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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METFORMIN AND SAXAGLIPTIN

Mahnoor Fatima * and S. Roshan

Azad College of Pharmacy, Moinabad, Hyderabad - 500075, Telangana, India.

Keywords:

RP-HPLC, Metformin, Saxagliptin, Antidiabeticdrug, Percentage purity

Correspondence to Author:
Mahnoor Fatima

Assistant Professor,
Azad College of Pharmacy,
Moinabad, Hyderabad - 500075,
Telangana, India.

E-mail: mahnoor.fatima17@gmail.com

ABSTRACT: The present study aims to develop a verse phase HPLC method for the simultaneous estimation of Metformin and Saxagliptin. In bulk and pharmaceutical dosage form and to validate the proposed method in accordance with ICH guidelines for the intended analytical application. Multiple trials were run to Metformin and Saxagliptin, get eluted with good peak symmetric properties. Mobile phase Phosphate buffer: Methanol: Acetonitrile (40:5:55), Column Hypersil silica and flow rate 1.0ml, detection wavelength at 233nm, column temperature 300C and diluents Water: Methanol (50:50). Column Hypersil silica and flow rate 1.0 ml, detection wavelength at 210 nm conditions were finalized as an optimized method. This method was validated for Stability Data, System suitability, Precision, Linearity, Accuracy, Robustness, Ruggedness, LOD, and LOQ. System suitability parameters were studied by injecting the standard five times, and results were well under the acceptance criteria. Linearity study was carried out between 25% to 150% levels, r^2 value found 0.999. By using the above method, assay of the marketed formulation was carried out 99.63% was present. So, this method can be used to estimate Metformin and Saxagliptin in Pharmaceutical dosage form for routine analysis purposes.

INTRODUCTION: Metformin is an oral antidiabetic drug and is a chemical, 1, 1-dimethyl biguanide hydrochloride [27.] It has high efficacy, safety profile, beneficial cardiovascular and metabolic effects and therapeutic benefit associated with other antidiabetic drugs. Hence, this drug is included in first-line therapy to treat patients with type 2 diabetes mellitus [28]. The main action of Metformin is to decrease fasting plasma glucose levels and is achieved by suppressing excessive hepatic glucose production and improving glucose clearance.

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It is the principal component in combination therapies intended for diabetes and is frequently used in high doses of about 500 to 850 mg [27]. Saxagliptin is also an oral antidiabetic drug and is belongs to new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs [29]. Chemical name is (1S, 3S, 5S) – 2 - [(2S) – 2 – amino – 2 - (3 - hydroxy-1-adamantyl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile [30]. It is used for the treatment of type II diabetes in combination with Metformin, a sulphonylurea [29].

HPLC [9, 13] is a modern technique; it is a much more reliable and reproducible method for standardizing both single and compound formulation. HPLC is a separation technique based on a stationary phase and a liquid mobile phase. Separations are achieved by partition, adsorption, or ion exchange process, depending upon the size of the stationary phase used. Reversed-phase HPLC (RP-HPLC or RPC)

has non-polar stationary and aqueous, moderately polar mobile phases. One common stationary phase is silica treated with RMe_2SiCl, where R is a straight-chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With these stationary phases, retention time is longer for more non-polar molecules, while polar molecules elute more readily. RPC [19] operates on the principle of hydrophobic forces, which originate from the high symmetry in the dipolar water structure and play the most important role in all processes in life science. RPC allows the measurement of these interactive forces. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent [1].

Literature Review: R. Pravin Kumar *et al.,* have developed a simple, economical, 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 rapid and sensitive, which can be used to estimate a combination of Metformin and saxagliptin in pharmaceutical dosage forms.

N. V. M. S. Bhagavanji *et al.,* developed an isocratic, reversed phase-liquid-chromatographic method was developed for the quantitative determination of Metformin and Saxagliptin in combined-dosage form. A thermo hypersil BDS C8 (250*4.6*5µ) column with mobile phase containing water pH 3.0 adjusted with orthophosphoric acid: methanol was used in the (70: 30, v/v) ratio. The flow rate was 1.0 mL/min, the column temperature was 30°C, and effluents were monitored at 241 nm. The retention times of Metformin and Saxagliptin were 4.7min and 6.8 min, respectively. The correlation coefficient for Metformin and Saxagliptin was found to be 1 and 0.999, respectively. The proposed method was validated concerning linearity, accuracy, precision, specificity, and robustness. Recovery of Metformin and Saxagliptin in formulations was found to be in the range of 98-103% and 99-103%, respectively confirming the non-interferences of the excipients in the formulation due to its simplicity, rapidness and high precision. The method was successfully applied to the estimation of Metformin and Saxagliptin in the combined dosage form.

Objective: In the present study, the combination of Metformin and Saxagliptin was selected. The extensive literature survey revealed that there are very few methods reported for the simultaneous estimation of these drugs in other combinations. Hence an attempt was made to develop a specific, precise, accurate, linear, simple, rapid, validated, and cost-effective HPLC method for the simultaneous estimation of Metformin and Saxagliptin in combined dosage forms.

MATERIALS AND METHODS: A simple reverse phase HPLC method was developed to determine Metformin [22] and Saxagliptin present in pharmaceutical dosage forms of 500 mg and 5mg. Column [9] used Hypersil Silica (250 x 4.6 mm, packed with 5µm) in an isocratic mode with mobile phase Buffer: Acetonitrile (55:45) was used. The flow rate was 1.0ml/ min, and effluent was monitored at 210.

Instrument:

HPLC System: Water alliance 2695 separation modules with PDA detector [22] connected to empower software.

Method Development: Based on drug solubility [11] and Pka Value following conditions has been used to develop the method for simultaneous estimation of Metformin and Saxagliptin [16]

Optimized Method:

Buffer: Accurately weighed 1.36gm of potassium dihydrogen phosphate in 1000ml of Volumetric flask (0.01M) add about 900ml of milli-Q water and sonicate to dissolve then makeup to 1000ml add 0.5ml of Triethylamine.PH 6.2 with dil Orthophosphoric acid solution.

Mobile Phase:

Buffer and methanol: water (40:5:55).

Chromatographic Conditions:

Flow rate: 1 ml/min

Column: Hypersil Silica, 250 x 4.6 mm, 5µ.

Detector wave length: 210nm

Column temperature: 30°C

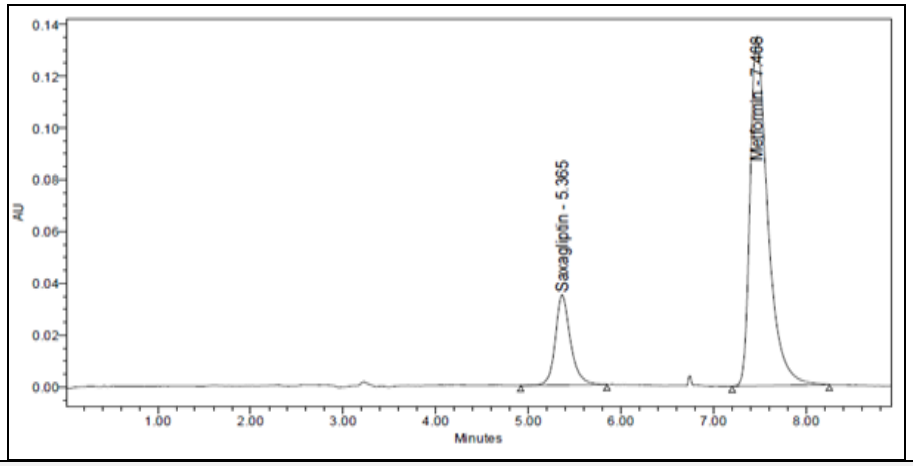
Injection volume: 10µL

Run time: 10 min

Diluent: Water: Methanol (50:50)

Standard Preparation: Accurately Weighed and transferred 50 mg of Metformin and 10mg of Saxagliptin working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 5 min and makeup to the final volume with diluents.

Sample Preparation: 5 tablets were weighed, and calculate the average weight of each tablet was; then the weight equivalent to 20 tablets was transferred into a 250 mL volumetric flask, 150mL of diluent added and sonicated for 25 min; further, the volume was made up with diluent and filtered. From the filtered solution, 1ml was pipetted out into a 10 ml volumetric flask and made upto 10ml with diluents [13].



S. no.	Peak name	RT	Area	%Area	UPS plate count	UPS tailing
1	Saxagliptin	5.365	397138	18.20	5537	1.18
2	Metformin	7.468	1785303	81.80	7946	1.61

FIG. 1: CHROMATOGRAM ASSAY

Assay Method: Assay of the marketed formulation was carried out by injecting sample [14] corresponding to equivalent weight into HPLC system [15-25]. And percent purity was found out by following formulae. Calculate the percentage purity of Metformin and Saxagliptin present in tablet using the formula: Spl area Std. Dil. Fac Avg. Wt of Tab Potency of Std

Assay = ---- X---- X ----X

Std area Spl. Dil. Fac L.C, Spl area – Sample Peak area, Std area – Standard Peak area, Std. Dil. Fac, Spl. Dil. Fac, Avg. Wt. of Tab, L.C, Potency of Std.

Selection of Solvent: Metformin and Saxagliptin are marketed as combined dosage formulations. different solvents are used for the solubility of drugs, it was found that both the drugs are freely soluble in acetonitrile [15].

Selection of Mobile Phase: The proposed method for estimation Metformin and Saxagliptin required adequate resolution of the two drug peaks in the chromatogram illustrated in **Fig. 1**. Several solvent systems were tried to obtain good optimum resolution.

Selection of Wavelength for Detection: Standard solutions of Metformin and Saxagliptin were scanned in the wavelength range of 200nm-400nm using Buffer, Methanol taken in the ratio 55:45 v/v as mobile phase.

Preparation of Buffer: Accurately weighed 1.36gm of Potassium dihydrogen orthophosphate was transferred into a 1000mlof Volumetric flask, about 900ml of milli-Q water was added and sonicate [9] to degassed and finally make up the volume with water. Finally, pH is adjusted to 6.2 with a dilute orthophosphoric acid solution.

Mobile Phase: Buffer Methanol were taken in the ratio 55:45v/v. Peaks of Metformin and Saxagliptin were well resolved with the solvent system of Buffer and Methanol in the ratio of 55:45 v/v.

Determination of Retention Time:

Standard Stock Solution of Metformin Accurately 50 mg of Metformin was weighed into a clean and dry 10 ml volumetric flask, dissolved with sufficient volume of diluent and sonicate for 5min. The volume made up to 10ml with diluent (5000µg/ml).

Standard Stock Solution of Saxagliptin: Accurately 10 mg of Saxagliptin was weighed into a clean and dry 10 ml volumetric flask, dissolved with sufficient volume of diluent and sonicate for 5min. The volume made up to 100ml with diluent (1000µg/ml).

Procedure: 2 ml of standard stock solution of Metformin (5000µg/ml) and 0.1ml of standard stock solution of saxgliptin (1000 µg/ml) are transferred in to a 10 ml volumetric flask and the volume made with diluent. The resulting solution was sonicated for 10 min and injected. The retention time, peak area and peak resolution were observed and the chromatogram is presented in **Fig. 1.**

TABLE 1: ASSAY METHOD DEVELOPMENT

Flow rate	1ml/min
Column	Hypersil Silica, 250 x 4.6 mm, 5µ.
Detector wave length	210nm
Column temperature	30°C
Injection volume	10µL
Run time	10 min
Diluent	Water: Methanol (50:50)
Mobile phase	Buffer: Methanol (55:45)

Validation of HPLC Method Developed for Simultaneous Determination of Metformin and Saxagliptinin Bulk and Tablet Dosage Form: The HPLC method developed was validated by performing the various method validation parameters like LOD, LOQ, linearity and range, precision, specificity, accuracy, robustness [17,] and system suitability parameters given in **Table 2, 3, 4, 5, 10, 11, 12.**

Precision Determination: The precision of the analytical method is determined by assaying a sufficient number of samples, and the relative standard deviation is calculated.

Method:

Preparation of Standard Stock Solution: Accurately weigh 50 mg of Metformin and 10mg of Saxagliptin and transferred into 10 ml volumetric flask and dissolved and volume was made up with diluents.

To 1 ml of the above solution is diluted to 10 ml with diluent to obtain the concentration of 1000 µg/ml of Metformin and 10µg/ml of Saxagliptin. Like six solutions were prepared individually and injected Six times into the HPLC and recorded the chromatograms.

Method Precision: Six Sample solutions were prepared individually from Metformin and Saxagliptin stock solution, as per methodology, and injected each solution into HPLC as given in **Table 2.**

Acceptance Criteria: % RSD Should not be more than 2.0%.

TABLE 2: RESULTS OF PRECISION

Sample no.	Sample (MET)	% Assay	Sample (SAX)	% Assay
1	394019	99.46	1789461	99.82
2	392734	99.14	1785736	99.77
3	391339	98.79	1782127	99.67
4	397992	100.47	1785378	100.52
5	392832	99.16	1783720	100.01
6	393064	99.22	1785882	99.89
AVG	393663	99.37	1785384	99.95
S.D	2288.2	0.57762	2464.5	0.30426
% RSD	0.581	0.58	0.138	0.30

TABLE 3: SYSTEM PRECISSION

System Precission	Metformin Areas	Saxagliptin Areas
1	393864	1789461
2	398285	1785736

3	397478	1782127
4	399012	1785378
5	389781	1783720
6	396097	1785882
AVG	395753	1785384
SD	3445.7	2464.58
%RSD	0.9	0.14

System Suitability: This parameter ensures that the analytical system is working properly and can give accurate and precise results. Solutions of Metformin and Saxagliptin were injected, and the parameter like theoretical plates per column and parameter of column was calculated from the following chromatogram given in **Fig. 4.**

TABLE 4: SYSTEM SUITABILITY PARAMETERS

Parameters	Metformin	Saxagliptin	Acceptance criteria
Theoretical plates	7715	5623	More than 2000
Tailing factor	1.23	1.65	Less than 2
Retention time	5.1	7.6	More than 2

Report: The system suitability parameters were determined for Metformin and Saxagliptin and were found to be within the acceptance criteria given in **Table 4.**

Specificity: This parameter was performed to assess and ensure that the impurities, degraded products and diluents do not affect the samples analyzed.

Metformin and Saxagliptin were injected into the system and chromatograms were recorded and presented below in **Fig. 2.**

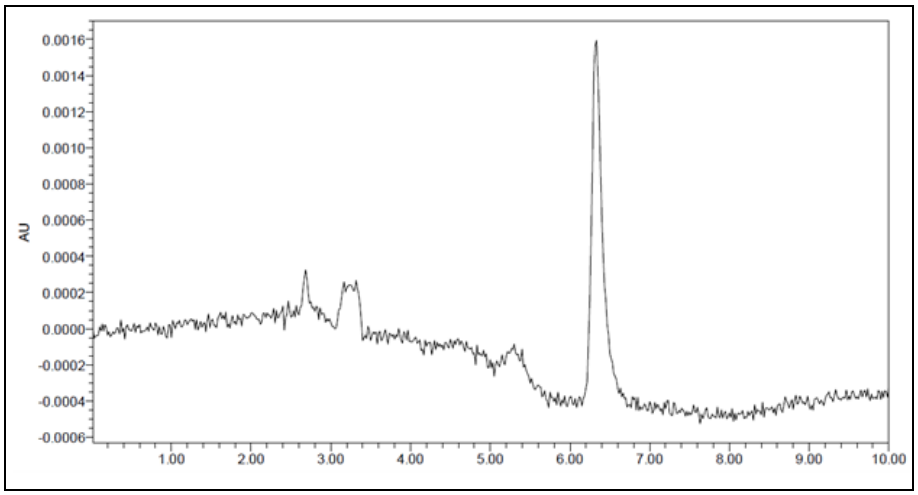


FIG. 2: CHROMATOGRAM OF DILUENTS

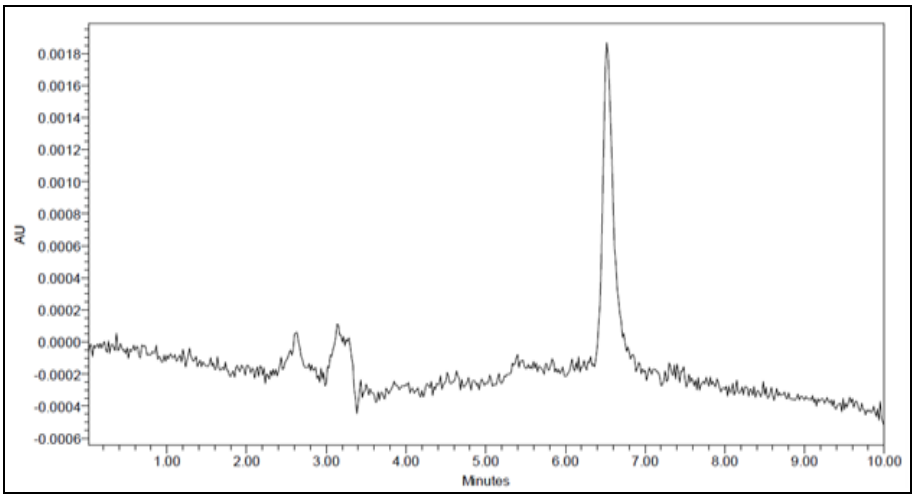
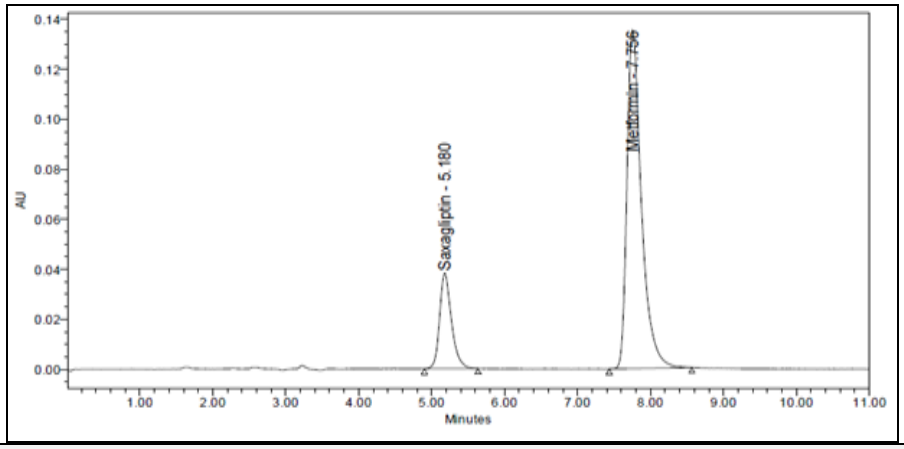


FIG. 3: CHROMATOGRAM OF PLACEBO



S. no.	Peak name	RT	Area	%Area	UPS plate count	UPS tailing
1	Saxagliptin	5.180	429400	18.69	5142	1.27
2	Metformin	7.756	1868275	81.31	7964	1.55

FIG. 4: CHROMATOGRAM OF METFORMIN AND SAXAGLIPTIN STANDARD

Report: The chromatograms of standards Metformin and Saxagliptin. Samples were fully resolved, and there is no interference from diluents, excipients, and impurities with the peaks of Metformin and Saxagliptin as shown in **Fig. 4.**

Limit of Detection: This parameter was performed by the developed HPLC method for the determination of Metformin and Saxagliptinand the chromatograms obtained are presented below.

TABLE 5: LOD REPORT OF METFORMIN AND SAXAGLIPTIN

Drug	Limit of Detection(µg/ml)
Metformin	0.48µg/ml
Saxagliptin	0.21µg/ml

Report: LOD for Metformin and Saxagliptin were found to be 0.48µg/ml and 0.21µg/ml, respectively, as shown in **Table 5.**

Limit of Quantification: LOQ parameter was performed to know the lowest amount of analyte in the sample, which can be determined and quantified with precision and accuracy.

TABLE 6: LOD REPORT OF METFORMIN AND SAXAGLIPTIN

Drug	Limit of Quantification(µg/ml)
Metformin	1.45µg/ml
Saxagliptin	0.65µg/ml

Report: The limit of quantification of Metformin and Saxagliptin were found to be 1.45µg/ml and 0.65µg/ml, respectively, as shown in **Table 6.**

Linearity: The linearity parameter was performed to ensure that the test results are directly proportional to the concentration of the analyte sample. For the linearity, 100% of each of the working standard solutions of Metformin and Saxagliptin were injected into the HPLC system.

The peak area and concentration were plotted to get a standard calibration curve. The correlation coefficient and regression coefficient were calculated. The results obtained are tabulated in **Table 7.**

Preparation of Linearity Solutions:

Preparation of 25% Solution (250ppm & 2.5ppm): from stock solution, 0.025 ml was taken in 10 ml volumetric flask, and volume was made up with diluents.

Preparation of 50% Solution (500ppm & 5ppm): from solution, 0.50 ml was taken in 10 ml volumetric flask, and volume were made up with diluent.

Preparation of 75% Solution (750ppm & 7.5ppm): from solution 0.75 ml was taken in 10 ml volumetric flask, and volume was made up with diluent.

Preparation of 100% Solution (1000ppm & 10ppm): from solution 1.0 ml was taken in 10 ml volumetric flask, and volume was made up with diluent.

Preparation of 125% Solution (1250ppm & 12.5ppm): from solution 0.125 ml was taken in 10 ml volumetric flask, and volume was made up with diluent.

Preparation of 150% Solution (1500ppm & 15ppm): from solution, 0.15 ml was taken in 10 ml volumetric flask, and volume was made up with diluent.

TABLE 7: LINEARITY SAMPLE

S. no.	Pipetted from stock (mL)	Volume of flask (mL)	Concentration in ppm(Met)	Concentration in ppm (Saxa)	%Linearity Level
1	0.025	10	250	2.5	25
2	0.5	10	500	5	50
3	0.75	10	750	7.5	75
4	1	10	1000	10	100
5	1.25	10	1250	12.5	125
6.	1.5	10	1500	15	150

TABLE 8: LINEARITY OF METFORMIN

S. no.	Concentration (µg/ml)	Area
1	250	527715
2	500	993507
3	750	1481077
4	1000	1960220
5	1250	2463740
6	1500	2962524

TABLE 9: LINEARITY OF SAXAGLIPTIN

Sl. no.	Concentration (µg/ml)	Area
1	2.5	116944
2	5	223826
3	7.5	322138
4	10	433036
5	12.5	548500
6.	15	656144

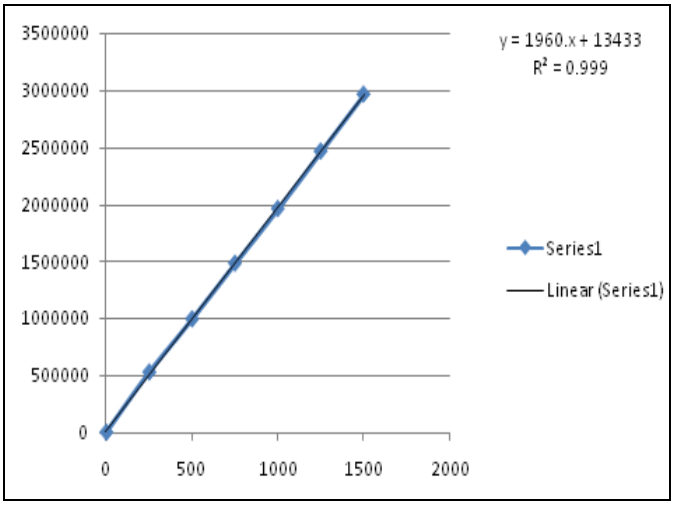


FIG. 5: LINEARITY CURVE FOR METFORMIN

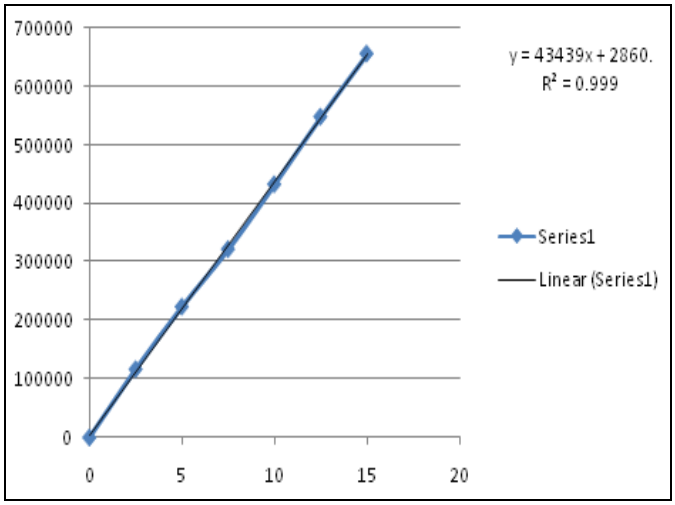


FIG. 6: LINEARITY CURVE FOR SAXAGLIPTIN

TABLE 10: CORRELATION COEFFICIENT AND % CURVE FITTING FOR METFORMIN AND SAXAGLIPTIN

Parameters	Results (n=6)	
	Metformin	Saxagliptin
Linearity range	250-1500 µg/ml	2.5-15µg/ml
Slope	1960	43439
Intercept	13433	2860
Correlation coefficient	0.999	0.999

The proposed method is found to be linear at a concentration of 250-1500 µg/ml for Metformin and 2.5-15µg/ml for Saxagliptin in **Fig. 5** and **6** and **Table 8, 9**.

The correlation coefficient and % curve fitting for Metformin and Saxagliptin were found to be 0.999, 99.9%, and 0.999, 99.9%, respectively, given in **Table 10,** which is well within the acceptance criteria limits.

Accuracy: This parameter is performed to determine the closeness of test results with that of the true value, which is expressed as % recovery as shown in **Fig. 7, 8, 9**. The difference between the theoretical added amount and the practically achieved amount is called the accuracy of the analytical method. Accuracy was determined at three different levels 50%, 100% and 150% of the target concentration in triplicate, as shown in **Table 11, 12.**

TABLE 11: RESULTS OF RECOVERY STUDIES FOR METFORMIN

Level %	No	Recovery (%)	Mean Recovery (%)	SD	RSD (%)
50%	1	100.49	100.44	0.0862	0.09
	2	100.34			
	3	100.50			
100%	1	99.99	99.97	0.0396	0.04
	2	99.93			
	3	100.00			
150%	1	99.13	99.41	0.2432	0.24
	2	99.54			
	3	99.56			

TABLE 12: RESULTS OF RECOVERY STUDIES FOR SAXAGLIPTIN

Level %	No	Recovery (%)	Mean Recovery (%)	SD	RSD (%)
50%	1	100.28	100.27	0.1207	0.12
	2	100.14			
	3	100.38			
100%	1	101.73	101.57	0.1886	0.19
	2	101.63			
	3				
		101.36			
150%	1	100.64	100.58	0.1480	0.15
	2	100.41			
	3	100.69			

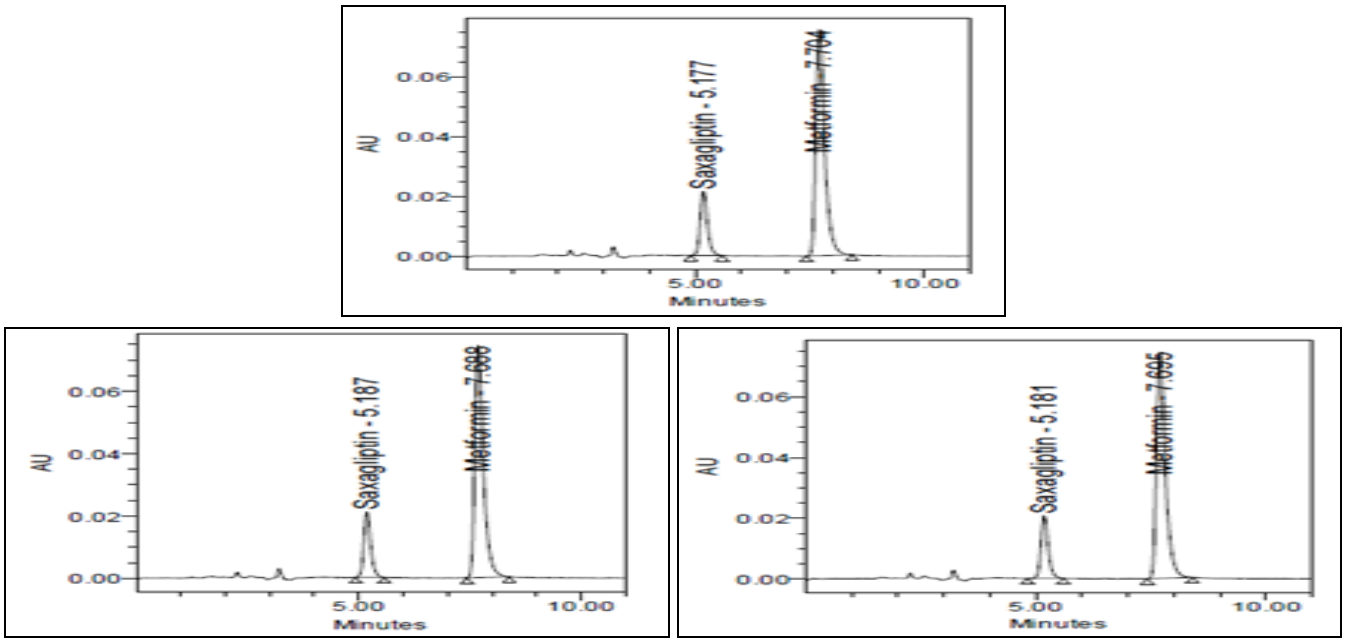


FIG. 7: CHROMATOGRAM FOR RECOVERY STUDIES-50% LEVEL 1, 2, 3.

PEAK NAME: SAXAGLIPTIN

S. no.	Peak name	RT	Area	UPS plate count	UPS tailing
1	Saxagliptin	5.177	198637	5653	1.23
2	Saxagliptin	7.181	198352	5807	1.20
3	Saxagliptin	5.187	198827	5864	1.22

PEAK NAME: METFORMIN

S. no.	Peak name	RT	Area	UPS plate count	UPS tailing
1	Metformin	7.688	989820	5653	1.52
2	Metformin	7.695	989558	5807	1.51
3	Metformin	7.704	898957	5864	1.51

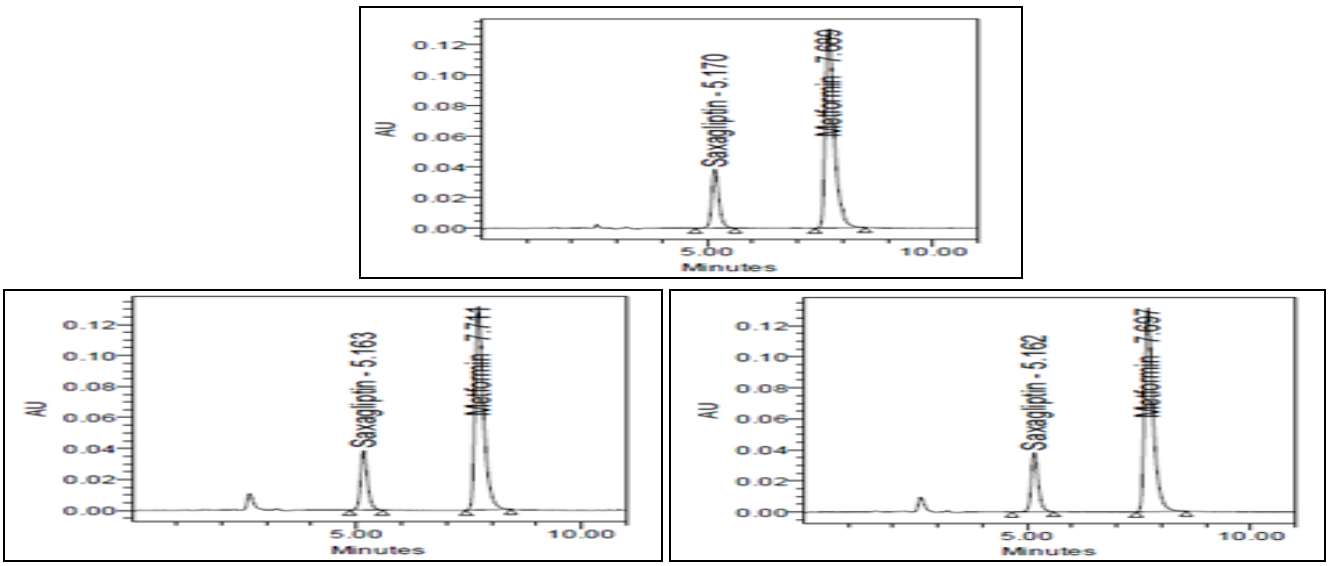


FIG. 8: CHROMATOGRAM FOR RECOVERY STUDIES-100% LEVEL 1, 2, 3

PEAK NAME: SAXAGLIPTIN

S. no.	Peak name	RT	Area	UPS plate count	UPS tailing
1	Saxagliptin	5.162	402984	5970	1.22
2	Saxagliptin	7.163	402620	5972	1.22
3	Saxagliptin	5.170	401547	5953	1.23

PEAK NAME: METFORMIN

S. no.	Peak name	RT	Area	UPS plate count	UPS tailing
1	Metformin	7.689	1788769	7953	1.61
2	Metformin	7.697	1767696	8244	1.57
3	Metformin	7.711	1759036	8293	1.55

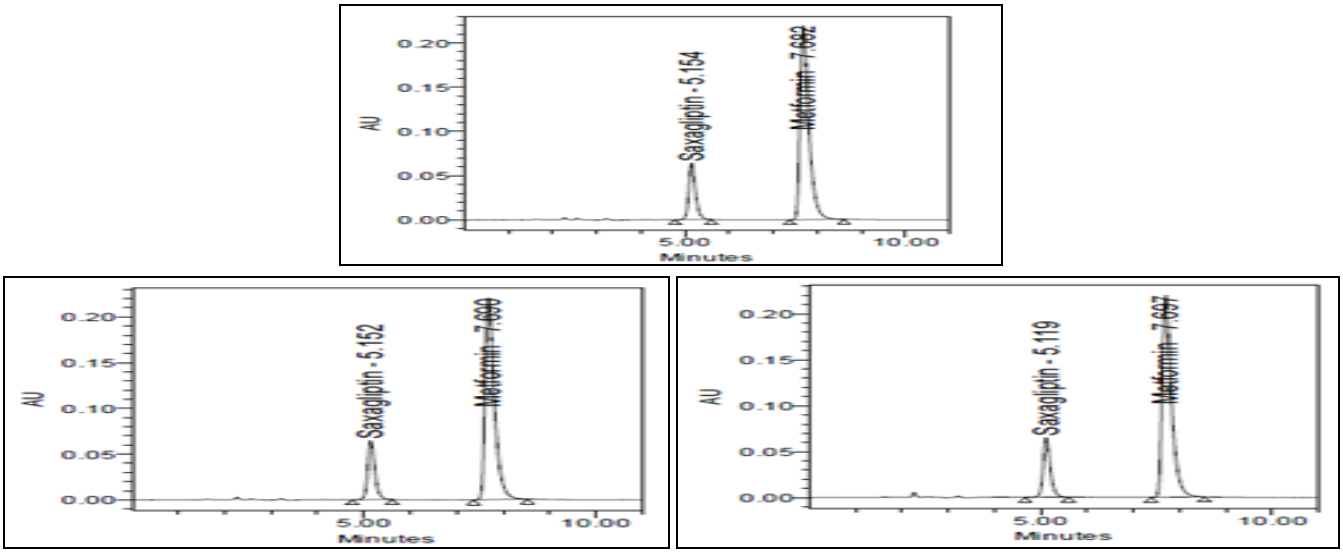


FIG. 9: CHROMATOGRAM FOR RECOVERY STUDIES-150% LEVEL 1, 2, 3

PEAK NAME: SAXAGLIPTIN

S. no.	Peak name	RT	Area	UPS plate count	UPS tailing
1	Saxagliptin	5.119	588837	5873	1.25
2	Saxagliptin	5.152	589100	5940	1.25
3	Saxagliptin	5.154	589323	5948	1.25

PEAK NAME: METFORMIN

S. no.	Peak name	RT	Area	UPS plate count	UPS tailing
1	Metformin	7.682	3060185	7577	1.64
2	Metformin	7.690	3071217	7598	1.64
3	Metformin	7.697	3071745	7537	1.63

Report: The mean percentage recovery for Metformin and Saxagliptin was found to be between 98.95-102.3% given in **Fig. 7**, 98.94-102.66 % given in **Fig. 8** and 98.98-101.66 % in **Fig. 9** respectively, which are well within the limit and hence the method was found to be accurate.

CONCLUSION: System suitability parameters were studied by injecting the standard five times, and results were well under the acceptance criteria. Linearity study was carried out between 25% to 150% levels, r^2 value found 0. 999. By using the above method, assay of the marketed formulation was carried out 99.63% was present. So, this method can be used for the estimation of Metformin and Saxagliptin in Pharmaceutical dosage form for routine analysis purposes.

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CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest concerning this article.

Authors' Contributions: Mahnoor Fatima conceived of and wrote the manuscript. Roshan S. edited the paper. Both authors read and approved the final manuscript.

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