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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR IMPURITIES OF SITAGLIPTIN BY RP-HPLC

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ABSTRACT:

A simple, precise, and accurate RP-HPLC method has been developed and validated for the impurities in SITAGLIPTIN tablets. A gradient separation was achieved using Kromasil C18, 4.6 ×250 mm, 5.0µm particle size column with a flow rate of 1.0 ml/minute and PDA Detector at 210 nm. The mobile phase-A consisted of a mixture of pH 4.5 Buffer and Acetonitrile in the ratio of 90:10, mobile phase-B consisted of mixture of methanol and acetonitrile in the ratio of 50:50 and column temperature is 40°C. Methanol is used as Diluent. The method was validated for specificity, linearity and accuracy. The specificity of the method was determined by assessing interference from placebo and stress testing the drug product (forced degradation). The method was linear and accurate. The method was found to be suitable for the related substance in SITAGLIPTIN tablets. Degradation products resulting from the stress studies did not interfere with the detection of SITAGLIPTIN peak in chromatogram, demonstrating the stability indicating power of method. The method is useful for the determination of following impurities Triazole impurity, Acid impurity, Dioxo impurity, Ketoamide impurity and Enamine impurity.

Key words: Sitagliptin, Triazole impurity, Acid impurity, Dioxo impurity, Ketoamide impurity, Enamine impurity, RP-HPLC.

INTRODUCTION:

Type 2 diabetes is a condition in which body either produces inadequate amounts of insulin to meet the demands of the body or insulin resistance has developed.

Sitagliptin is an oral anti diabetic agent which act as dipeptidyl peptidase-4 inhibitor used for the management of type 2 diabetes mellitus.it is used in conjugation with diet and exercise to improve glycaemic control in patients with type 2 diabetes mellitus. The effect of this medication leads to glucose dependent increases in insulin and decreases in glucagon to improve control of blood sugar.

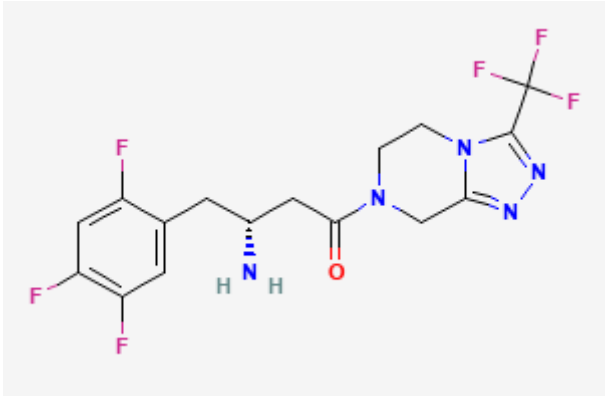

Fig.1.Structure of Sitagliptin

The development of Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) methods for analysing impurities in SITAGLIPTIN is essential for ensuring the purity, safety, and efficacy. Impurities can affect the drug's performance and patient safety. RP-HPLC provides a powerful and precise analytical tool for detecting and quantifying these substances, which is crucial in drug development and quality control.

MATERIALS AND METHODOLODY:

Samples and Standard: The SITAGLIPTIN working standard and tablets were provided by laboratory. The grade of the material used were listed in below table.

Table-1 Chemicals utilized

S.NO	Chemicals/Reagents	Make	Grade
3.	Acetonitrile	Supelco	HPLC Grade
4.	Methanol	Supelco	HPLC Grade
5.	Orthophosphoric acid	Supelco	Lab reagent Grade
6.	Potassium dihydrogen phosphate	Supelco	Reagent Grade
7.	Water	Millipore	Milli-Q
8.	Ammonium formate	SRL	Reagent Grade

Equipment's: Instruments used for the present study:

HPLC Model: Waters 2695 alliance.

Analytical balance (Sartorius), pH meter (Hanna), centrifuge (Remi), rotary shaker (Remi), Sonicator (Samarth), Vacuum/ Pressure pump (Borosil)), were used for this work.

Chromatographic parameters:

Liquid chromatography equipped with PDA detector

Table-2 Chromatographic conditions

Column	Kromasil C18,4.6 ×250 mm, 5.0µm
Wavelength	210nm
Column temperature	40°C
Flow rate	1.0 ml/min
Run time	35.0 minutes
Injection volume	10µl

Table-3 Gradient program

Time (in minutes)	% Mobile Phase- A (%)	% Mobile phase- (%)
0.0	85	15
10	75	25
15	50	50
20	40	60
30	20	80
31	85	15
35	85	15

Mobile phase preparation:

Preparation of buffer solution: Accurately weigh and transfer about 1.0g of potassium dihydrogen phosphate and 10g of ammonium formate into 1000 ml water and dissolve, adjust pH of the solution to 4.5 with dilute Orthophosphoric acid and mix well. Filter through 0.22µ membrane filter.

Preparation of Mobile Phase-A: Buffer: Acetonitrile (90:10), mix well and sonicate to degas.

Preparation of Mobile Phase-B: Methanol: Acetonitrile (50:50), mix well and sonicate to degas.

Diluent: Methanol

Preparation of Blank solution: Use diluent as blank solution.

Preparation of Standard solution: weigh and transfer accurately 57.0 mg of Sitagliptin working standard into a 50 ml volumetric flask and add 30ml diluent. Sonicate to dissolve and makeup the mark with diluent. Pipette out 5ml of aforementioned solution into a 50ml volumetric flask and dilute to volume with diluent. Pipette out 1ml of aforementioned solution into a 50ml volumetric flask and dilute to volume with diluent.

Preparation of Placebo solution: Accurately weigh and transfer placebo blend equivalent to 100mg of Sitagliptin into a 100 ml volumetric flask, add 70ml of diluent to it and keep om a rotary shaker for 10 minutes, sonicate for 30 min at 20°C with intermittent shaking and dilute to volume with diluent and mix well. Centrifuge a portion of solution at 3500 RPM for 15 minutes.

Preparation of Sample solution: Weigh 20 tablets, take the tablets into a mortar and crush to fine powder with pestle. Accurately weigh and transfer the crushed powder equivalent to 100mg of Sitagliptin into a 100ml volumetric flask, add 70ml of diluent to it and keep on rotary shaker for 10 minutes, sonicate for 30minutes at 20°C with intermittent shaking and dilute to volume with diluent and mix well. Centrifuge a portion of solution at 3500 RPM for 15 minutes at room temperature.

RESULTS AND DISCUSSIONS :

Method development:

The system was set up by equilibrating the column with mobile phase and then injecting the solution to determine the necessary parameters of the system, a single injection of blank and single injection of Sitagliptin standard solution was performed.

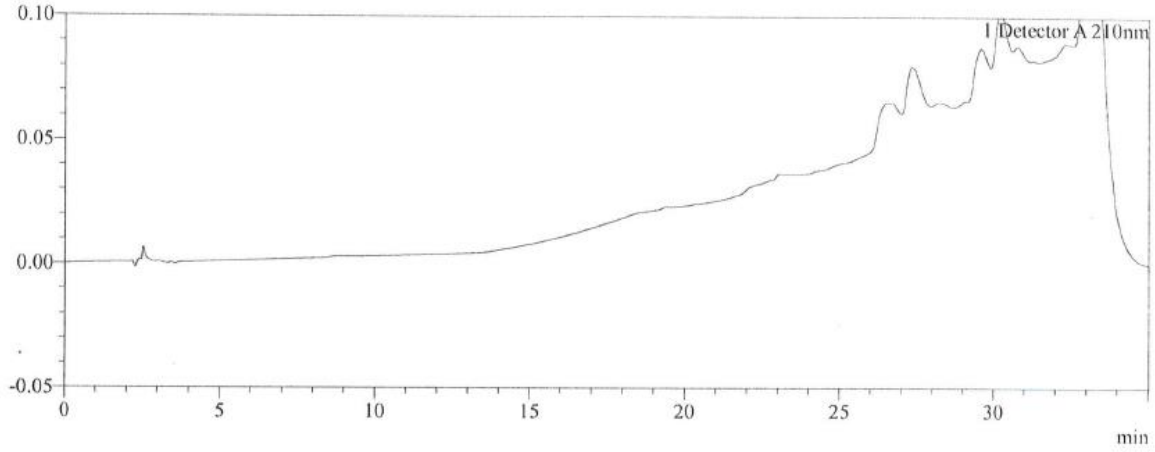

Fig.2 Blank solution chromatogram

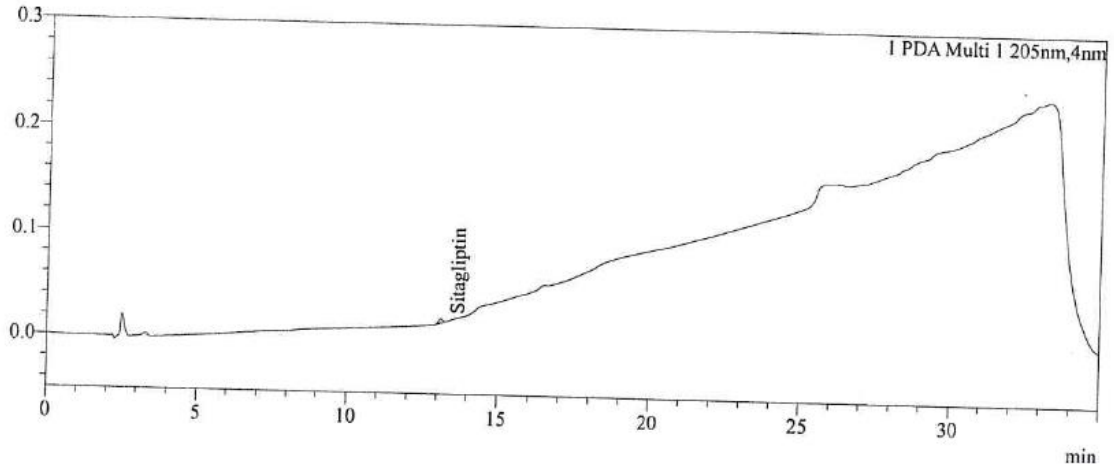

Fig.3 Standard solution chromatogram

Method Validation:

The developed and optimised HPLC method was validated according to ICH guidelines.

System suitability: The system suitability parameters were determined by preparing standard solution of Sitagliptin and the solution were injected six times after injecting a blank solution.

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2.

All the system suitable parameters of Sitagliptin were passed and were within the limits.

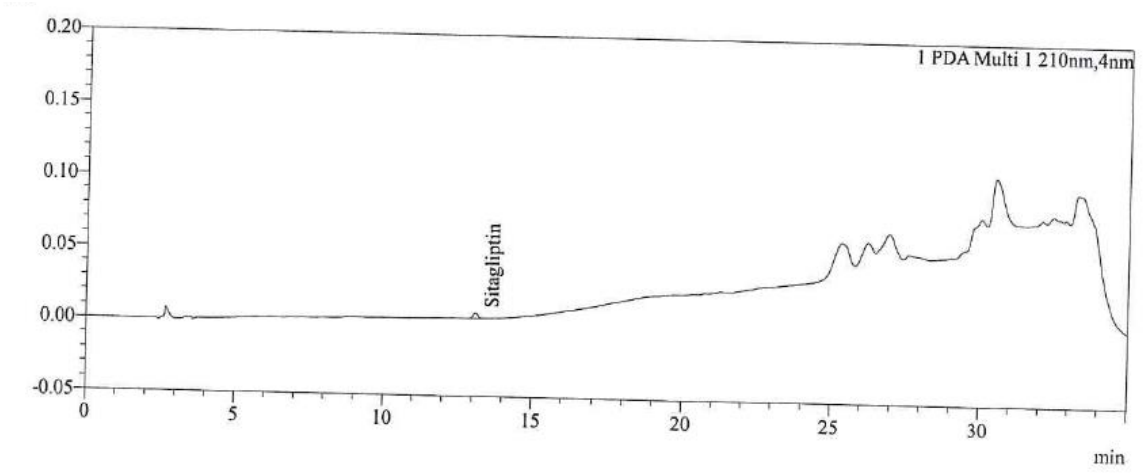

Fig.4 system suitability chromatogram

Specificity:

Blank, placebo and impurity interferences solution were injected and compared, there is no peak found in the blank. Placebo and impurity at the retention time of drug. So, the method developed was specific and do not have any interference.

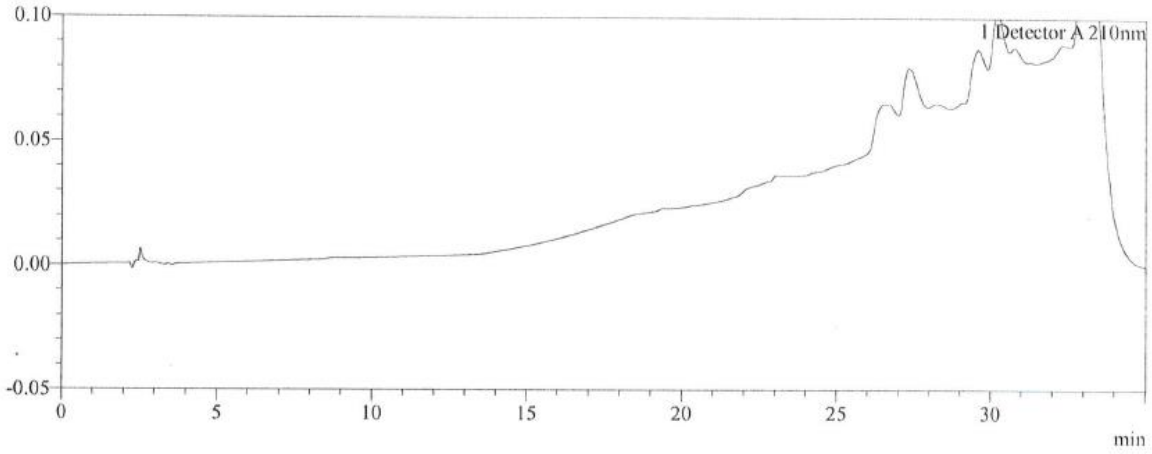

Fig.5: Blank chromatogram

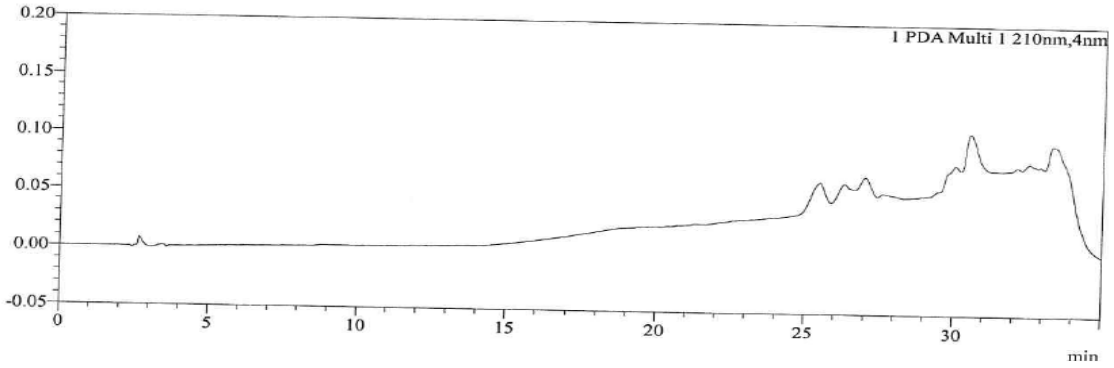

Fig.6: placebo chromatogram

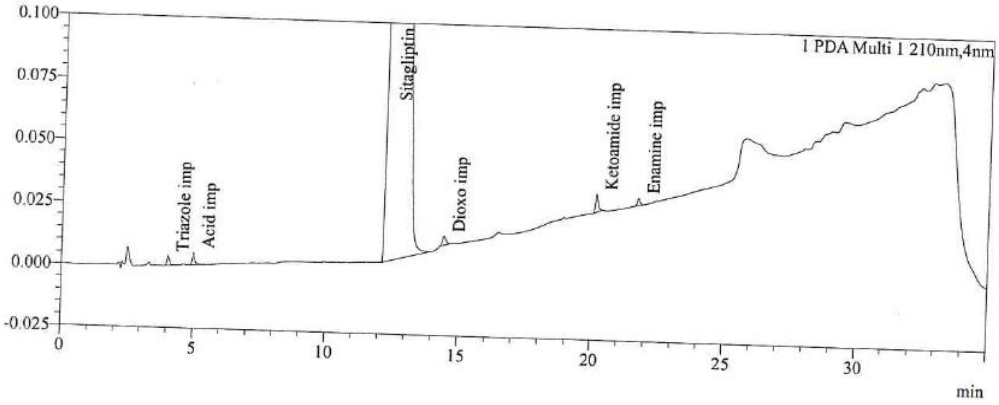

Fig.7: Impurity interference chromatogram

Linearity:

Linearity of detector was established by plotting a graph of concentration and responses of Sitagliptin. The detector response was found to be linear. Results were tabulated in table

Table-4 linearity table

S.No.	Description	RT	RRT	Slope	r^2 Value	RRF
1	Sitagliptin	12.3	1.00	3754	0.999	1.00
2	Triazole impurity	4.0	0.33	6835	0.999	0.41
3	Acid impurity	5.0	0.41	12124	0.999	0.72
4	Dioxo impurity	14.5	1.18	11085	0.992	0.66
5	Ketoamide impurity	20.1	1.63	20802	0.996	1.24
6	Enamine impurity	21.7	1.76	9565	0.999	0.57

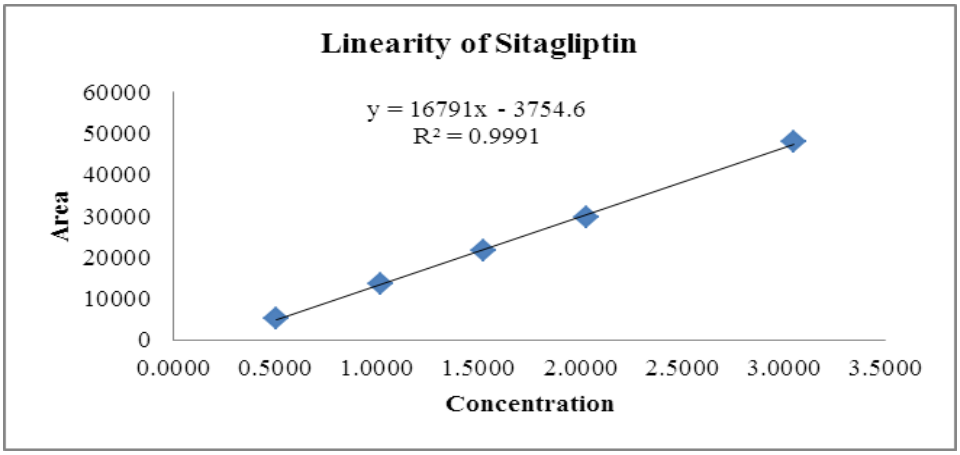

Fig.8: Calibration curve of Sitagliptin

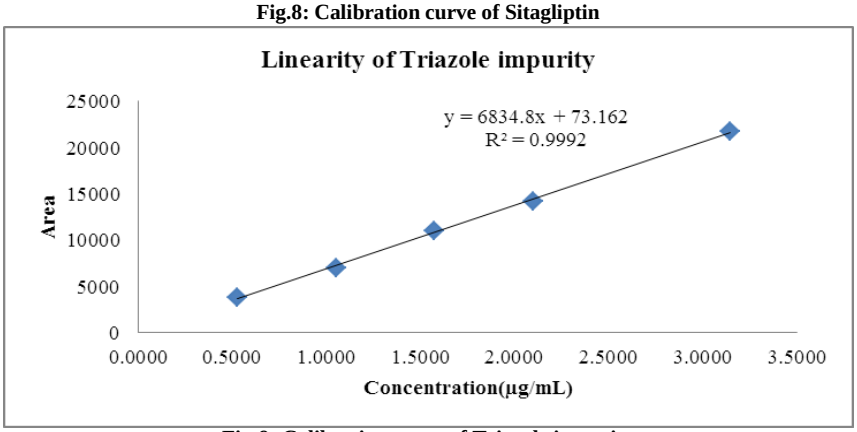

Fig-9: Calibration curve of Triazole impurity

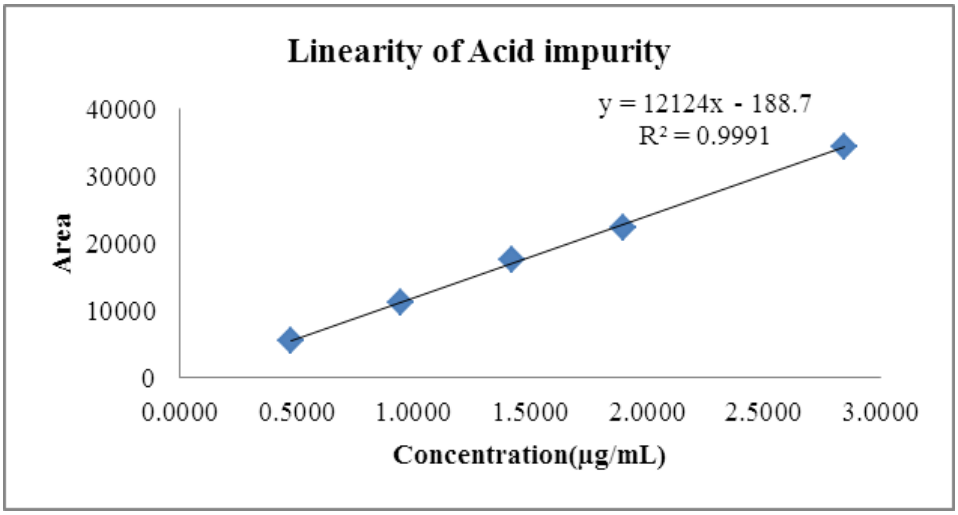

Fig.10: calibration curve of acid impurity

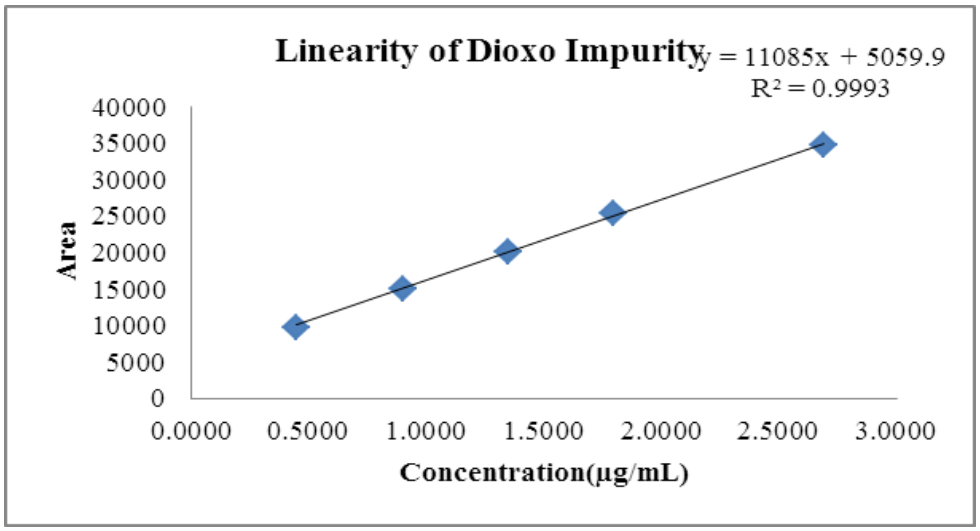

Fig.11: calibration curve of Dioxo impurity

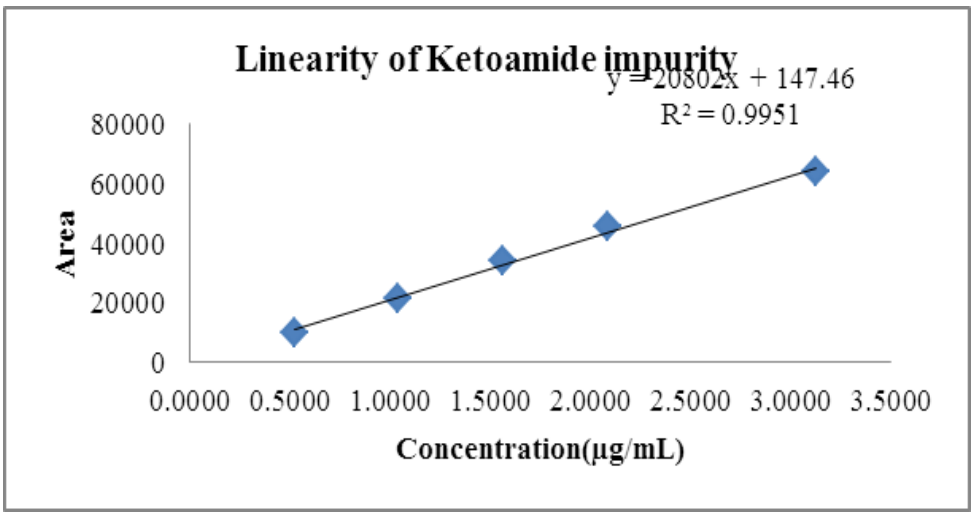

Fig.12: calibration curve of Ketoamide impurity

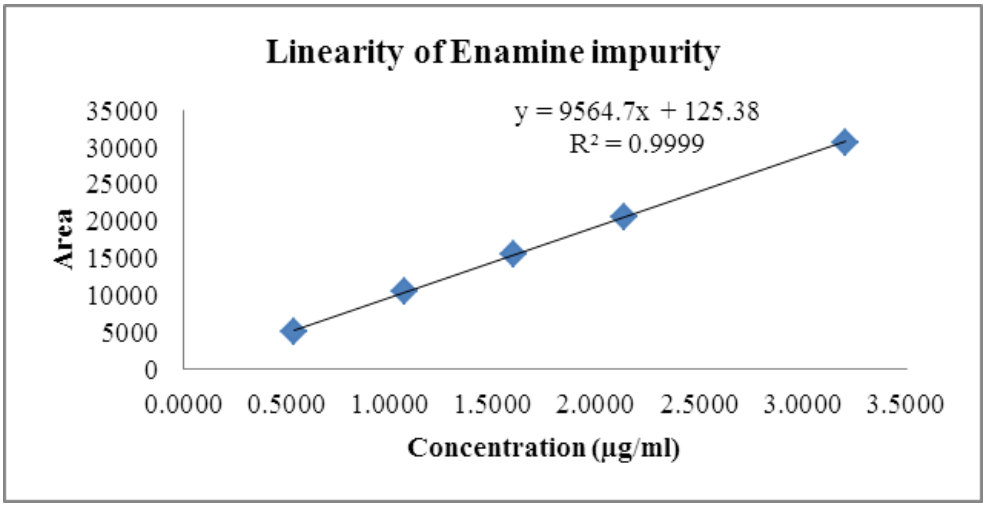

Fig.13: calibration curve of Enamine impurity

Report: The method was found to be linear.

Accuracy:

A series of sample solutions were prepared in duplicate by spiking the Sitagliptin and their impurities and injected into HPLC system and analysed.

Table-5 accuracy table

% of Conc	Dilution scheme from each stock solution in mL						Final Volume (mL)	Concentration for Known imp (µg/mL)	Concentration for Sitagliptin (µg/mL)
	Triazole Imp	Acid Imp	Dioxo Imp	Ketoamide Imp	Enamine	Sitagliptin			
25%	0.25	0.25	0.25	0.25	0.25	0.25	50	0.5	0.5
50%	0.5	0.5	0.5	0.5	0.5	0.5	50	1.0	1.0
75%	0.75	0.75	0.75	0.75	0.75	0.75	50	1.5	1.5
100%	1	1	1	1	1	1	50	2.0	2.0
150%	1.5	1.5	1.5	1.5	1.5	1.5	50	3.0	3.0

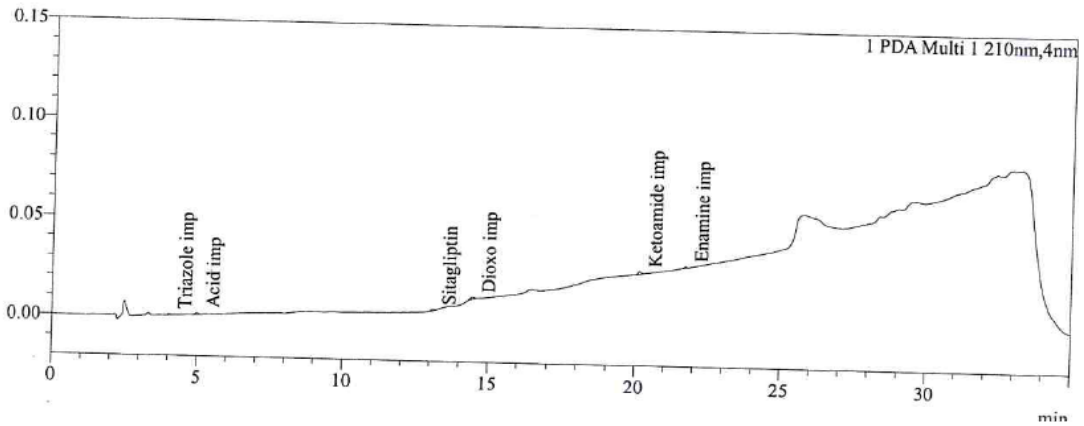

Fig.14: 25% specification level solution

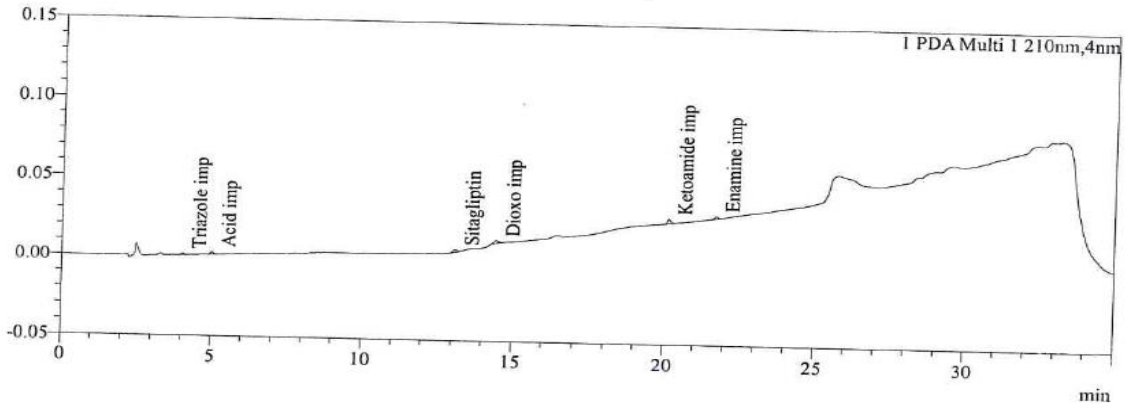

Fig.15: 50% specification level solution

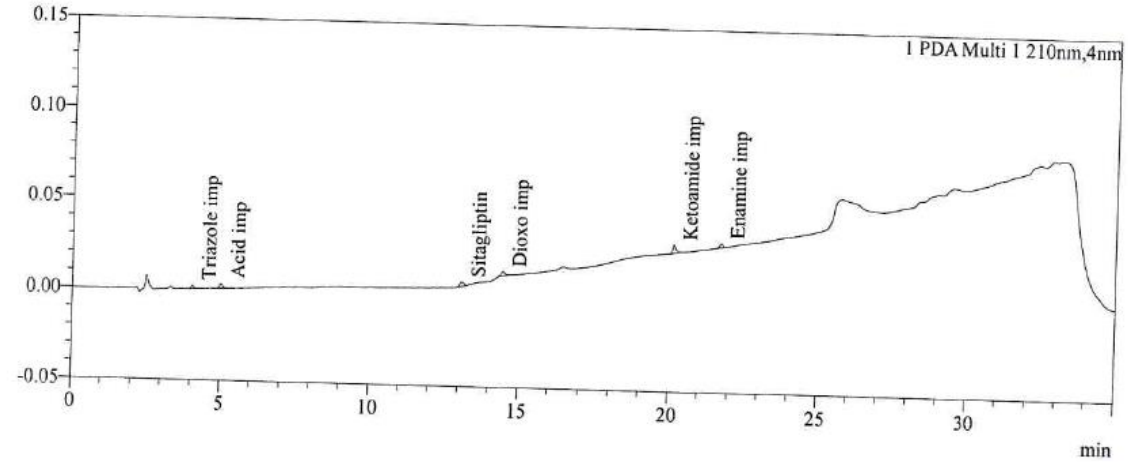

Fig.16: 75% specification level

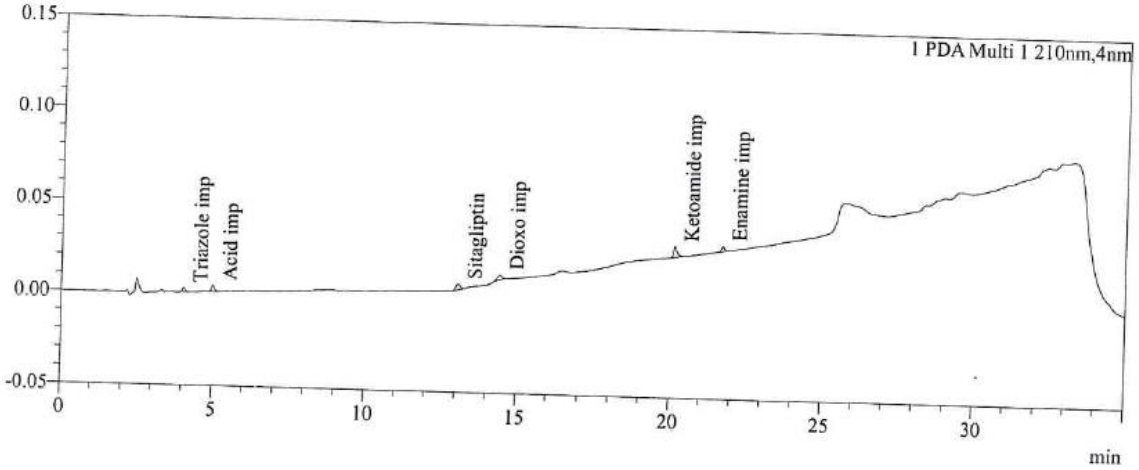

Fig.17: 100% specification level solution

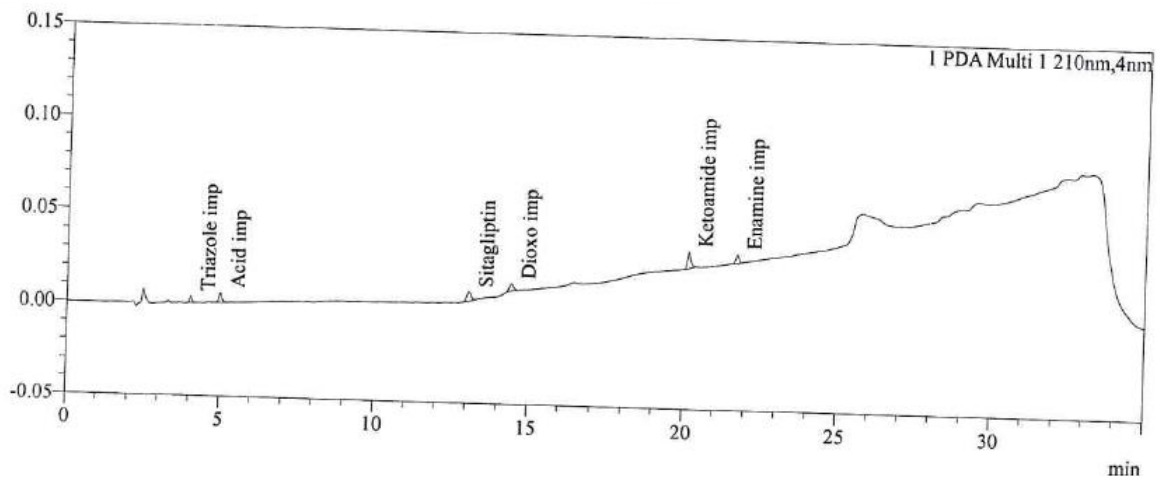

Fig.18: 150% specification level solution

Results: method was found to be accurate.

Forced degradation:

Sitagliptin drug product were subjected to following stress conditions:

Acid hydrolysis: 5N HCL/60°C/1Hour

Base hydrolysis: 2N NaOH/60°C/1Hour and 5N NaOH/60°C/1Hour

Oxidation: 10% Hydrogen peroxide/50°C/1Hour

Water: 5ml water/50°C/1Hour

Table-6 Forced degradation data

Stress Condition	Triazole imp	Acid imp	Dioxo imp	Ketoamide imp	Enamine imp	Unknown maximum	Total imp
As such sample	ND	ND	ND	ND	ND	ND	NA
5 N Acid stress	3.19	2.20	ND	ND	ND	0.87	7.04
5 N Base stress	5.71	5.97	ND	ND	ND	0.03	11.71
Oxidation	0.03	0.02	ND	ND	ND	0.08	0.14
Water	0.01	ND	ND	ND	ND	0.02	0.03

Table- 7 peak purity table

S.No.	Description	Retention time (minutes)	Peak purity
1	Sitagliptin	13.08	0.9999
2	Triazole impurity	4.28	0.9999
3	Acid impurity	5.30	0.9999
4	Dioxo impurity	15.72	0.9998
5	Ketoamide impurity	20.81	0.9999
6	Enamine impurity	22.48	0.9999

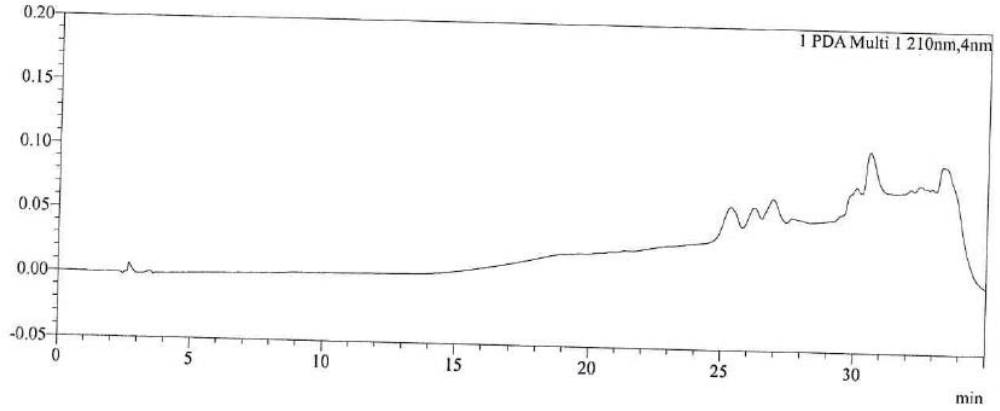

Fig.19: blank chromatogram

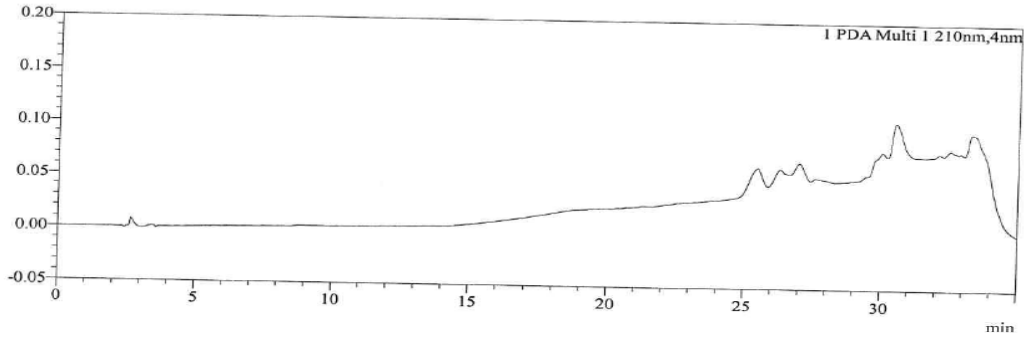

Fig.20: placebo chromatogram

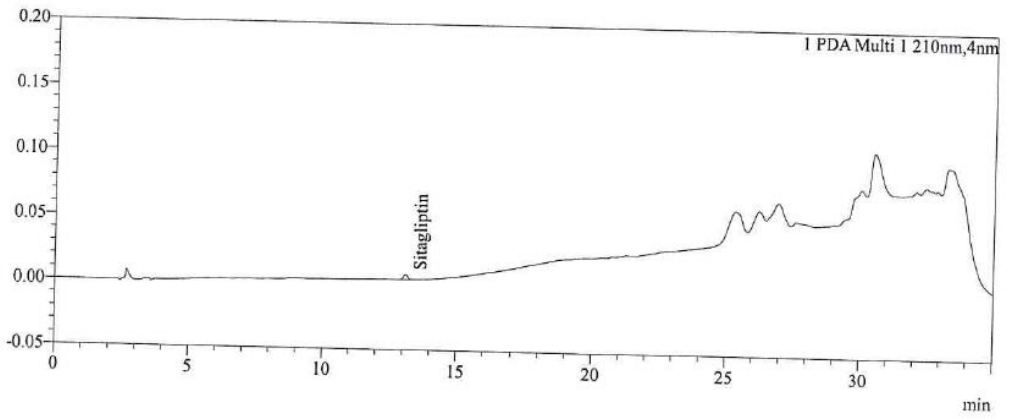

Fig.21: standard chromatogram

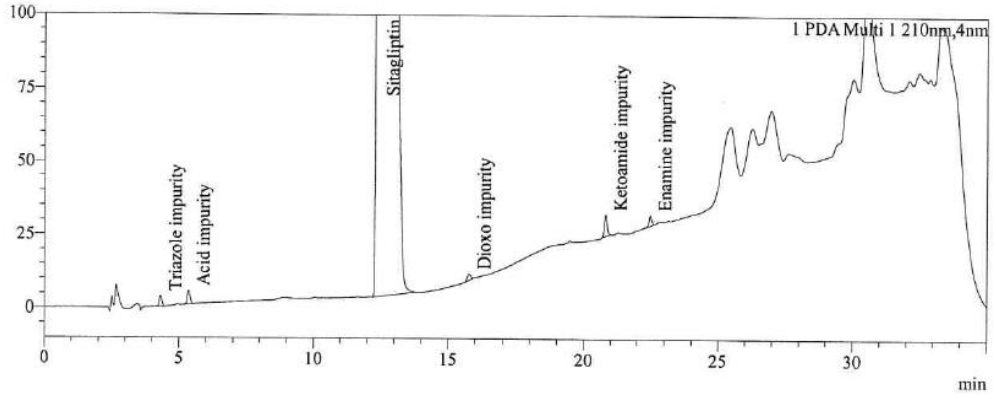

Fig.22: system suitability chromatogram

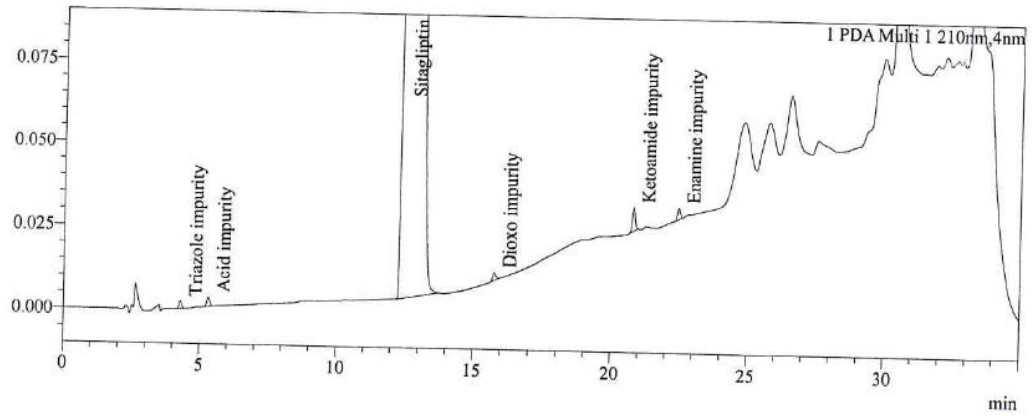

Fig.23: spiked sample chromatogram

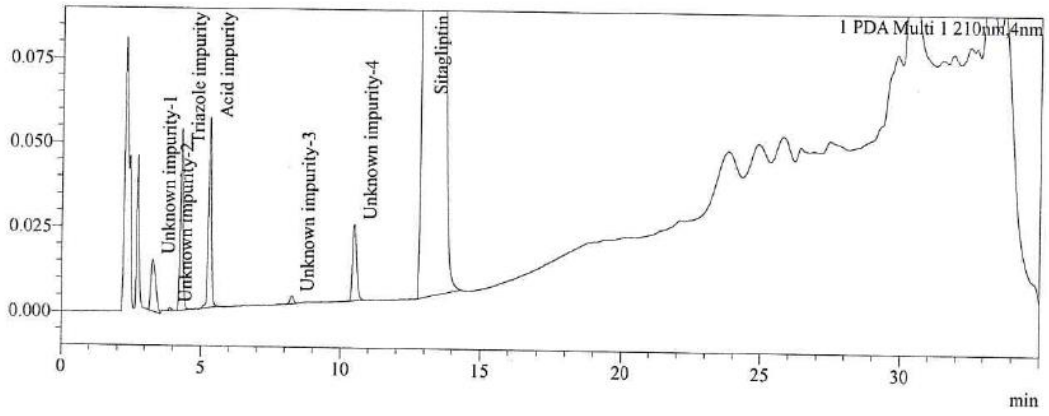

Fig.24: acid stress sample chromatogram

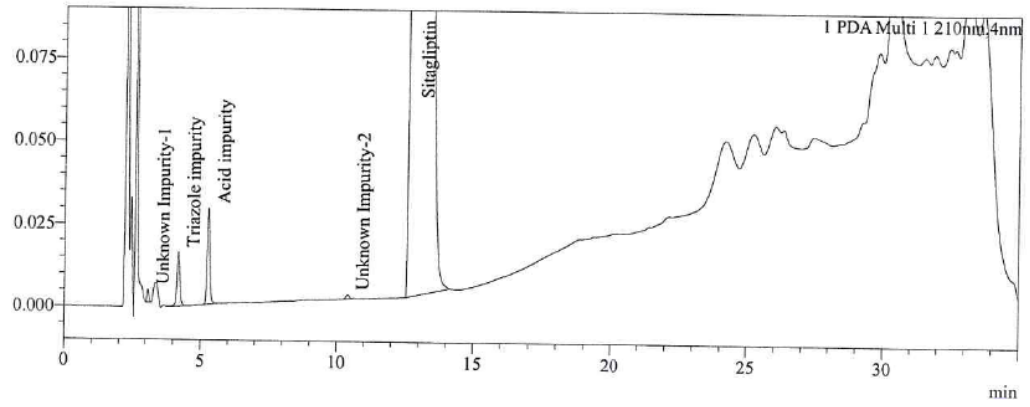

Fig.25: base stress sample chromatogram

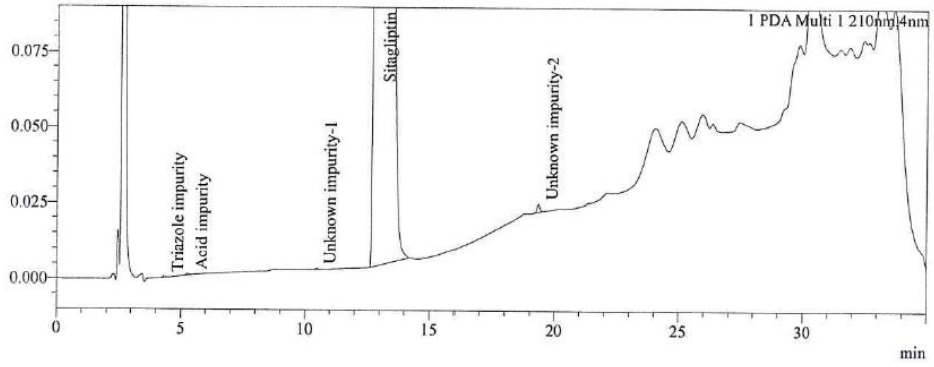

Fig.26: peroxide stress sample chromatogram

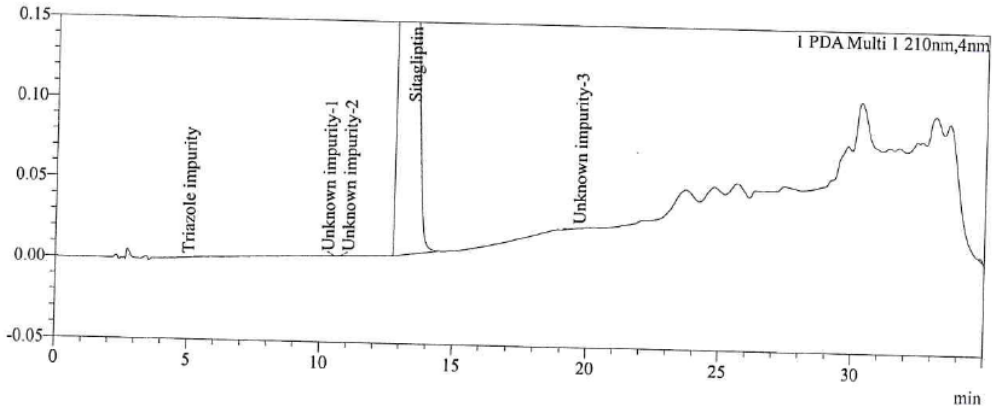

Fig.27: water stress sample chromatogram

Acceptance criteria

The method is considered selective if: The analytes of interest are free of interference from the blank and placebo and from each other. Peaks are considered to be pure and positively identified if purity angle met the criteria. Peak purity pass= The purity angle is less than the purity threshold.

CONCLUSION:

A simple, accurate method was developed for the estimation of related substances of Sitagliptin tablets. Retention time of Sitagliptin, Triazole impurity, acid impurity, Dioxo impurity, Ketoamide impurity and enamine impurity were found to be 4.0min, 5.0 min, 14.5 min, 20.1 min and 21.7 min. %Recovery was obtained as 100%. The developed method was validated as per described in the ICH Q2B guidelines like system suitability, specificity, linearity, accuracy and recovery. The proposed method has the uses of and requires less expensive reagents. In forced degradation studies all main peak purity angles were founded less than peak purity threshold so this method is defined as stability indicating method. Hence this method can be used for routine analysis.

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