



Research Article

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Stability indicating quantitative RP-HPLC method development and validation for simultaneous determination of metformin hydrochloride and saxagliptin in bulk and combined tablet dosage form

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ABSTRACT

A simple and precise stability indicating RP-HPLC method was developed and validated for the simultaneous determination of Metformin Hydrochloride and Saxagliptin in pure drug and combined tablet dosage form. Chromatography was carried out on Hypersil ODS C₁₈ (250x4.6mm, 5μ particle size) analytical column using a mobile phase of Phosphate buffer (KH₂PO₄) adjusted to pH 5.0 with dilute orthophosphoric acid, acetonitrile and methanol in the ratio of 25:50:25 % v/v/v at a flow rate of 1.0 ml/min. The analyte was monitored using PDA detector at 211 nm. The retention time was found to be 2.246 min and 4.516 min for Metformin Hydrochloride and Saxagliptin respectively. Linearity was observed in the concentration range of 125-750 μg/ml and 1.25-7.5 μg/ml for both Metformin Hydrochloride and Saxagliptin with correlation coefficient of 0.999 respectively. The mean % recoveries obtained for Metformin Hydrochloride and Saxagliptin were found to be 99.62-99.93% and 99.66-99.80% respectively. Stress testing which covered acid hydrolysis, base hydrolysis, peroxide, photolytic (UV light), neutral and thermal degradation was performed to prove the specificity of the proposed method and degradation was achieved. The developed method has been statistically validated according to ICH guide lines and found to be simple, precise and accurate with the prescribed values. Thus the proposed RP-HPLC method was successfully applied for the stability indicating simultaneous estimation of Metformin Hydrochloride and Saxagliptin in routine quality control analysis in bulk and marketed formulations.

Keywords: Metformin Hydrochloride, Saxagliptin, RP-HPLC, Forced degradation, Method validation.

INTRODUCTION

Saxagliptin:

Chemically, (as shown in figure 1) it is (1s, 3s, 5s)-2-[(2s)-2-Amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile. It has a molecular formula of C₁₈H₂₅N₃O₂ and molecular weight of 315.41 g/mol. Saxagliptin is a new oral hypoglycemic agent, dipeptidyl peptidase-4 (DPP-4) inhibitor and antidiabetic for the treatment of type 2 diabetes. DPP-4 inhibitors are a class of compounds that work by affecting the action of natural hormones in the body called incretins. Incretins decrease blood sugar by increasing consumption of sugar by the body, mainly through increasing insulin production in the pancreas, and by reducing production of sugar by the liver. The inhibition of DPP-4 increases levels active of glucagon like peptide 1 (GLP-1), which inhibits glucagon production from pancreatic alpha cells and increases production of insulin from pancreatic beta cells [1-7].

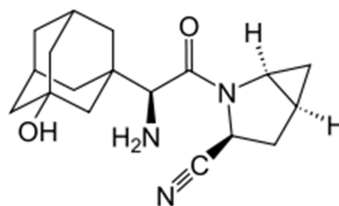


Fig.1: Chemical structure of Saxagliptin

Metformin Hydrochloride:

Chemically, (as shown in fig.1) it is (3-(diamino methylidene)-1, 1-dimethylguanidine; hydrochloride. It has molecular formula of $C_4H_{12}ClN_5$ and molecular weight is 165.62 g/mol. Metformin is an oral antihyperglycemic agent (type 2 diabetes) belongs to class of biguanides and useful for treating non-insulin-dependent diabetes mellitus (NIDDM). Metformin decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization. These effects are mediated by the initial activation by metformin of AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats.

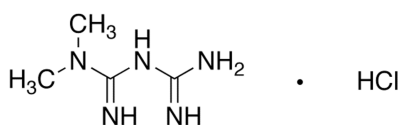


Fig. 2: Chemical structure of Metformin Hydrochloride

Literature survey reveals that few analytical methods were reported like RP-HPLC methods [8-14], GC method [15], HPTLC methods [16-17] and spectrophotometric methods [18-19] in single or in combination with other drugs in pharmaceutical dosage forms. However there was no stability indicating method reported for this drug combination and hence the present study was aimed to develop a simple, fast, economical, selective, accurate, precise and sensitive stability indicating RP-HPLC method for the simultaneous determination of Metformin Hydrochloride and Saxagliptin in bulk and combined tablet dosage forms suitable for routine quality control analysis.

EXPERIMENTAL SECTION

Chemicals:

Metformin Hydrochloride and Saxagliptin were obtained as gift samples from Dr.Reddy's Laboratories, Hyderabad. HPLC grade water, methanol and acetonitrile were purchased from E.Merck. Chem. ltd., Mumbai. All the chemicals used were of analytical reagent grade (E.Merck). Fixed dose combination tablet formulations (Kombiglyze XR) containing 500 mg of Metformin and 5 mg of Saxagliptin (manufactured by Bristol Myers Squibb India Pvt. Ltd, Mumbai) were procured from local pharmacy market.

Instrumentation:

Quantitative HPLC was performed on Waters technologies 2695 series and PDA detector module equipped with auto injector using empower software. A reverse phase Hypersil ODS C_{18} (250x 4.6mm, particle size 5 μ m) analytical column was used. Weighing was done on shimadzu balance (AX 200) and pH adjustments were done using pH meter (ELICO LI 615) was used.

Chromatographic conditions:

Chromatographic separation and analysis were carried out on Hypersil ODS C_{18} column (250x4.6mm, 5 μ particle size) column. The optimized mobile phase consisting of Phosphate buffer (pH adjusted to 5.0 with dilute orthophosphoric acid), acetonitrile and methanol in the ratio of 25:50:25 %v/v/v. Flow rate was maintained at 1.0 ml/min and run time for 7 min. Prior to sample injection, column was saturated with mobile phase for 30 min and injection volume of 10 μ l was injected using auto sampler mode. The detection response was measured at 211 nm at ambient temperature (30 $^{\circ}$ C).

Preparation of Phosphate buffer:

Accurately weighed and transferred a quantity of 1.36 gm of potassium dihydrogen orthophosphate into a 1000 ml of volumetric flask. Added about 900 ml of HPLC grade water and degas to sonicate for 5 min and finally made up the volume with water and then pH of the solution was adjusted to 5.0 with dilute ortho phosphoric acid. The buffer was subjected to filtration using 0.45 μ membrane filter.

Preparation of mobile phase:

Phosphate buffer (adjusted to pH 5.0 with dilute OPA), acetonitrile and methanol were taken in the ratio of 25:50:25 %v/v/v and mixed well and then degassed by subjecting to sonication for 10 minutes and filtered under vacuum filtration.

Preparation of diluent:

Mobile phase was used as diluent.

Preparation of Standard solutions:

Accurately weighed and transferred 50 mg of Metformin and 5 mg of Saxagliptin reference standards into a 10 ml and 100 ml clean dry volumetric flasks separately and added 3/4th volume of diluent, sonicated for 5 min and made up to the final volume with diluent. From the above stock solution, 1.0 ml was pipette out in to a 10 ml volumetric flask and then made up to the final volume with diluent to obtained concentration of 500µg/ml of Metformin and 5µg/ml of Saxagliptin working standard solutions respectively.

Preparation of sample solutions:

20 tablets were weighed and determined the average weight and then crushed to a fine powder. The tablet powder equivalent to 500 mg of Metformin and 5 mg of Saxagliptin was weighed accurately and transferred into a 100 ml volumetric flask, 70 ml of diluent was added and sonicated for 10 min and then made up the volume with diluent and filtered. From the filtered solution, 1.0 ml was pipette out into a 10 ml volumetric flask and made up to the mark with diluent to obtained concentration of 500µg/ml of Metformin and 5µg/ml of Saxagliptin working sample solutions. Injected 10 µl of filtered portion of the sample and standard preparation into the chromatographic system. Record the responses for the major peaks. Calculated the content of Metformin and Saxagliptin present in each tablet.

Method validation:

System suitability:

System suitability test should be carried out to verify that the analytical system is working properly and can give accurate and precise results. Standard solutions were prepared as per the test method and injected five times into the chromatographic system. The system suitability parameters were evaluated for tailing factor, retention times and theoretical plates of standard chromatograms. The results obtained are reported.

Accuracy:

The accuracy of an analytical method is the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The study was performed by making three different concentrations at 50%, 100% and 150% levels using standard addition method where sample preparations were spiked with known amount of standard and then each concentration was injected three times into the chromatographic system. The accuracy of an analytical method should be established across its range.

System Precision and Method precision:

The system precision was carried out to ensure that the analytical system is working properly by injecting standard solution preparations six times into the HPLC and the retention time and peak areas were measured and % RSD was calculated. In method precision, a homogenous sample containing of Metformin and Saxagliptin of a single batch were analyzed six times and % RSD was calculated.

Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of compounds that may be expected to present, such as impurities, degradation products and matrix components. The specificity of the method was assessed by comparing the Chromatograms obtained from the drug standards with that of obtained from the tablet preparations. The retention times of the drug standards and the drug from sample preparations were same, so the method was specific without interference from excipients in the tablets.

Linearity:

The linearity of an analytical method was carried out to check its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Different concentrations of standard solutions were prepared by diluting aliquots (0.25- 1.50 ml) of standard stock solution (5000µg/ml of Metformin and 50 µg/ml for Saxagliptin) in to 10 ml volumetric flasks to obtained concentrations in the range of 125-750 µg/ml for Metformin and 1.25-7.5µg/ml for Saxagliptin and then injected each concentration into the chromatographic system and the chromatograms were recorded.

Robustness:

The robustness of the proposed method was determined by analyzing aliquots from homogenous lots by differing physical parameters like mobile phase composition, flow rate and column temperature. The standard and sample solutions were injected into the chromatograph at varied conditions of flow rate ± 0.2 ml/min, mobile organic phase composition, mobile phase buffer pH ± 0.2 units and column temperature by ± 5 °C.

Limit of Detection (LOD)

Limit of detection is the lowest concentration of the analyte that can be detected by injecting decreasing amount, not necessarily quantity by the method, under the stated experimental conditions. The minimum concentration at which the analyte can be detected is determined from the linearity curve by applying the formula.

$$\text{LOD} = 3.3 S_a/b.$$

S_a is the standard deviation of intercept

b is the slope of calibration curve

Limit of Quantification (LOQ)

Limit of quantification is the lowest concentration of the analyte in a sample that can be estimated quantitatively by injecting decreasing amount of drug with acceptable precision and accuracy under the stated experimental conditions of the method. Limit of quantitation can be obtained from linearity curve by applying the following formula.

$$\text{LOQ} = 10 S_a/b$$

S_a is the standard deviation of intercept

b is the slope of calibration curve

Ruggedness (Intermediate precision):

It is carried out by injecting standard solution preparations six times into the chromatographic system on two different days. %RSD was determined for retention time and peak areas of standard and sample solutions of Metformin and Saxagliptin.

Forced degradation (stress testing) studies:

Stress testing of the drug substance can help identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule and to develop and validate the stability indicating power of the procedures used.

Acid degradation studies:

To 1.0 ml of stock solution of Metformin and Saxagliptin, 1.0 ml of 2N Hydrochloric acid solution was added and refluxed for 30 min at 60°C and then neutralized the solution with 1.0 ml of 2N NaOH solution. The resultant solution was suitably diluted with diluent in a 10ml volumetric flask to obtain 500µg/ml & 5µg/ml solutions of Metformin and Saxagliptin respectively. Then 10 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample.

Base degradation studies:

To 1.0 ml of stock solution of Metformin and Saxagliptin, 1.0 ml of 2N sodium hydroxide solution was added and refluxed for 30 min at 60°C and then neutralized the solution with 1.0 ml of 2N HCL solution. The resultant solution was suitably diluted with diluent in a 10 ml volumetric flask to obtain 5000µg/ml & 5µg/ml solution of Metformin and Saxagliptin respectively. Then 10 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample.

Oxidation (Peroxide) studies:

To 1.0 ml of stock solution of Metformin and Saxagliptin, 1.0ml of 20% Hydrogen peroxide (H₂O₂) was added separately and kept for 30 min at 60°C. The resultant solution was suitably diluted with diluent in a 10 ml volumetric flask to obtain 500µg/ml & 5µg/ml solution of Metformin and Saxagliptin respectively. Then 10 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample.

Thermal degradation studies:

The standard stock solution was placed in an oven at 105 °C for 6 hrs. For HPLC study, the resultant solution was then further diluted with diluent in a 10 ml volumetric flask to obtain 5000 µg/ml & 5 µg/ml solutions of Metformin and Saxagliptin respectively and 10 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of the sample.

Photolytic studies:

It is carried out by exposing 1.0 ml of stock solution of Metformin and Saxagliptin to UV light, by keeping the beaker in UV Chamber for 7 days or 200 Watt hours/m² in photo stability chamber. The resultant solution was diluted with diluent in a 10 ml volumetric flask to obtain 500 µg/ml & 5 µg/ml solutions of Metformin and Saxagliptin respectively and 10 µl solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample.

RESULTS AND DISCUSSION

Saxagliptin is not official in any pharmacopoeia and there is no stability indicating RP-HPLC method was reported for simultaneous estimation with Metformin in bulk and pharmaceutical dosage forms. Hence author has planned to develop and validate stability indicating RP-HPLC method for simultaneous estimation of Metformin and Saxagliptin in combined tablet dosage form.

From this study, it was found that a simple, precise, accurate, sensitive and efficient stability indicating RP-HPLC method has been developed and validated for the estimation of Metformin and Saxagliptin in pharmaceutical dosage form. Chromatographic separation were carried out on Hypersil ODS C₁₈ column (250x 4.6mm, 5 µ particle size) using mobile phase composed of Phosphate buffer (KH₂PO₄) adjusted to pH 5.0 with dilute orthophosphoric acid, acetonitrile and methanol in the ratio of 25:50:25 % v/v/v at a flow rate of 1.0 ml/min using PDA detection at 211 nm. The retention time of Metformin and Saxagliptin were found to be 2.246 min and 4.516 min respectively.

System suitability chromatogram is shown in figure 3 and results are shown in table 1. Linearity was evaluated in the concentration range of 125-750 µg/ml for Metformin and 12.5-7.5 µg/ml for Saxagliptin respectively. The calibration curves were described by the equation $y = 10843.36x + 452.39$ and $y = 47925.54x + 2576.8$ with correlation coefficient of 0.99983 and 0.99962 for Metformin and Saxagliptin respectively as shown in figure 4 and figure 5 respectively. The standard and sample chromatograms in the specificity studies are shown in figure 6 and figure 7. The chromatograms of Limit of detection (LOD) and limit of quantification (LOQ) are shown in figure 8 and figure 9. Accuracy data is shown in table 2. The validation summary parameters and assay results obtained from the marketed formulation are shown in table 3 and table 4. The results of robustness studies are shown in table 5 and table 6. The %RSD in precision, accuracy and robustness studies were found to be less than 2 %, indicating that the method was precise, accurate and robust. The stress testing chromatograms for both Quinapril and HCTZ are shown from figure 10 to figure 15 and results are shown in table 7 and table 8.

Table-1: System suitability studies

S. No	System suitability parameters	Metformin HCL	Saxagliptin
1	Tailing factor(T _f)	1.47	1.12
2	Resolution (R _s)	12.46	
3	Retention time(Min)	2.246	4.516
4	Theoretical plates(N)	3638	7506

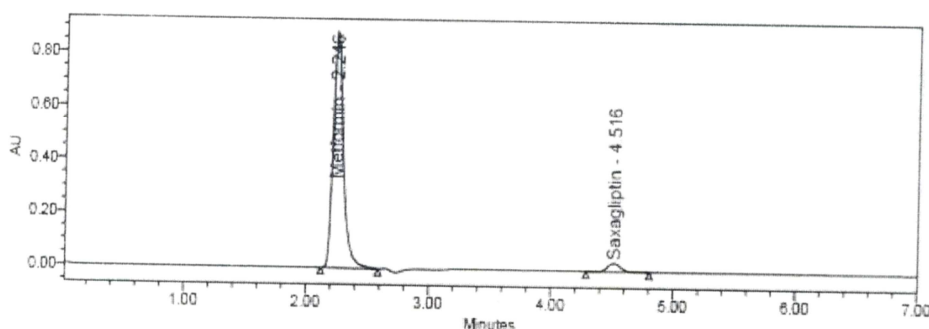


Fig. 3: Typical chromatogram of system suitability solution

Table-2: Accuracy (recovery) studies

Sample	Level	Peak area*	Mean % Recovery $\pm$ SD
Metformin HCL	50%	2548327	99.84 $\pm$ 0.49
	100%	5101386	99.93 $\pm$ 0.42
	150%	7634469	99.62 $\pm$ 0.84
Saxagliptin	50%	118232	99.73 $\pm$ 0.66
	100%	236612	99.80 $\pm$ 0.6
	150%	354419	99.66 $\pm$ 0.54

*Mean of three determinations

Linearity

The calibration curve was found to be linear over the concentration range of 125-750 μ g/ml for Metformin and 1.25-7.5 μ g/ml for Saxagliptin. The correlation coefficient was found to be 0.999 for both Metformin and Saxagliptin respectively.

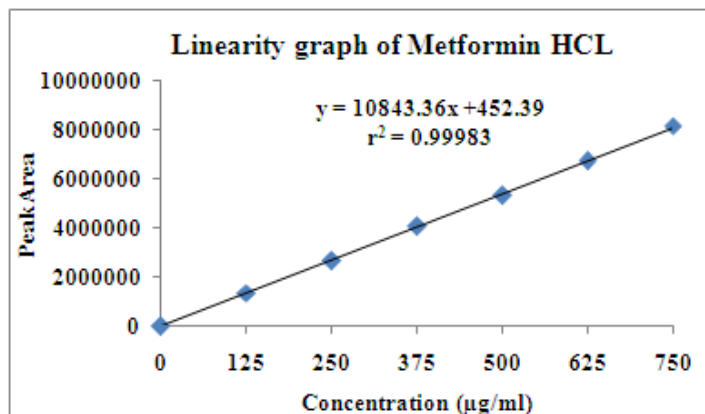
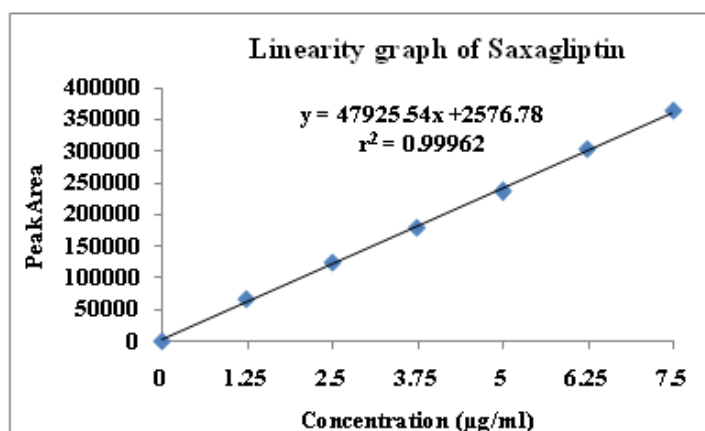

Fig.4: Linearity Graph of Metformin HCL (125-750 μ g/ml)

Fig.5: Linearity Graph of Saxagliptin (1.25-7.5 μ g/ml)

Table -3: Validation summary parameters of the proposed RP-HPLC Method

Parameter	Metformin HCL	Saxagliptin
Regression equation	$y = 10843.36x + 452.39$	$y = 47925.54x + 2576.78$
Correlation coefficient	0.99983	0.99962
LOD (μ g/ml)	1.25	0.17
LOQ (μ g/ml)	4.38	0.59
System precision (% RSD)	0.52	0.26
Method precision (% RSD)	0.63	0.38
% Assay	99.74-99.94%	99.62-99.92%

Table 4: Results of Assay in Marketed Formulation

Brand	Drug	Standard peak area	Sample peak area	Labelled amount (mg/tab)	Amount found (mg/tab)	% Assay	%RSD*
Kombiglyze XR	Metformin HCL	5084435	5098717	500	499.35	99.87	0.54
	Saxagliptin	236147	236701	5	4.99	99.83	0.36

*Mean of six determinations

Specificity studies:

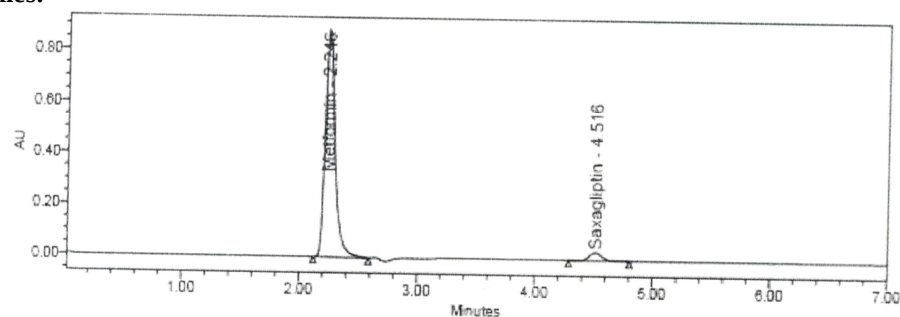


Fig.6: Typical chromatogram of standard

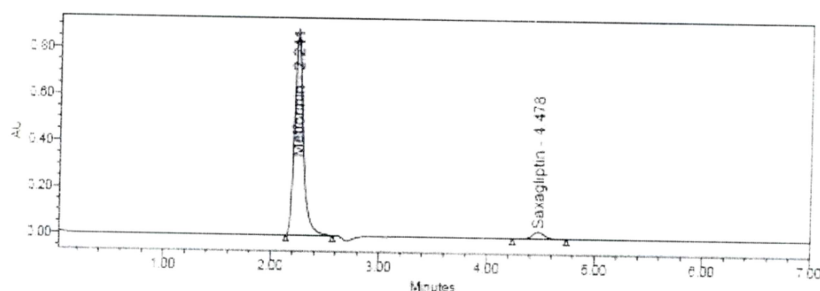


Fig.7: Typical chromatogram of sample

Limit of detection (LOD) and Limit of Quantification (LOQ):

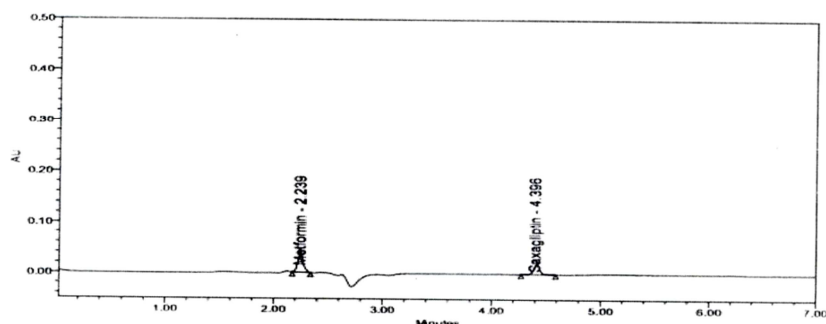


Fig.8: Typical chromatogram of Limit of detection (LOD)

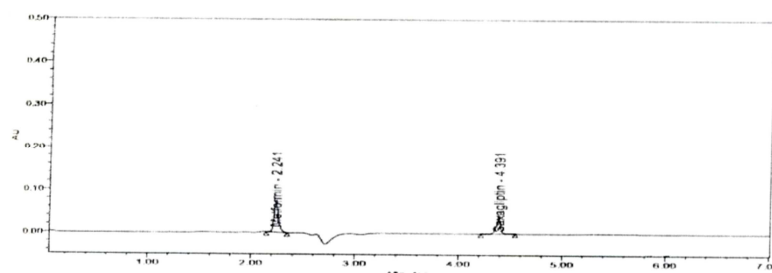


Fig.9: Typical chromatogram of Limit of Quantification (LOQ)

Table-5: Results of robustness study of Metformin HCl

S. No.	Parameter	Change Level	Metformin HCl			
			Retention time (min)	Peak area	USP Tailing	USP Plate count
1.	Flow rate (± 0.2 ml/min)	0.8	2.564	5083219	1.48	3472
		1.0	2.240	5056675	1.41	3728
		1.2	1.927	475624	1.23	4826
2.	Mobile organic phase composition ($\pm 10\%$ v/v)	30:40:30	2.487	509144	1.37	3741
		25:50:25	2.252	506783	1.45	3874
		20:60:20	2.076	487462	1.41	3918
3.	Temperature ($\pm 5^\circ\text{C}$)	25	2.174	504642	1.52	3562
		30	2.287	507418	1.37	3622
		35	2.316	499342	1.47	3974

Robustness:

The developed method is robust with deliberate changes with variation of mobile organic phase composition, flow rate and column oven temperature for both Metformin HCl and Saxagliptin.

Table-5: Results of robustness study of Saxagliptin

S. No.	Parameter	Change Level	Saxagliptin			
			Retention time (min)	Peak area	USP Tailing	USP Plate count
1.	Flow rate (± 0.2 ml/min)	0.8	5.012	255741	1.24	6921
		1.0	4.520	234809	1.16	7482
		1.2	4.084	218725	1.08	8837
2.	Mobile organic phase composition ($\pm 10\%$ v/v)	30:40:30	4.176	268241	1.19	7281
		25:50:25	4.582	247164	1.14	7554
		20:60:20	3.924	226467	1.11	7665
3.	Temperature ($\pm 5^\circ\text{C}$)	25	4.233	22692	1.16	7432
		30	4.467	237814	1.14	7506
		35	4.548	216482	1.12	7964

Forced degradation studies:

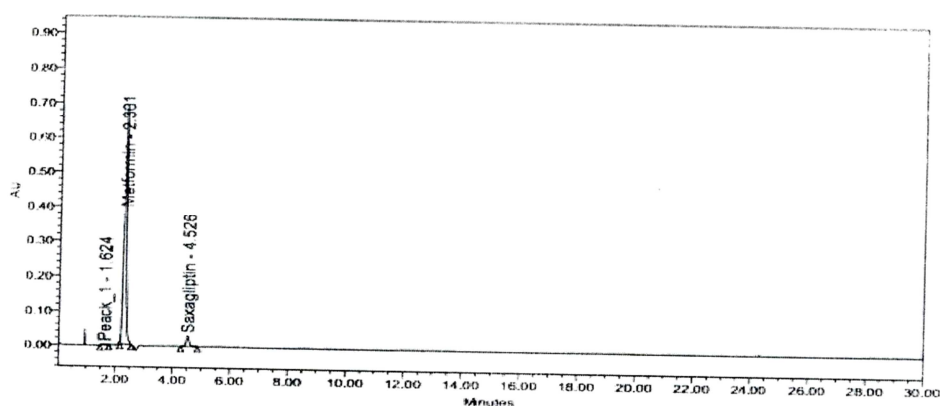


Fig.10: Chromatogram of Acid hydrolysis

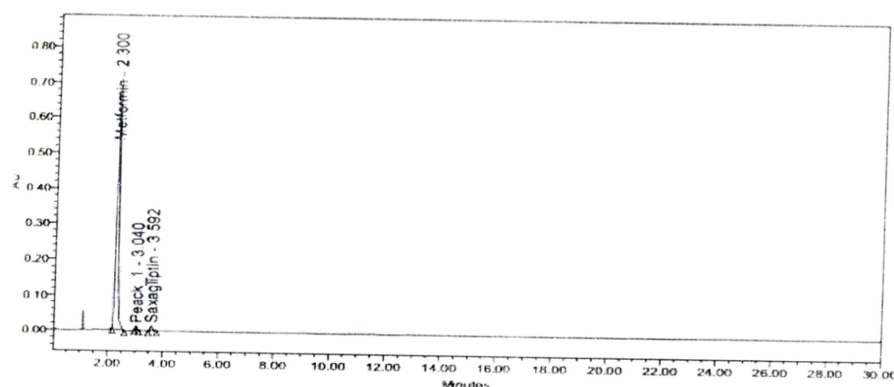


Fig.11: Chromatogram of Base hydrolysis

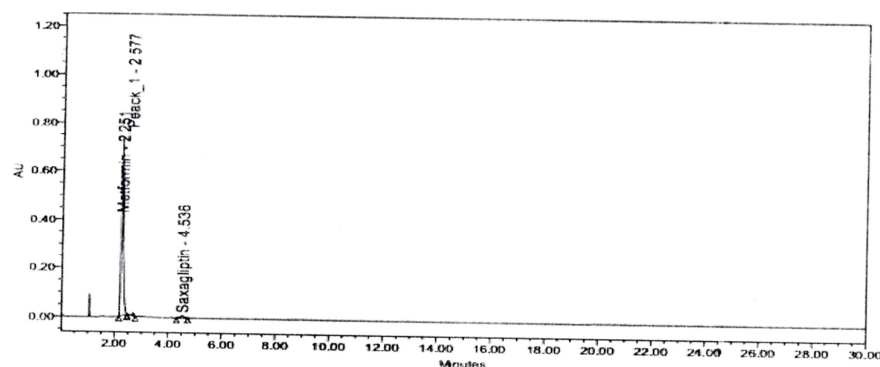


Fig.12: Chromatogram of Oxidation (peroxide) degradation

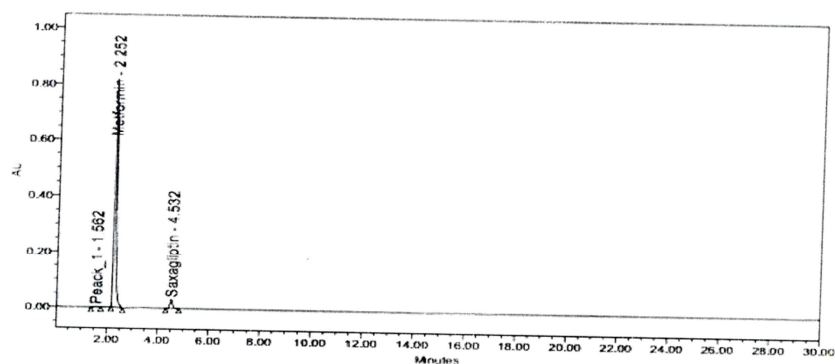


Fig.13: Chromatogram of Thermal degradation

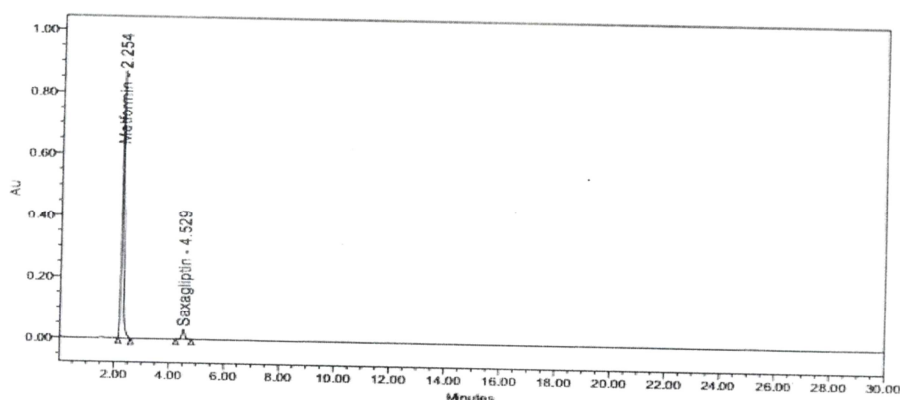


Fig.14: Chromatogram of UV Exposure

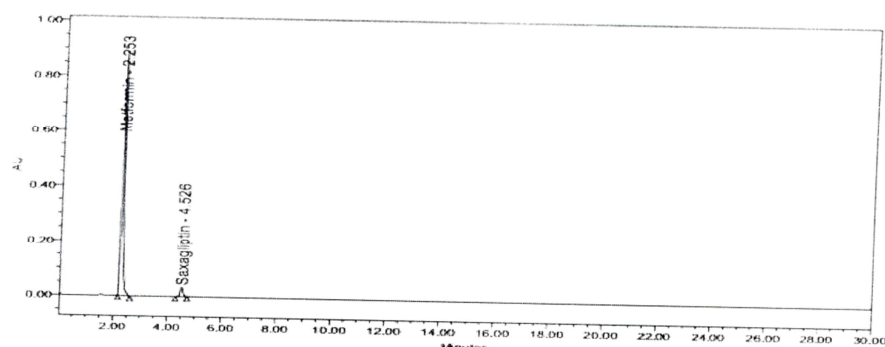


Fig.15: Chromatogram of Neutral degradation

Table-7: Degradation Study of Metformin HCl

S. No.	Condition	Retention time	Peak Area	Degradation % Assay	% Net degradation
1	Acid degradation Hydrolysis	2.381	4762049	92.18	7.69
2	Base Hydrolysis	2.300	4762278	93.22	6.65
3	Oxidation (peroxide) degradation	2.251	4814105	94.24	5.63
4	Thermal degradation	2.252	4856398	95.06	4.81
5	UV Exposure	2.254	5012491	97.74	2.13
6	Neutral degradation	2.253	5083206	97.08	2.79

Table-8: Degradation Study of Saxagliptin

S. No.	Condition	Retention time	Peak Area	Degradation % Assay	% Net degradation
1	Acid degradation Hydrolysis	4.256	215482	92.37	7.46
2	Base Hydrolysis	3.592	218584	93.70	6.13
3	Oxidation (peroxide) degradation	4.536	221304	94.86	4.97
4	Thermal degradation	4.532	222816	95.51	4.32
5	UV Exposure	4.529	230110	97.64	2.19
6	Neutral degradation	4.528	232401	97.62	2.21

CONCLUSION

From this study it is concluded that the proposed Stability Indicating RP-HPLC method was found to be simple, accurate, precise, rapid and useful for routine analysis of Metformin HCl and Saxagliptin in bulk & Pharmaceutical dosage form. The statistical parameters and recovery studies were carried out and reported. The obtained results were satisfactory as per ICH guidelines.

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