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# STABILITY-INDICATING RP-HPLC METHOD AND ITS VALIDATION FOR ANALYSIS OF METFORMIN HYDROCHLORIDE & SAXAGLIPTIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

**Naidu Srinivasa Rao*, Dr. K. Venkataramana, and P.Anitha**

Department of Pharmacy, Acharya Nagarjuna University, Andhra Pradesh, India.

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***Correspondence for Author**
**Naidu Srinivasa Rao**
Department of Pharmacy, Acharya Nagarjuna University, Andhra Pradesh, India.

## ABSTRACT

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Metformin hydrochloride & Saxagliptin in pharmaceutical dosage forms. Chromatographic separation of MET & SAXA was achieved on Inertsil C8, 150mm x 4.6mm, 5µm and the mobile phase containing $P^H$ 2.5 buffer & Acetonitrile in the ratio of 70:30 v/v. The flow rate was 1.0 ml/min, detection was carried out by absorption at 229nm using a photodiode array detector at ambient temperature. The RT of Metformin hydrochloride and Saxagliptin is found to be 2.8 and 5.2 min. The drugs were exposed to thermal, photolytic, hydrolytic, acid, alkali, and oxidative stress and the stressed samples were analyzed by use of the proposed method & chromatograms from the stressed samples, obtained by use of the photodiode-array detector. The linearity of the method was excellent over the range 50-740µg/ml and 0.5-7.5 µg/ml for MET & SAXA respectively. The correlation coefficient was 0.999. The proposed method was validated according to ICH guidelines. And it was found to be suitable and accurate method for quantitative analysis of Dosage form and study of its stability.

**KEYWORDS:** High performance liquid chromatography, forced degradation, Metformin hydrochloride, Saxagliptin.

## INTRODUCTION

Saxagliptin (rINN) is (1S,3S,5S)-2-((2S)-Amino(3-hydroxytricyclo(3.3.1.13,7)dec-1-yl)acetyl)-2-azabicyclo (3.1.0) hexane-3-carbonitrile an orally active hypoglycemic (anti-

diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs and Metformin hydrochloride is N,Ndimethyl imidocar boni-midic diamide is used in the treatment of type 2 diabetes. Structures are shown in fig 1 and 2 respectively. Saxagliptin works to competitively inhibit the enzyme dipeptidyl peptidase 4 (DPP-4) affecting the action of natural hormones in the body called incretins they are able to potentiate the secretion of insulin and suppress the release the glucagon by the pancreas. This drives blood glucose levels to normal. Metformin hydrochloride activates AMP-activated proteinkinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats; activation of AMPK is required for Metformin hydrochloride inhibitory effect on the production of glucose by liver cells.

Metformin is official in IP[11] and USP,[12] while Sitagliptin is not official in any pharmacopoeias. Literature survey reveals, UV,[1] HPLC[2] methods for analysis of Metformin as single and combined dosage forms with other drugs and UV,[3] HPLC[4] methods for analysis of Sitagliptin as single component systems. Few method are reported Simultaneous determination of Metformin hydrochloride and Saxagliptin by reverse phase HPLC[5-10] in pharmaceutical dosage forms. As one or two Stability HPLC method have been reported for the determination of Saxagliptin and Metformin hydrochloride an attempt was made to report a simple, sensitive, validated and economic method for the determination of Saxagliptin and Metformin hydrochloride

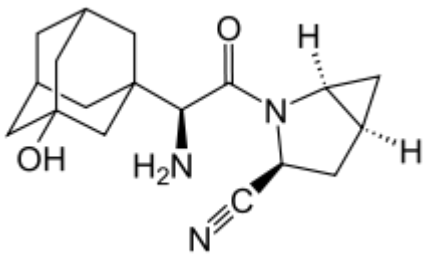


**Fig:1 Saxagliptin**

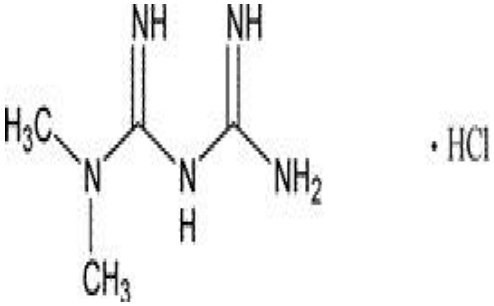


**Fig:2 Metformin hydrochloride**

## MATERIALS AND METHODS

### Instruments Used

All analytical works performed on Waters model LC-20AD dual pump, a Waters model DGU-20A degasser, Waters model SPD-M20A photo diode array (PDA) detector and a

Waters model SIL-20A HT auto injector, Empower2 solution version software, Inertsil C8 column (150 X 4.6mm, 5 µm particle size) as stationary phase, a calibrated electronic single pan balance Shimadzu (AUX-220), a pH meter of Elico (LI-120) and ultrasonic cleaner (SONICA) were also used during the analysis.

**Reagents and chemicals**

Analytically pure Metformin hydrochloride and Saxagliptin were obtained as gift samples from Hyderabad, India. Tablet (Kombiglyze) was purchased from the local market. Buffers, ACN and all other chemicals were analytical grade.

**Method Development**

**Preparation of Buffer:** Weigh 1.50gm of Sodium Hexane-1-sulphonic acid is dissolved into 1lt Water adjust pH-2.5 with OPA.

**Mobile phase:** Prepare a mixture of Buffer and ACN in the ratio of (70:30). Filter and degas.

**Chromatographic condition** Use suitable High Performance Liquid Chromatography equipped with UV-visible detector.

| | |
|---|---|
| Column | : Inertsil C8, 150mm x 4.6mm, 5µm. |
| Wavelength | : 229 nm |
| Injection Volume | : 10µL |
| Column Temperature | : Ambient |
| Flow rate | : 1.0 mL/min. |

Retention time of Metformin hydrochloride is about 3.0-4.0 min and Saxagliptin is about 5.0-6.0min.

**Preparation of Diluent:** Used mobile phase as diluents.

**Preparation of standard solution of Metformin hydrochloride:** Weigh accurately about 500 mg of Metformin hydrochloride working standard is taken into 100ml volumetric flask. Add 70 mL of diluent, sonicate to dissolve and dilute to volume diluent. Further dilute 5 mL to 50 mL with the diluent.

**Preparation of standard solution of Saxagliptin:** Weigh accurately about 10mg of Saxagliptin working standard are taken into 200ml volumetric flask. Add 70 mL of

diluent, sonicate to dissolve and dilute to volume diluent. Further dilute 5mL to 50mL with the diluent.

**Preparation of Sample solution:** Weigh 10tablets and crush the tablets weigh powder then take tabletspowder equivalent to metformin and saxagliptin of sample into a 200 mL volumetric flask. Add 70 mL of diluent, sonicate to dissolve and dilute to volume diluent. Further dilute 5 mL to 50 mL with the diluent. Filter through 0.45µ Nylon syringe filter.

**Procedure** Inject 10µl of Standard preparation five times and Sample preparation in the Chromatograph. Record the chromatograms and measure the peak responses for Metformin hydrochloride & Saxagliptin. The System suitability parameters should be met. From the peak responses, calculate the content of Metformin hydrochloride & Saxagliptin in the sample. The results are shown in "Fig:3" & "Fig:4" and "Table:1"

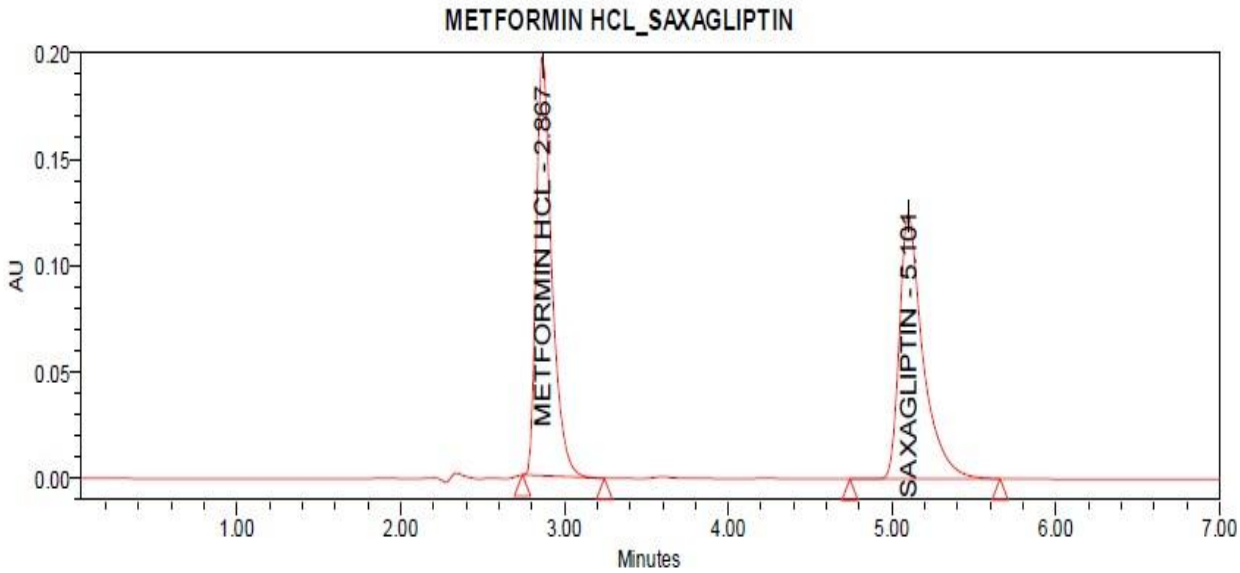


**Fig: 3 Standard chromatogram of Metformin & Sitagliptin.**

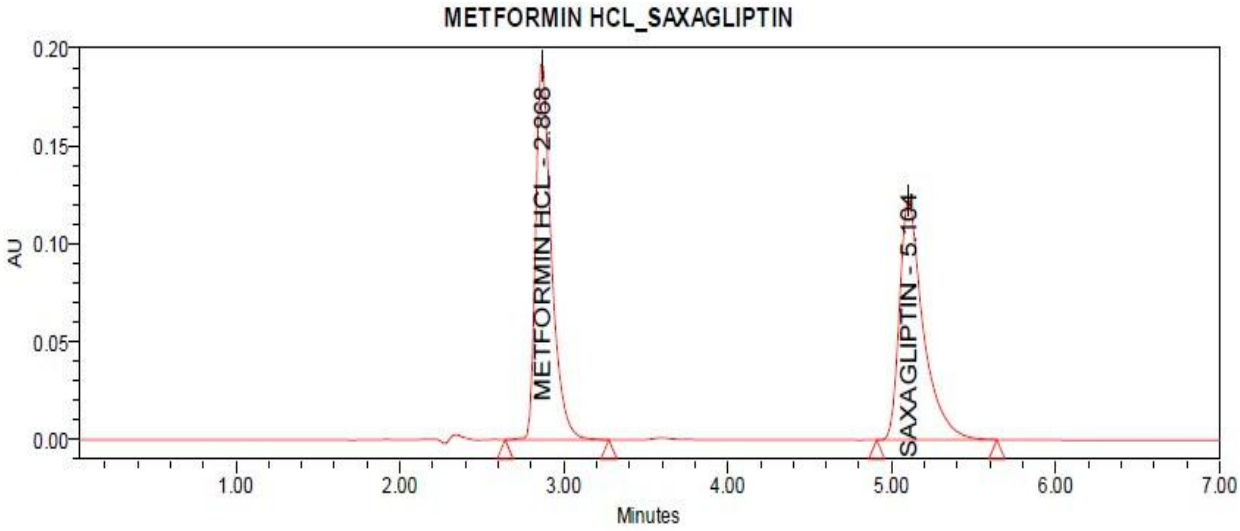


**Fig: 3 Sample chromatogram of Metformin & Sitagliptin.**

**Table 1. Assay results of Metformin hydrochloride and Saxagliptin in combined dosage form.**

| Drug | Label claim | % Drug found ± SD | % RSD |
|---|---|---|---|
| Metformin hydrochloride | 500 mg | 99.8 | 0.18 |
| Saxagliptin | 5 mg | 99.6 | 0.23 |

**Evaluation of system suitability**

1. Relative Standard Deviation of five replicate injections of Standard preparation for Metformin hydrochloride & Saxagliptin peaks should not be more than 2.0%.
2. The tailing factor for Metformin hydrochloride & Saxagliptin peaks should be more than 2.0 and plate count will be not less than 3000.

The results are shown in "Table:2".

**Table: 2 System suitability parameters.**

| Parameter | Metformin hydrochloride | Saxagliptin |
|---|---|---|
| USP tailing factor | 1.50 | 1.65 |
| Theoretical plates | 3481 | 6692 |
| %RSD for Areas | 0.48 | 0.335 |
| %RSD for RT | 0.10 | 0.13 |

**Method validation**

This method described above had been validated as per the ICH guidelines for the parameters like accuracy, linearity, precision, detection limit, quantitation limit and robustness. And the results were summarized below.

**System suitability**

The system suitability was assessed using five replicate analyses of drugs at concentration of 500µg/mL for MET and 5µg/mL for TEL by increasing the injection volumes 10-50µL.

**Specificity**

Specificity studies were carried for both pure drug and drug product by comparing the plots with blank and placebo. Peak purity tests were also carried out to show that the analyte chromatographic peak is not attributable to more than one component as the impurities are not available by purity index data.

**Forced Degradation studies**

Forced degradation study was carried out by treating the sample under the following conditions. Sample Stock solution is from Method Precision sample flask.

**a) Acid degradation**

5 ml of the above stock solution was transferred into 100ml volumetric flask and added 60ml of diluent, treated with 5.0ml of 5N hydrochloric acid and heated at 60°c for 10 minutes, and cooled, neutralized with 5ml of 5N sodium hydroxide and diluted to volume with diluent and was analyzed as per the test method.

**b) Alkali degradation**

5 ml of the above stock solution was transferred into 100ml volumetric flask and added 60ml of diluent, treated with 5.0ml of 5N sodium hydroxide and heated at 60°c for 10 minutes, and cooled, neutralized with 5ml of 5N hydrochloric acid and diluted to volume with diluent and was analyzed as per the test method.

**c) Peroxide degradation**

5 ml of the above stock solution was transferred into 100ml volumetric flask and added with 60ml diluent was treated with 5 ml of 30% v/v solution of hydrogen peroxide and heated at 60°c for 10 minutes, cooled and diluted to volume with diluent and was analyzed as per the test method.

**d) Reduction**

5 ml of the above stock solution was transferred into 100ml volumetric flask and added with 60ml diluent was treated with 5 ml of 1N solution of sodium bicarbonate and heated at 60°c for 10 minutes, and cooled, made to volume with diluent and was analyzed as per the test method.

**e) Photolytic degradation**

Sample was exposed to 1.2 Million lux hours of light and analyzed the exposed sample as per test procedure.

**f) Thermal degradation**

Sample was kept in hot air oven at 60°C for 1 hour. Treated sample was analyzed as per the test method.

The results are shown in "Fig:5" to "Fig:10" and "Table:3" & "Table:4".

**Table: 3 Data for forced degradation studies of Metformin Hydrochloride.**

| Parameter | Area count | %label claim | % degradation |
|---|---|---|---|
| Control | 1289594 | 100.4 | - |
| Acid | 1021542 | 79.5 | 20.9 |
| Alkali | 1025578 | 79.3 | 21.1 |
| Peroxide | 1066442 | 80.2 | 20.2 |
| Reduction | 1037231 | 80.5 | 20.5 |
| Thermal | 1029321 | 80 | 20 |
| Photolytic | 1031751 | 80.3 | 20.7 |

**Table: 4 Data for forced degradation studies of Saxagliptin.**

| Parameter | Area count | %label claim | % degradation |
|---|---|---|---|
| Control | 1232568 | 100.7 | - |
| Acid | 981325 | 80.7 | 20 |
| Alkali | 969984 | 80.6 | 20.1 |
| Peroxide | 1037923 | 80.5 | 20.2 |
| Reduction | 980952 | 72.6 | 28.1 |
| Thermal | 983006 | 79.4 | 21.3 |
| Photolytic | 985308 | 79.8 | 20.9 |

**Chromatograms for forced Degradation Studies**

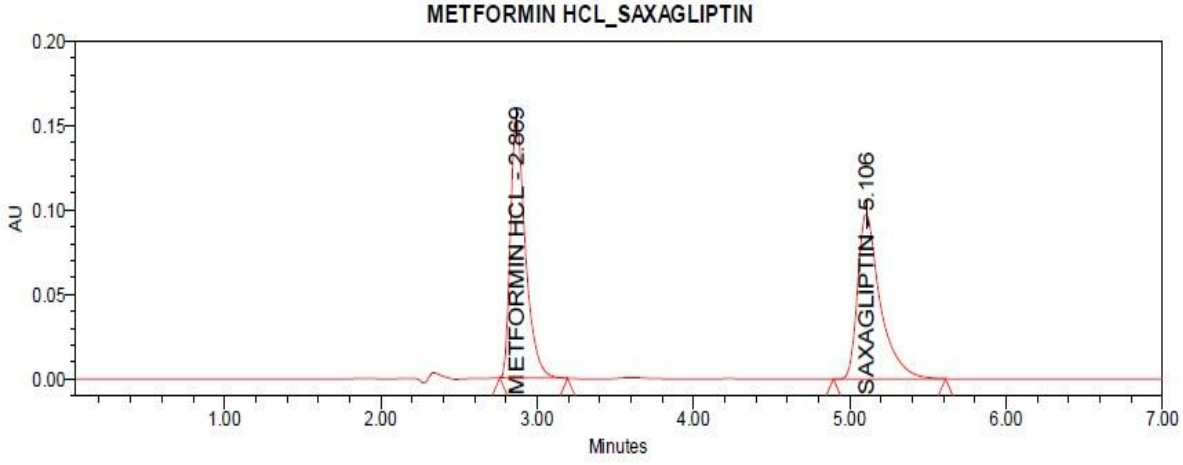


**Fig:5 Acid Degradation Chromatogram for Metformin Hcl & Saxagliptin.**

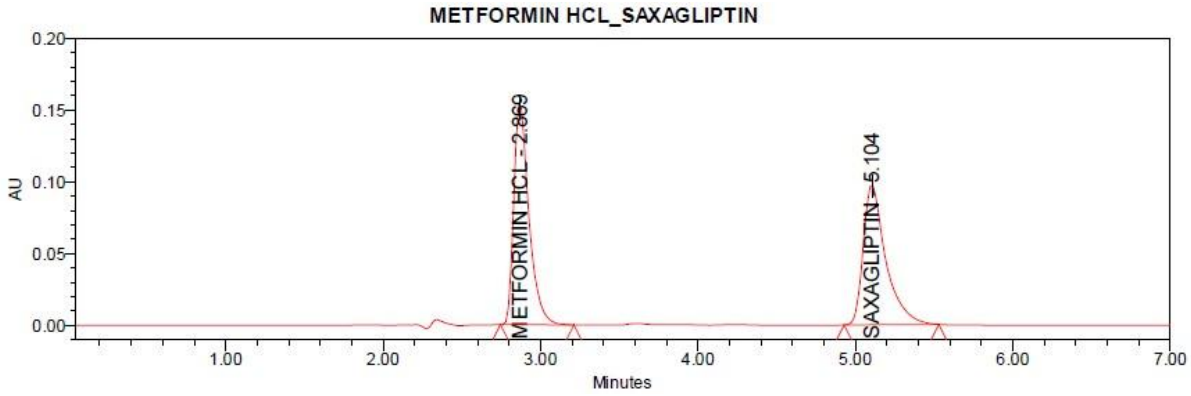


**Fig:6 Alkali Degradation Chromatogram for Metformin Hcl & Saxagliptin.**

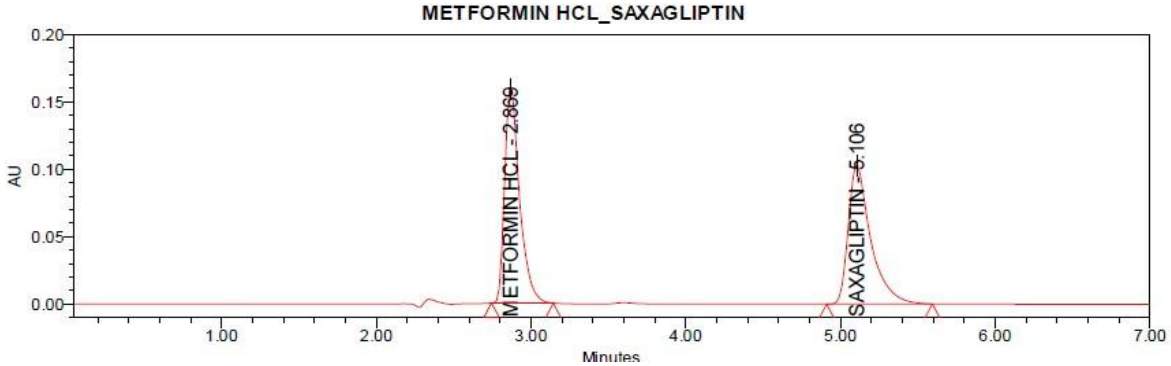


**Fig:7 Peroxide Degradation Chromatogram for Metformin Hcl & Saxagliptin.**

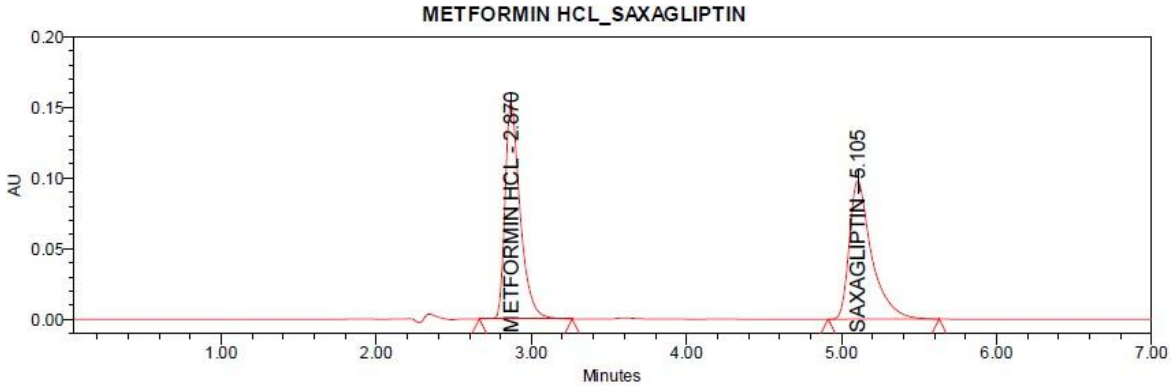


**Fig:8 Reduction Degradation Chromatogram for Metformin Hcl & Saxagliptin.**

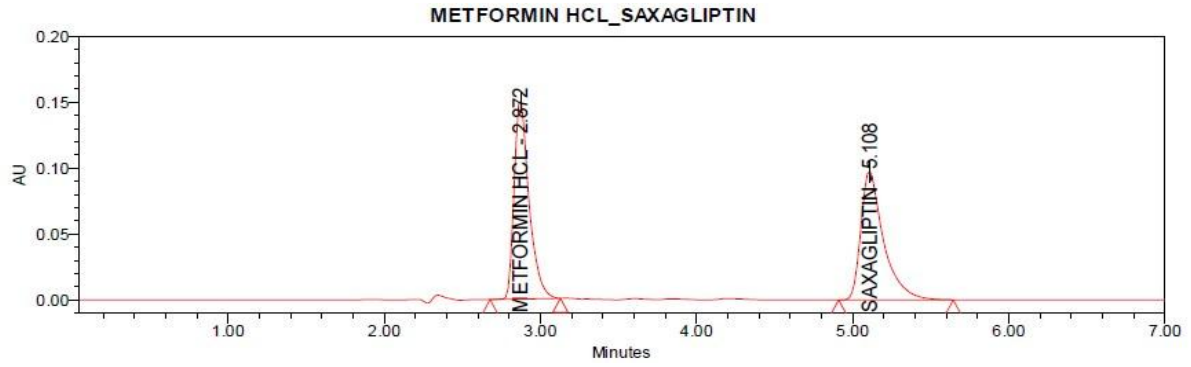


**Fig:9 Thermal Degradation Chromatogram for Metformin Hcl & Saxagliptin.**

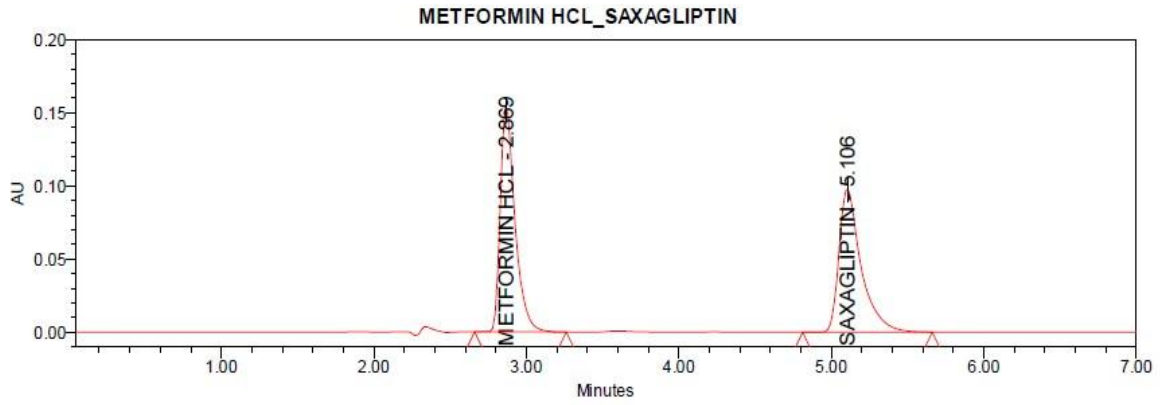


**Fig:10 Photolytic Degradation Chromatogram for Metformin Hcl & Saxagliptin.**

**Linearity**

The linearity responses in the concentration range of 80-730 µg/mL for MET and 8-70 µg/mL for SITA was determined. And the co-relation coefficient was NLT 0.99.

**Table 5: Linearity of Metformin hydrochloride.**

| Concentration (µg/ml) | Area |
|---|---|
| 50 | 122309 |
| 100 | 257527 |
| 200 | 495746 |
| 261 | 648447 |
| 300 | 760044 |
| 400 | 972351 |
| 500 | 1240768 |
| 600 | 1512721 |
| 740 | 1835172 |

**Table 6: Linearity of Saxagliptin.**

| Concentration (µg/ml) | Area |
|---|---|
| 0.5 | 110969 |
| 1.0 | 260032 |
| 2.0 | 477758 |
| 2.5 | 621817 |
| 3.0 | 728045 |
| 4.0 | 933008 |
| 5.0 | 1196868 |
| 6.0 | 1463862 |
| 7.5 | 1772508 |

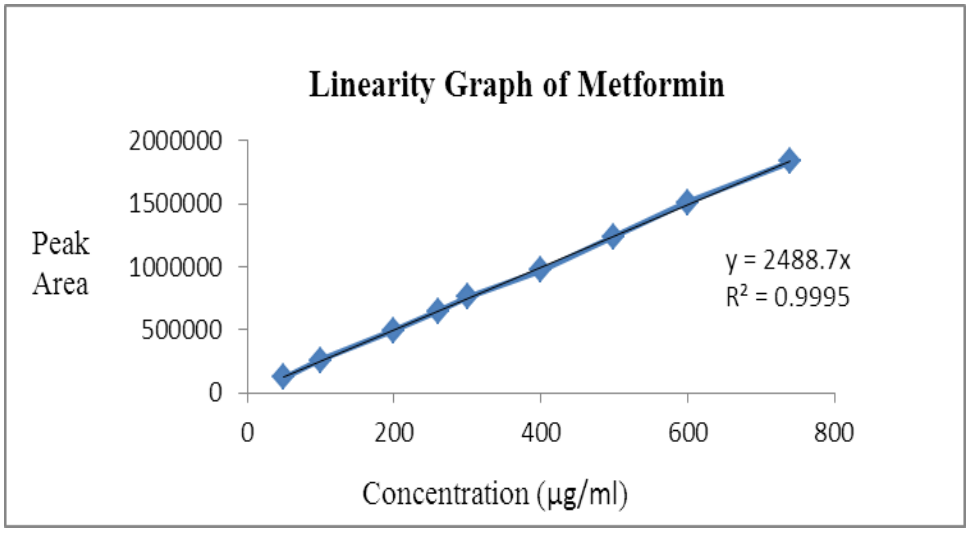


**Fig: 11 Linearity graph of Metformin Hcl.**

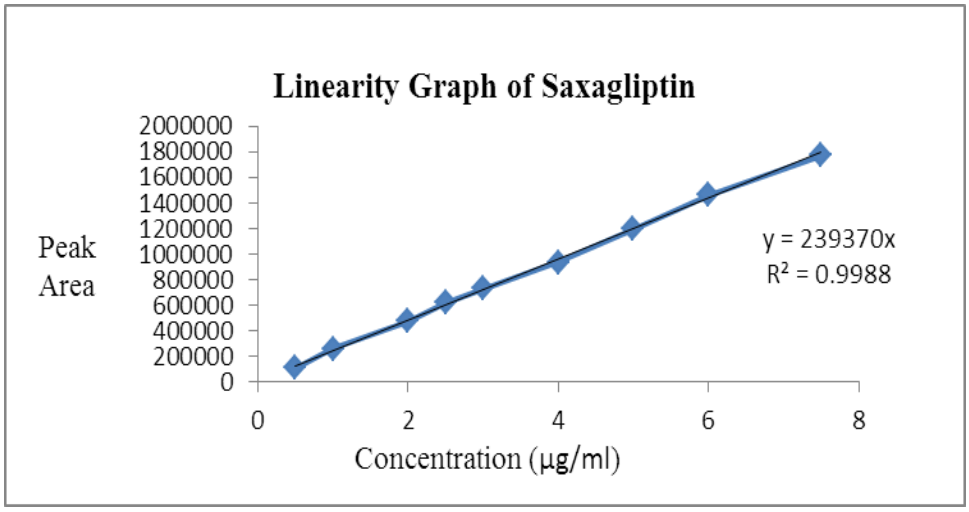


**Fig:12 Linearity graph of Saxagliptin.**

**Precision**

Precision was measured in terms of repeatability of application and measurement. Study was carried out by injecting six replicates of the standard at a concentration of 500µg/mL for MET and 5µg/mL for SITA. And the RSD calculated from replicates of assay values NMT 2.0%.

**Table 7: Precision data of the proposed method.**

| Drug | Concentration added, µg mL | Intra-day precision | | Inter-day precision | |
|---|---|---|---|---|---|
| | | Mean amount found, µg mL | % RSD (n = 6) | Mean amount found, µg mL-1 | % RSD (n = 6) |
| Metformin hydrochloride | 500 | 499.66±0.72 | 0.32 | 499.81±0.27 | 0.52 |
| Saxagliptin | 5 | 4.9.25±0.54 | 0.24 | 4.87±0.53 | 0.38 |

**Accuracy**

Accuracy (Recovery) of the method was determined by spiking 50, 100 and 150% of working standard at a concentration of 500µg/mL for MET and 5µg/mL for SAXA. Samples were injected in triplicate across its range according to the assay procedure. The RSD calculated from replicates of assay values NMT 2.0% and the percentage recovery was in between 99% to 102%.

**Recovery**

| S.NO | Level | % Recovery for Metformin hydrochloride | %Recovery for Saxagliptin |
|---|---|---|---|
| 1 | 50% | 99.8 | 99.6 |
| 2 | 100% | 100.1 | 98.9 |
| 3 | 150% | 99.5 | 99.6 |

**Detection and quantitation limits**

The LOD and LOQ values were determined by the formulae LOD = 3.3 s/ m and LOQ = 10 s/m (Where, s is the standard deviation of the responses and m is mean of the slopes of the calibration curves).

**Robustness**

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as flow rate (1± 0.1 mL/min), wavelength (±1nm), organic phase (± 10%) and ph(±0.2)

**RESULT AND DISCUSSION**

A reversed-phase column procedure was proposed as a suitable method for the simultaneous determination of Saxagliptin and Metformin hydrochloride in combined dosage forms. The chromatographic conditions were optimized by changing the mobile phase composition, $p^H$, and buffers used in the mobile phase. Different ratios were experimented to optimize the mobile phase. Finally a mixture of $P^H$2.5 buffer & Acetonitrile in the ratio of 70:30 v/v was used. A typical chromatogram obtained by using the above mentioned mobile phase from 10µl of the assay preparation is illustrated below. The retention times of Saxagliptin and Metformin hydrochloride were 5.1 and 2.8 min, respectively. The linearity of the method was tested from 0.5-7.5 µg/ml for Saxagliptin and 50-750µg/ml for Metformin hydrochloride. Correlation coefficients for the regression line were 0.9965 and 0,9998 for Saxagliptin and Metformin hydrochloride

respectively. The accuracy of the method was studied by recovery experiments. The recovery was determined at three levels, viz. 50%, 100%, and 150% of the selected concentrations. Three samples were prepared for each recovery level. The recovery values for Saxagliptin and Metformin hydrochloride ranged from 99.5- 100.4%, respectively. The precision of the method was determined from one lot of combined dosage form. To determine the robustness of the developed method experimental conditions were purposely altered and RSD of the peak areas of Saxagliptin and Metformin hydrochloride were found not greater than 2.0 illustrates the robustness of the method.

## CONCLUSION

A simple specific stability-indicating HPLC method has been developed for the quantification of Metformin Hydrochloride and Saxagliptin. This method has been validated and found to be specific, precise, accurate, linear, robust, and linear for the detection and quantification of Metformin Hydrochloride and Saxagliptin. This method exhibited an excellent performance in terms of sensitivity and speed and it helps in simultaneous estimation of Metformin Hydrochloride and Saxagliptin in pharmaceuticals i.e., in combination drugs. This method is suitable for routine analysis and quality control of pharmaceuticals.

## ACKNOWLEDGEMENTS

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