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

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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 & Sitagliptin Phosphate in pharmaceutical dosage forms. Chromatographic separation of MET & SITA was achieved on Zodiac C18 , 150mm x 4.6mm, 5µm and the mobile phase containing TEA buffer & Methanol in the ratio of 80:20 v/v. The flow rate was 1.0 ml/min, detection was carried out by absorption at 224nm using a photodiode array detector at ambient temperature. The RT of Metformin hydrochloride and Sitagliptin Phosphate is found to be 3.6 and 5.3 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 80-730µg/ml and 8-70 µg/ml for MET & SITA 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, Sitagliptin Phosphate.

INTRODUCTION

SITAGLIPTIN PHOSPHATER)-4-oxo-4-[3-(trifluromethyl)-5, 6-dihydro[1,2,4]triaz-olo[4,3a]pyrazin-7(8H) yl]-1-(2,4,5-trifluorophenyl)butan-2-amine and Metformin hydrochloride is N,Ndimethyl imidocar boni-midic diamide are used in the treatment of type 2 diabetes. Structures are shown in fig 1 and 2 respectively. SITAGLIPTIN PHOSPHATE works to competitively inhibit the enzyme dipeptidyl peptidase 4 (DPP-4). This enzyme breaks down the incretins GLP-1 and GIP inactivation, 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 Sitagliptin Phosphate by reverse phase HPLC[5-10] in pharmaceutical dosage forms. As one or two Stability HPLC method have been reported for the determination of Sitagliptin Phosphate and Metformin hydrochloride an attempt was made to report a simple, sensitive, validated and economic method for the determination of Sitagliptin Phosphate and Metformin hydrochloride

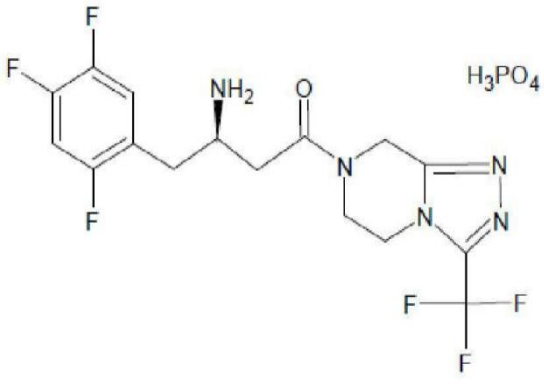

Fig:1 Sitagliptin Phosphate

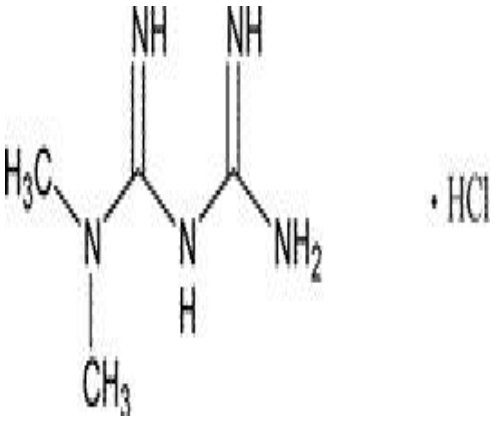

Fig:2 Metformin hydrochloride

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, Zodiac C18 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 Sitagliptin Phosphate were obtained as gift samples from Dr. Reddy's laboratories, Hyderabad, India. Tablet (Zanumet) was purchased from the local market. Buffers, Methanol and all other chemicals were analytical grade.

Method Development

Preparation of Buffer: Pipette out 1ml Tri ethyl amine is dissolved into 1lt Water adjust pH-2.5 with OPA.

Mobile phase: Prepare a mixture of Buffer and Methanol in the ratio of (80:20%v/v). Filter and degas.

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

Column	: Zodiac C18, 150mm x 4.6mm, 5µm.
Wavelength	: 224 nm
Injection Volume	: 20µL
Column Temperature	: Ambient
Flow rate	: 1.0 mL/min

Retention time of Metformin hydrochloride is about 2.0-3.0 min and Sitagliptin Phosphate is about 4.0-5.0min.

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 Sitagliptin Phosphate: Weigh accurately about 50mg of Sitagliptin Phosphate working standard are taken into 100ml 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 5 tablets equivalent of sample into a 250 mL volumetric flask. Add 70 mL of diluent, sonicate to dissolve and dilute to volume diluent. Further dilute 5 mL to 100 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 & Sitagliptin Phosphate. The System suitability parameters should be met. From the peak responses, calculate the content of Metformin hydrochloride & Sitagliptin Phosphate in the sample. The results are shown in "Fig:3" & "Fig:4" and "Table:1".

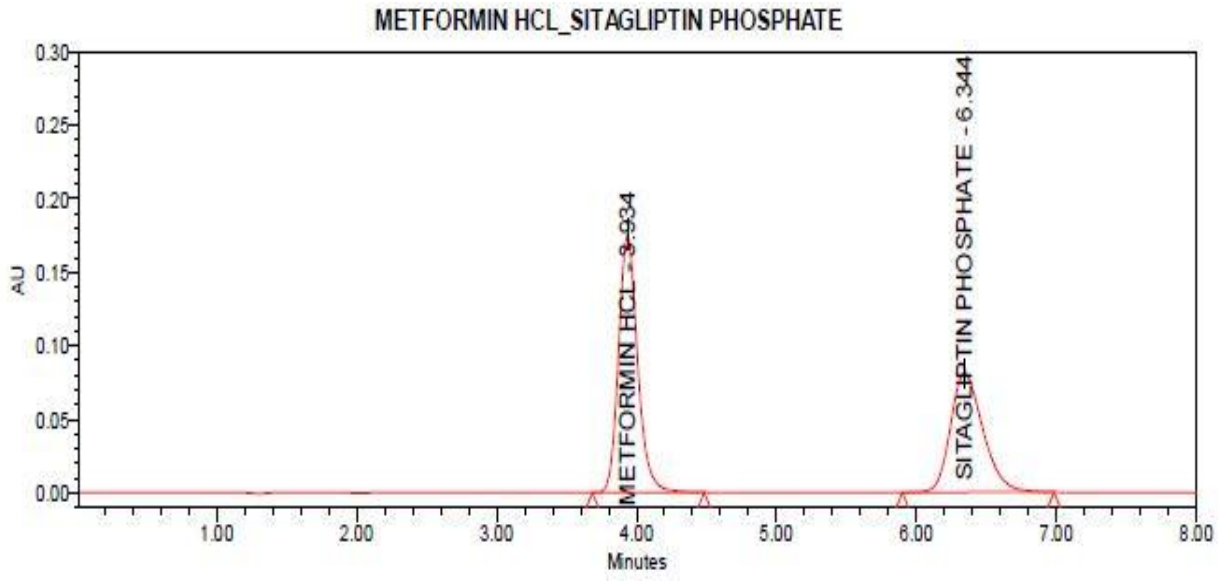

Fig: 3 Standard chromatogram of Metformin & Sitagliptin.

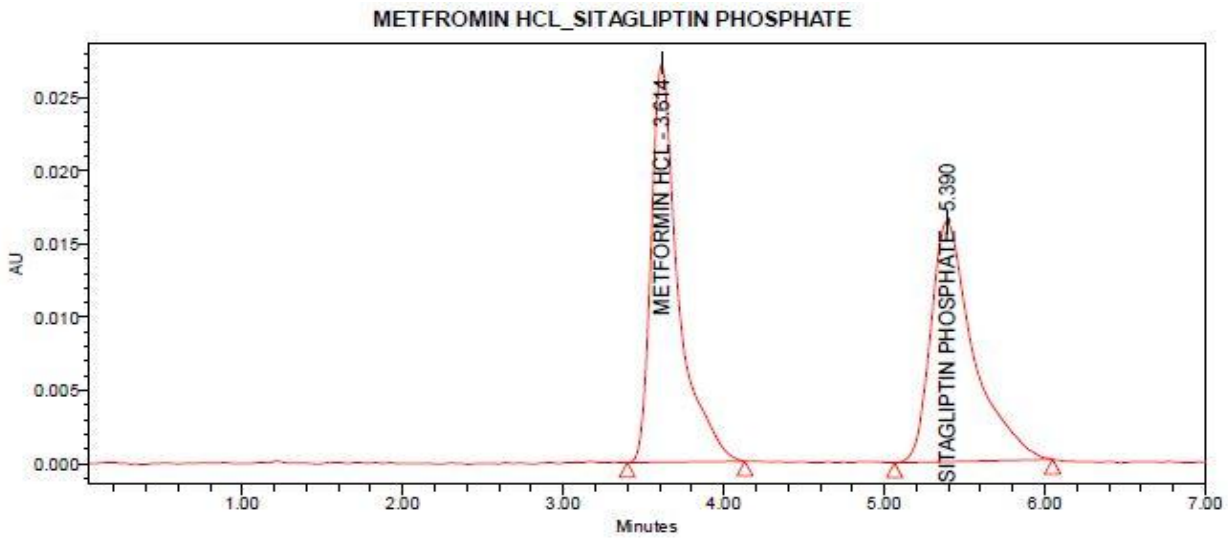

Fig: 3 Sample chromatogram of Metformin & Sitagliptin.

Table 1. Assay results of Metformin hydrochloride and Sitagliptin Phosphate in combined dosage form.

Drug	Label claim	% Drug found ± SD	% RSD
Metformin hydrochloride	500 mg	99.5	0.10
Sitagliptin Phosphate	50 mg	99.4	0.13

Evaluation of system suitability

1. Relative Standard Deviation of five replicate injections of Standard preparation for Metformin hydrochloride & Sitagliptin Phosphate peaks should not be more than 2.0%.
2. The tailing factor for Metformin hydrochloride & Sitagliptin Phosphate 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	Sitagliptin Phosphate
USP tailing factor	1.76	1.656
Theoretical plates	3481	3187
%RSD for Areas	0.48	0.44
%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 50µg/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	320015	99.9	0.1
Acid	240747	76	23.9
Alkali	244610	76.7	23.2
Peroxide	257542	79	20.9
Reduction	206358	71.1	28.8
Thermal	243384	78.9	21
Photolytic	240601	78.2	21.7

Table: 4 Data for forced degradation studies of Sitagliptin Phosphate.

Parameter	Area count	%label claim	% degradation
Control	302099	100.4	-
Acid	215726	73.1	27.3
Alkali	220850	74.6	25.8
Peroxide	240860	79.3	21.1
Reduction	196461	73.6	26.8
Thermal	222156	79	214.
Photolytic	224849	78.6	21.8

Chromatograms for forced Degradation Studies

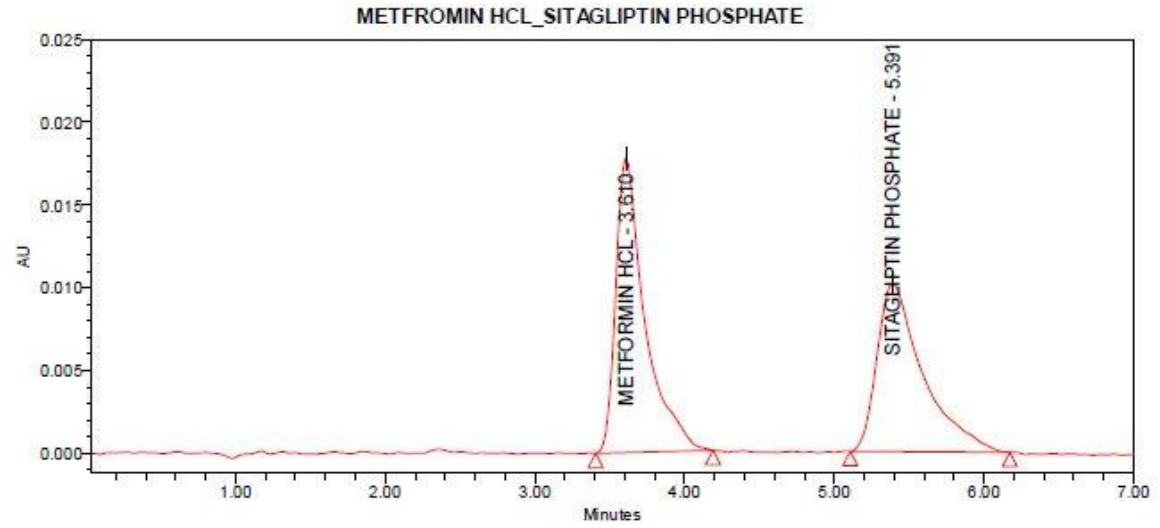

Fig:5 Acid Degradation Chromatogram for Metformin Hcl & Sitagliptin phosphate.

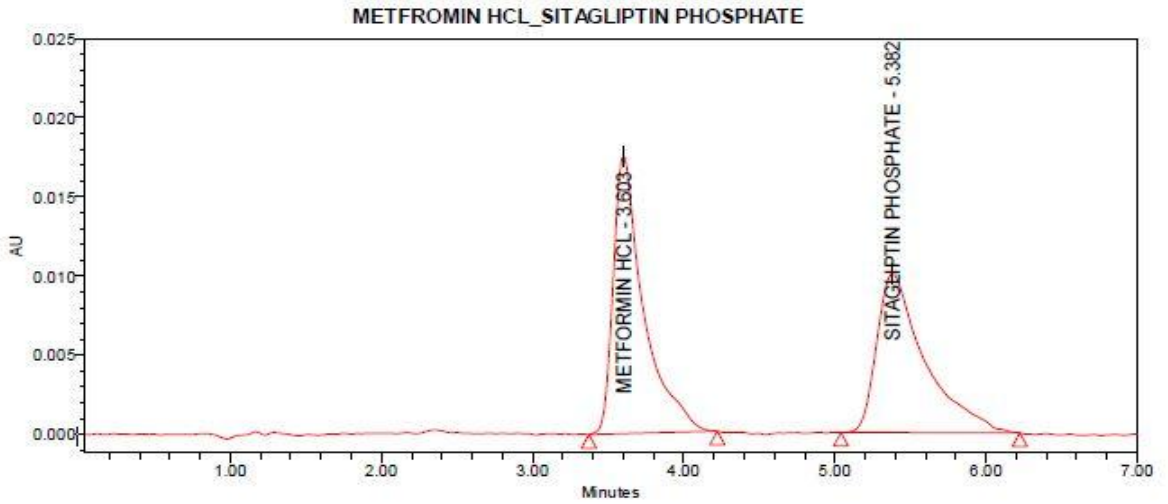

Fig:6 Alkali Degradation Chromatogram for Metformin Hcl & Sitagliptin phosphate.

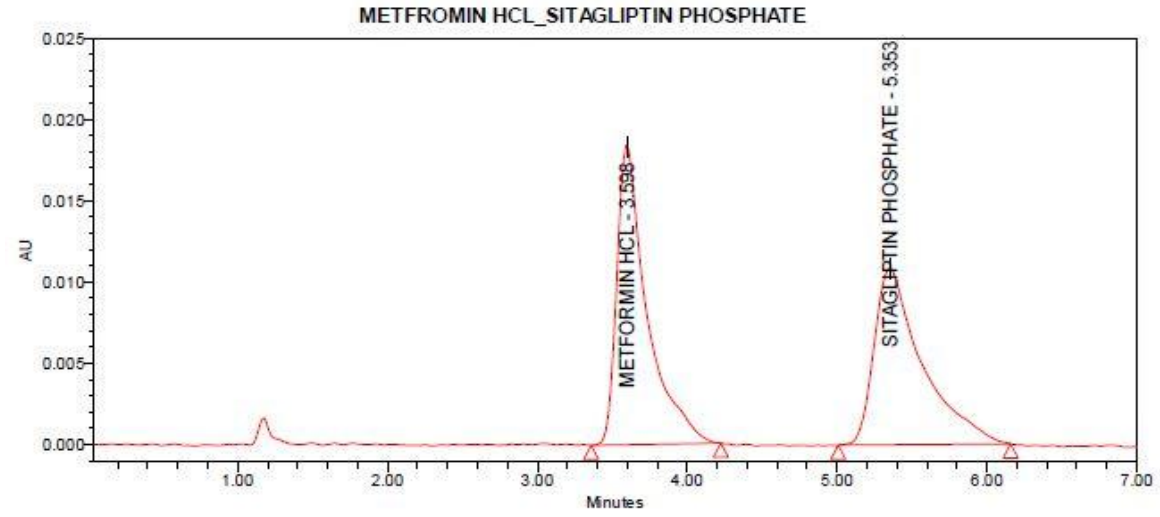

Fig:7 Peroxide Degradation Chromatogram for Metformin Hcl & Sitagliptin phosphate.

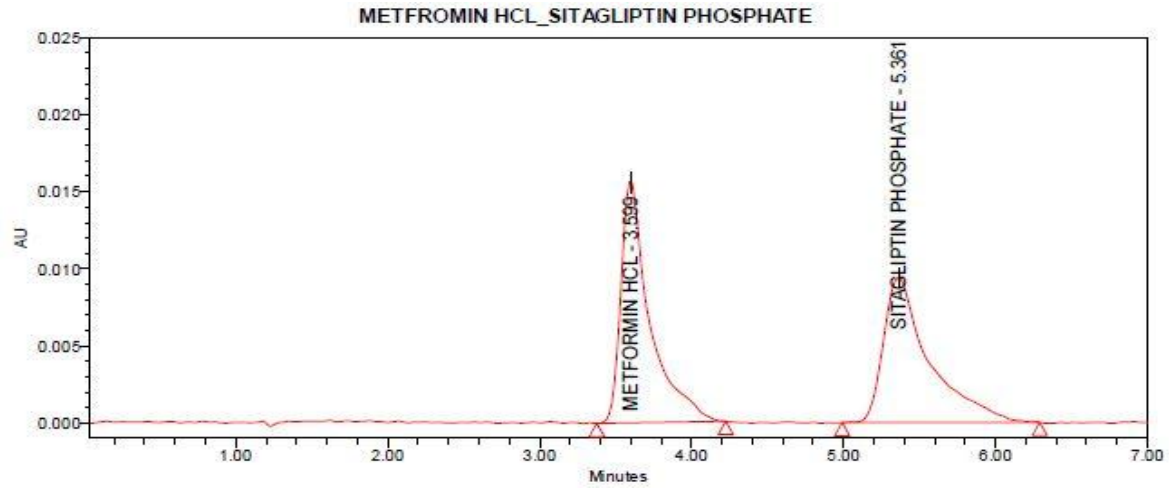

Fig:8 Reduction Degradation Chromatogram for Metformin Hcl & Sitagliptin phosphate.

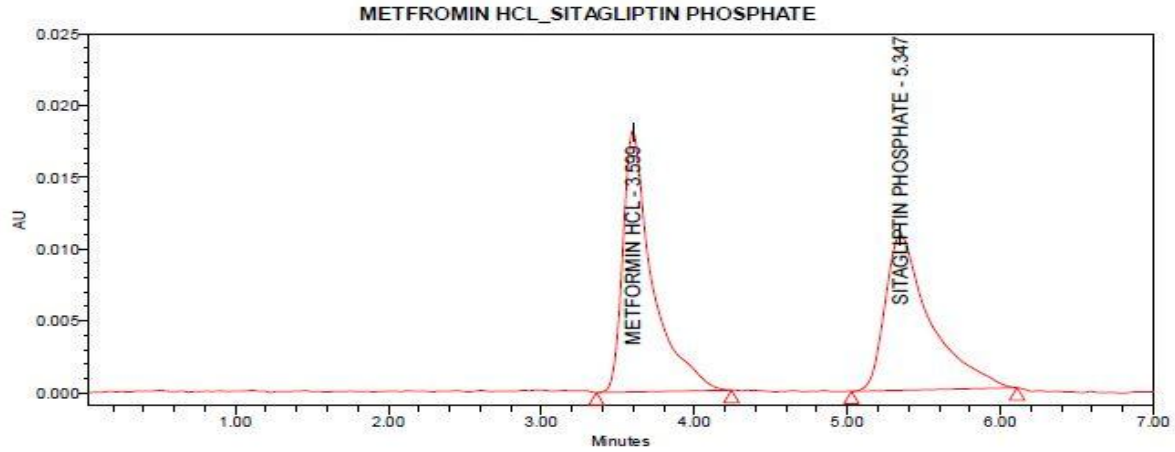

Fig:9 Thermal Degradation Chromatogram for Metformin Hcl & Sitagliptin phosphate.

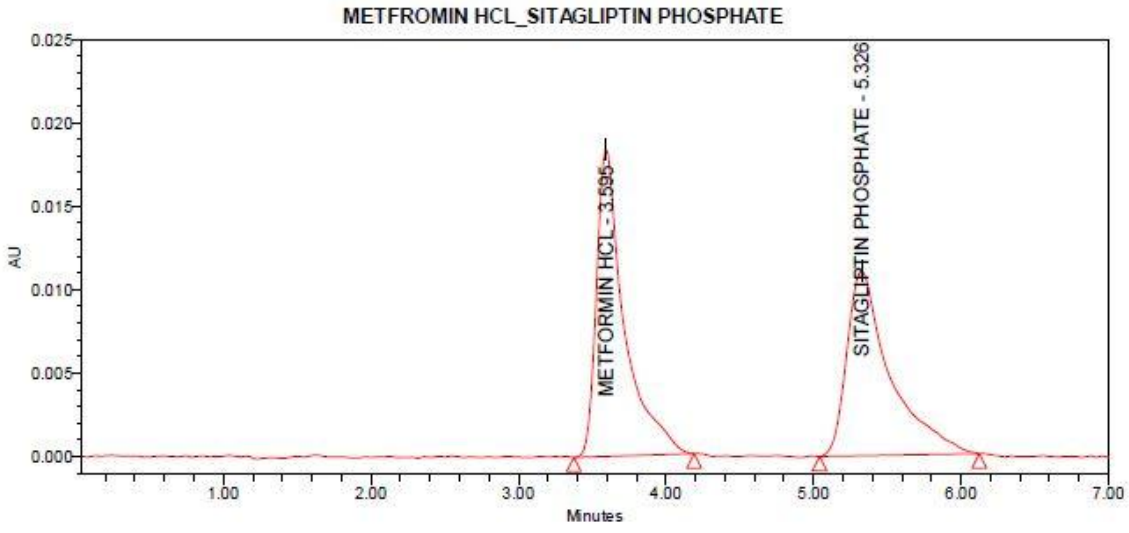

Fig:10 Photolytic Degradation Chromatogram for Metformin Hcl & Sitagliptin phosphate.

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
80	71160
200	142422
250	173569
320	212315
460	285794
510	319751
580	353148
730	432300

Table 6: Linearity of Sitagliptin Phosphate.

Concentration (µg/ml)	Area
8	54170
21	112524
27	135819
33	165519
45	219041
51	244968
56	267540
70	328640

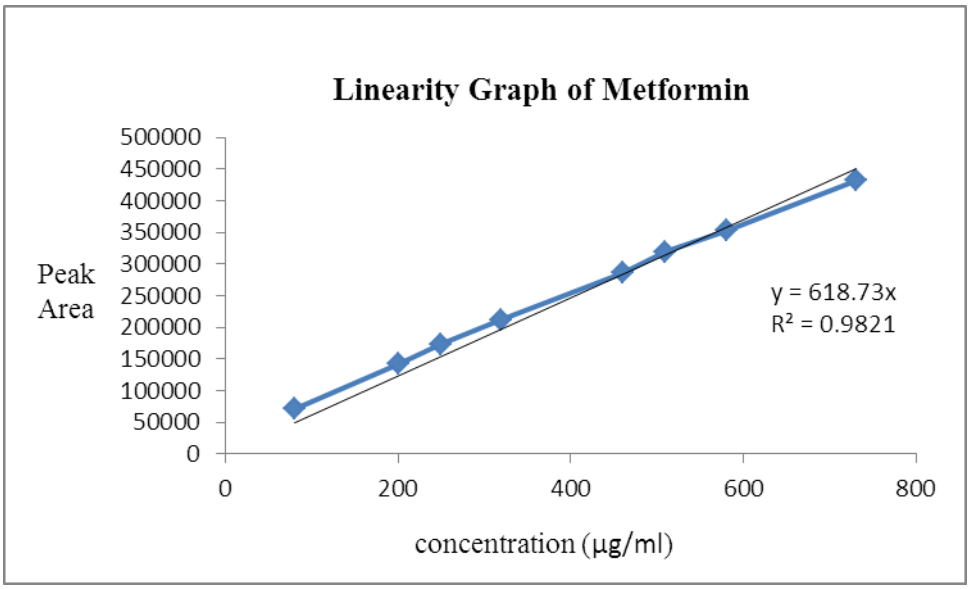

Fig: 11 Linearity graph of Metformin Hcl.

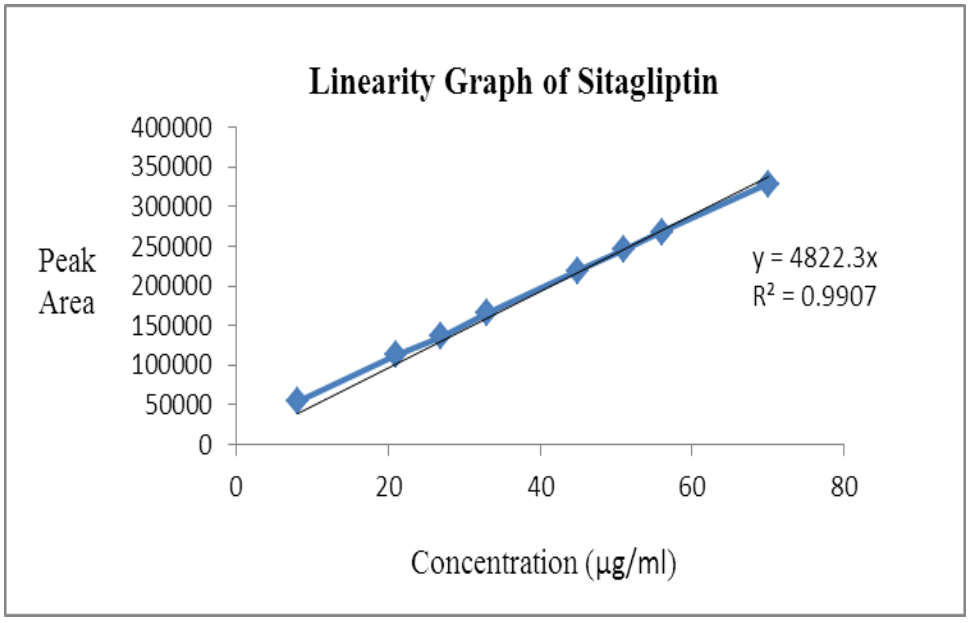

Fig:12 Linearity graph of Sitagliptin Phosphate.

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 50µg/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	499.56±0.63	0.23	499.81±0.27	0.25
Sitagliptin Phosphate	50	49.05±0.45	0.42	48.47±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 50µg/mL for SITA. 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 Sitagliptin Phosphate
1	50%	99.1	99.4
2	100%	100.4	98.3
3	150%	99.9	99.4

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 Sitagliptin Phosphate 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 TEA buffer & Methanol in the ratio of 80:20 v/v was used. A typical chromatogram obtained by using the above mentioned mobile phase from 20µl of the assay preparation is illustrated below. The retention times of Sitagliptin Phosphate and Metformin hydrochloride were 3.6 and 5.3 min, respectively. The linearity of the method was tested from 8-70µg/ml for Sitagliptin Phosphate and 80-730µg/ml for Metformin hydrochloride. Correlation coefficients for the regression line were 0.9956 and 0,9993 for Sitagliptin Phosphate 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 Sitagliptin Phosphate and Metformin hydrochloride ranged from 99.8- 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 Sitagliptin Phosphate 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 Sitagliptin Phosphate. 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 Sitagliptin Phosphate. This method exhibited an excellent performance in terms of sensitivity and speed and it helps in simultaneous estimation of Metformin Hydrochloride and Sitagliptin Phosphate in pharmaceuticals i.e., in combination drugs. This method is suitable for routine analysis and quality control of pharmaceuticals.

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