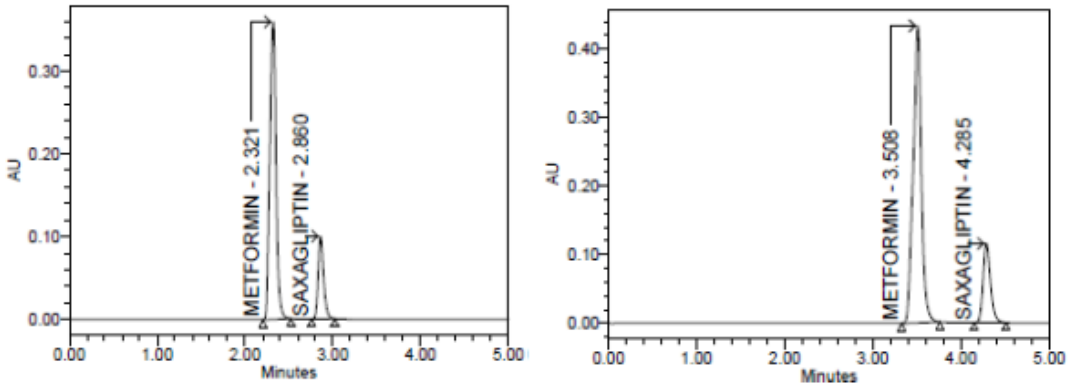

Figure 12: Chromatograms for increased and decreased flow rate.

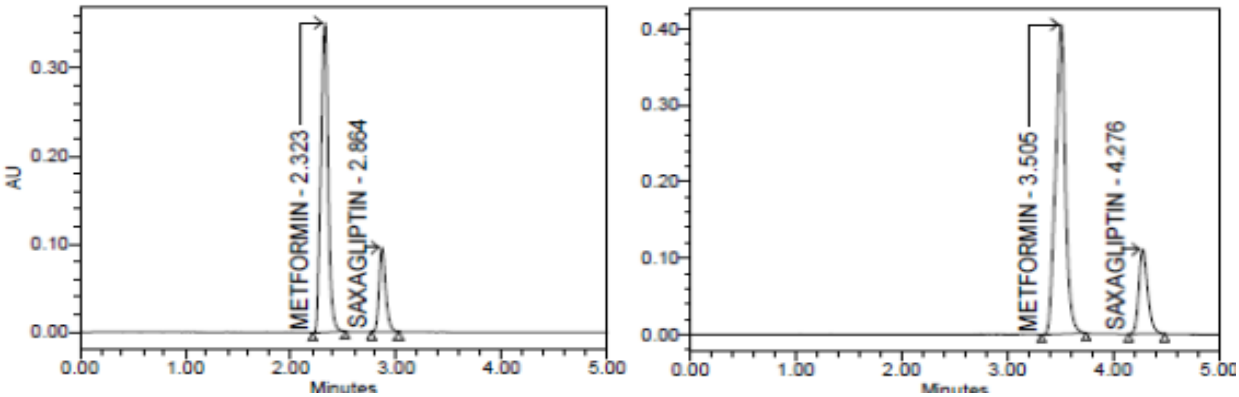

Figure 13: Chromatograms for increased and decreased temperature.

System Suitability: System suitability parameters were determined by six replicate injections of standard solutions. Retention times, theoretical plate counts, and tailing factors were within acceptable limits, confirming system suitability (Table 9, Figure 14).

Table 9: System suitability parameters.

Parameter	Metformin	Saxagliptin	Acceptance criteria
Retention time	2.787	3.436	+-10
Theoretical plates	5071	11220	>2500
Tailing factor	1.02	1.23	<2.00
% RSD	0.2	0.4	<2.00

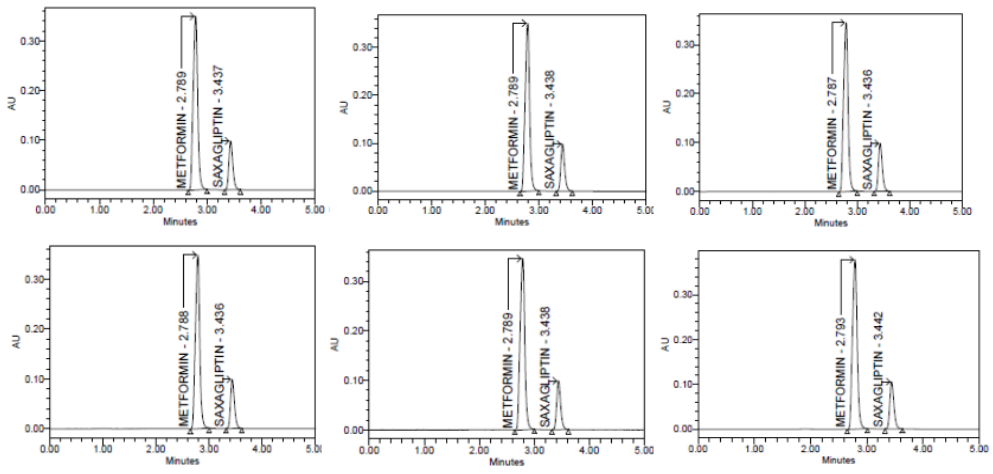

Figure 14: System suitability chromatography of Metformin and Saxagliptin.

CONCLUSION

In this study, a novel, simple, precise, and economical RP-HPLC method was successfully developed and validated for the simultaneous estimation of Metformin and Saxagliptin in bulk and pharmaceutical dosage forms. The method fulfilled all validation parameters as per ICH Q2(R1) guidelines, demonstrating excellent linearity, accuracy, precision, specificity, sensitivity, and robustness. Short retention times and good peak resolution ensured rapid and efficient analysis, making the method suitable for routine quality control of formulations. Compared to existing analytical approaches, this method provides a reliable, cost-effective, and practical alternative for the accurate quantification of Metformin and Saxagliptin in combined dosage forms.

Abbreviations

KH_2PO_4 – Potassium dihydrogen phosphate

OPA – Ortho Phosphoric Acid

MET- Metformin

SAX – Saxagliptin

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