RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF SITAGLIPTIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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***ABSTRACT** A simple, rapid, precise, accurate and sensitive reverse phase liquid chromatographic method has been developed for the determination of Sitagliptin in bulk and pharmaceutical dosage form dosage form. The chromatographic method was standardized using Develosil ODS HG-5 RP C18, 5µm, 15cm x 4.6mm i.d. column with UV detection at 255 nm and (0.05M) Phosphate Buffer : Acetonitrile with 30:70 (pH-2.8) ratio at a flow rate of 1.0 ml/ min. The proposed method was successfully applied to the determination of Sitagliptin in bulk and pharmaceutical dosage form. The method was linear over the range of 30-70µg/ml. The recovery was in the range of 98% to 102% and limit of detection was found to be 0.09 µg/ml and quantification was found to be 0.027 µg/ml. Different analytical performance parameters such as precision, accuracy, limit of detection, limit of quantification and robustness were determined according to International Conference on Harmonization (ICH) guidelines.*
***Keywords:** RP-HPLC, Sitagliptin, Method development and validation, ICH Guidelines.*

I.INTRODUCTION

Sitagliptin, sold under the brand name Januvia among others, is an medication used to treat diabetes mellitus type 2. It is generally less preferred than metformin or a sulfonylurea. It is taken by mouth. It is also available within a single pill as metformin/sitagliptin.[1-4] Common side effects include headaches, swelling of the legs, and upper respiratory tract infections. Serious side effects may include angioedema, low blood sugar, kidney problems, pancreatitis, and joint pain.[5-8] Whether use in pregnancy or breastfeeding is safe is unclear. It is in the dipeptidyl peptidase-4 (DPP-4) inhibitor class and works by increasing the production of insulin and decreasing the production of glucagon by the pancreas. Sitagliptin is used to treat diabetes mellitus type 2.[9-12] It is generally less preferred than metformin or sulfonylureas. It is taken by mouth. It is also available within a single pill as metformin/sitagliptin.[13-16] Adverse effects from sitagliptin are similar to placebo, except for rare nausea, common cold-like symptoms, and photosensitivity. It does not increase the risk of diarrhea. No significant difference exists in the occurrence of hypoglycemia between placebo and Sitagliptin. In those taking sulphonylureas, the risk of low blood sugar is increased[17-18].

The IUPAC Name of Sitagliptin is (3R)-3-amino-1-[3-(trifluoromethyl)-5H,6H,7H,8H-[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one.[19-20]

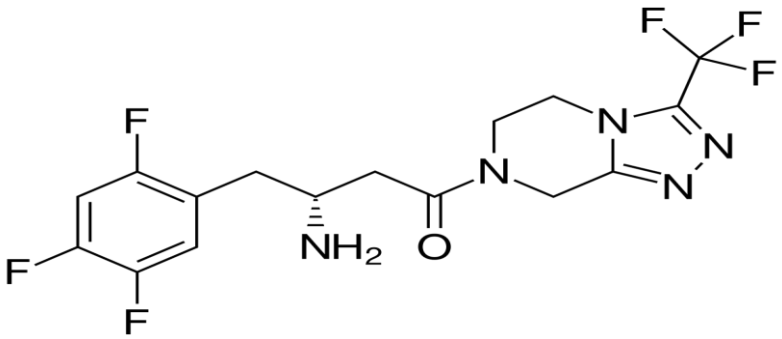


Fig 1: Chemical Structure of Sitagliptin

II.MATERIALS AND METHODS
METHOD DEVELOPMENT

HPLC Instrumentation & Conditions:

The HPLC system employed was HPLC with Empower2 Software with Isocratic with UV-Visible Detector.

Standard & sample preparation for UV-spectrophotometer analysis :

25 mg of Sitagliptin standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.2 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase. The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Sitagliptin, so that the same wave number can be utilized in HPLC UV detector for estimating the Sitagliptin. While scanning the Sitagliptin solution we observed the maxima at 255nm. The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450.

Optimized Chromatographic Conditions:

Column: Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.

Mobile Phase : Acetonitrile : (0.05M) Phosphate buffer (pH-2.8) in 70:30.

Flow Rate : 1.0ml/minute

Wave length : 255 nm

Injection volume : 20µl
Run time : 08 mins.
Column temperature : Ambient
Sampler cooler : Ambient

Mobile Phase Preparation :

Mobile phase was prepared by taking Acetonitrile : (0.05M) Phosphate buffer (pH-2.8) (70:30v/v). Mobile phase was filtered through 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min.

Sample & Standard Preparation for the Analysis :

25 mg of Sitagliptin standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

Method Validation

Accuracy:

Recovery study : To decide the exactness of the proposed strategy, recuperation thinks about were completed by including diverse sums (80%, 100%, and 120%) of unadulterated medication of Sitagliptin were taken and added to the pre-dissected detailing of fixation 50µg/ml. From that rate recuperation esteems were ascertained. The outcomes were appeared in Table-3.

Precision :

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Sitagliptin (API) the percent relative standard deviations were calculated for Sitagliptin is presented in the Table-4.

Intermediate Precision :

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Sitagliptin revealed that the proposed method is precise.

Linearity and Range :

Linearity range was found to be 30-70µg/ml for Sitagliptin. The correlation coefficient was found to be 0.999, the slope was found to be 11266 and intercept was found to be 50416 for Sitagliptin.

Method Robustness :

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature ($\pm 2^0$C), Wavelength of detection (±2nm) & Acetonitrile content in mobile phase (±2%) studied to determine the

robustness of the method are also in favour of (Table-7, % RSD < 2%) the developed RP-HPLC method for the analysis of Sitagliptin (API).

LOD & LOQ :

The limit of detection (LOD) is the lowest concentration of analyte in a sample which can be detected, but not quantitated. LOD is a limit test that specifies whether an analyte is above or below a certain value. Signal-to-noise ratio of three-to-one is used to determine LOD.

The Limit of Quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. Signal-to-noise ratio of ten-to-one is used to determine LOQ.

Estimation of Sitagliptin in Pharmaceutical Dosage Form

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 100 mg of drugs were transferred to 100 ml volumetric flask, and 70 ml of HPLC grade methanol was added and solution was sonicated for 15 minutes, there after volume was made up to 100 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with HPLC grade methanol. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (3.5 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system. The solution prepared was injected in five replicates into the HPLC system and the observations were recorded. A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-8.

Stability Studies

Acid Degrdation :

An accurately weighed 10 mg of pure drug was transferred to a clean & dry round bottom flask. 30 ml of 0.1 N HCl was added to it and it was refluxed in a water bath at 60^0C for 4 hours. Allowed to cool to room temperature. The sample was then neutralized using dilute NaOH solution & final volume of the sample was made up to 100ml with water to prepare 50 µg/ml solution. It was injected into the HPLC system against a blank of mobile phase (after optimizing the mobile phase compositions). This experiment was repeated several times using same concentration of HCl (0.1N) and observed its degradation profile. The typical chromatogram shown below is the degradation profile of Sitagliptin in 0.1N HCl.

Basic Hydrolysis :

An accurately weighed 10 mg of pure drug was transferred to a clean & dry round bottom flask. 30 ml of 0.1N NaOH was added to it. & it was refluxed in a water bath at 60^0C for 4 hours. Allowed to cool to room temperature. The sample was than neutralized using 2N HCl solution & final volume of the sample was made up to 100ml to prepare 100 µg/ml solution. It was injected into the HPLC system against a blank of mobile phase after optimizing the mobile phase compositions. This experiment was repeated several times using same concentration of NaOH such as 0.1N to observe its degradation profile. The chromatogram shown below is the degradation profile of Sitagliptin in 0.1N NaOH.

Thermal Degradation :

Accurately weighed 10 mg of pure drug was transferred to a clean & dry round bottom flask. 30 ml of HPLC water was added to it. Then, it was refluxed in a water bath at 60^0 c for 6 hours uninterruptedly. After the reflux was over, the drug became soluble and the mixture of drug & water was allowed to cool to room temperature. Final volume was made up to 100 ml with HPLC water to prepare 100 µg/ml solution. It was injected into the HPLC system against a blank of mobile phase.

Photolytic Degradation :

Approximately 10 mg of pure drug was taken in a clean & dry Petri dish. It was kept in a UV cabinet at 254 nm wavelength for 24 hours without interruption. Accurately weighed 1 mg of the UV exposed drug was transferred to a clean & dry 10 ml volumetric flask. First the UV exposed drug was dissolved in methanol & made up to the mark with mobile phase to get 100 µg/ml solution. Finally this solution was injected into the HPLC system against a blank of mobile phase and chromatogram was obtained.

Oxidation With (3%) H_2O_2 :

Accurately weighed 10 mg. of pure drug was taken in a clean & dry 100 ml volumetric flask. 30 ml of 3% H_2O_2 and a little methanol was added to it to make it soluble & then kept as such in dark for 24 hours. Final volume was made up to 100 ml. using water to prepare 100 µg/ml solution. The above sample was injected into the HPLC system.

III.RESULT AND DISCUSSION

Method Development

UV Spectrophotometric Method :

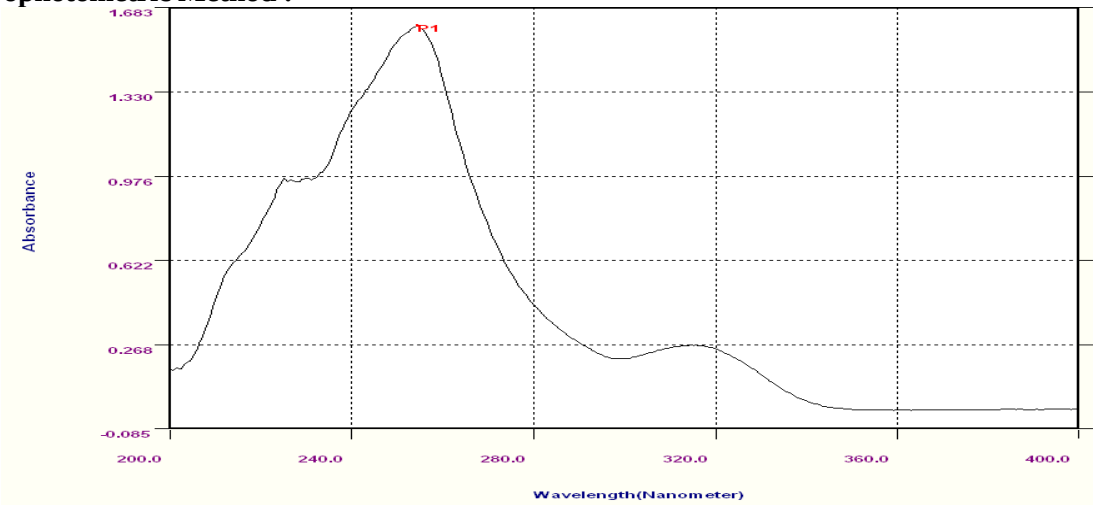
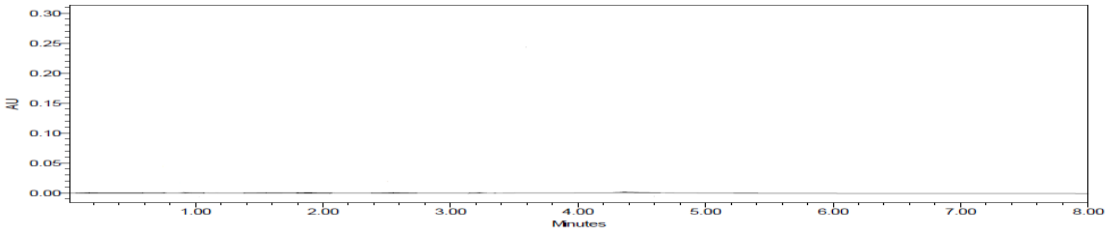


Fig 2: UV Spectrum

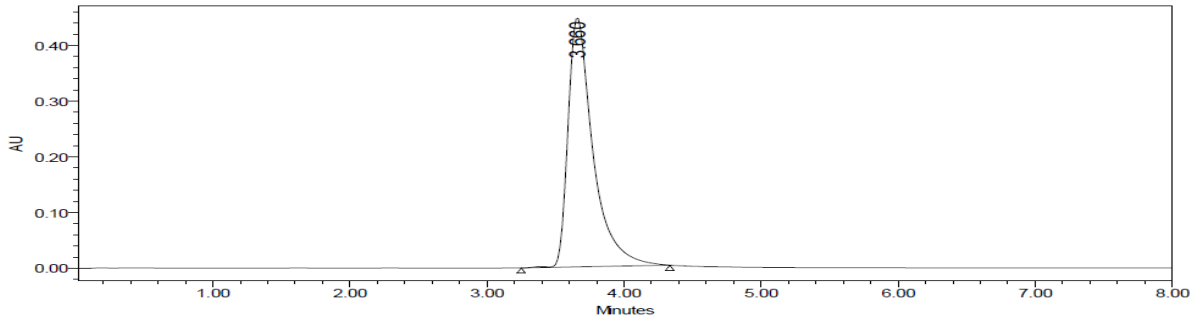
Table-1: Trials for Method Development

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Methanol : Acetonitrile = 50 : 50	1.0ml/min	255nm	Very Low response	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Methanol : Acetonitrile = 70 : 30	1.0ml/min	255nm	Low response	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Phosphate Buffer : Methanol = 35:65 (pH-5.2)	1.0ml/min	255nm	Tailing peaks	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Phosphate Buffer : Acetonitrile = 30:70 (pH-4.8)	1.0ml/min	255nm	Resolution was not good	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Phosphate Buffer : Acetonitrile = 60:40 (pH-3.6)	1.0ml/min	255nm	Tailing peak	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	(0.05M) Phosphate Buffer : Acetonitrile = 30:70 (pH-2.8)	1.0ml/min	255nm	Nice and Good peak	Method accepted

Optimized Condition :



Chromatogram for Blank Preparation



Optimized Condition

Table 2: Peak Results

S.No.	Drug Name	Rt	Peak Area	Theoretical Plates	Tailing Factor
1	Sitagliptin	3.660	5652284	5634	1.58

Method Validation
Accuracy:

Table-3: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	40	40.141	502647	100.352	Mean= 100.3947% S.D. = 0.071319 % R.S.D.= 0.071038
S_2 : 80 %	40	40.191	503214	100.477	
S_3 : 80 %	40	40.142	502656	100.355	
S_4 : 100 %	50	50.044	614215	100.088	Mean= 99.98533% S.D. = 0.183045 % R.S.D.= 0.183071
S_5 : 100 %	50	49.887	612451	99.774	
S_6 : 100 %	50	50.047	614254	100.094	
S_7 : 120 %	60	60.192	728547	100.32	Mean= 100.311% S.D. = 0.408574 % R.S.D.= 0.407308
S_8 : 120 %	60	59.939	725698	99.898	
S_9 : 120 %	60	60.429	731211	100.715	

Precision :
Repeatability

Table-4: Repeatability Results of Precision

HPLC Injection Replicates of Sitagliptin	Retention Time (Min)	Peak Area (AUC)
Replicate – 1	3.649	5674158
Replicate – 2	3.684	5654715
Replicate – 3	3.687	5665841
Replicate – 4	3.688	5654578
Replicate – 5	3.688	5652284
Replicate – 6	3.687	5641487
Average		**5657177**
Standard Deviation		**11369.72**
% RSD		**0.200979**

Intermediate Precision:

Table-5: Results of Intra day & Inter day

Conc. Of Sitagliptin (API) (µg/ml)	Observed Conc. Of Sitagliptin (µg/ml) by the proposed method			
	Intra day		Inter day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
40	40.05	1.09	39.89	1.08
50	50.08	0.95	49.54	0.76
60	60.09	0.97	59.86	0.94

Linearity and Range

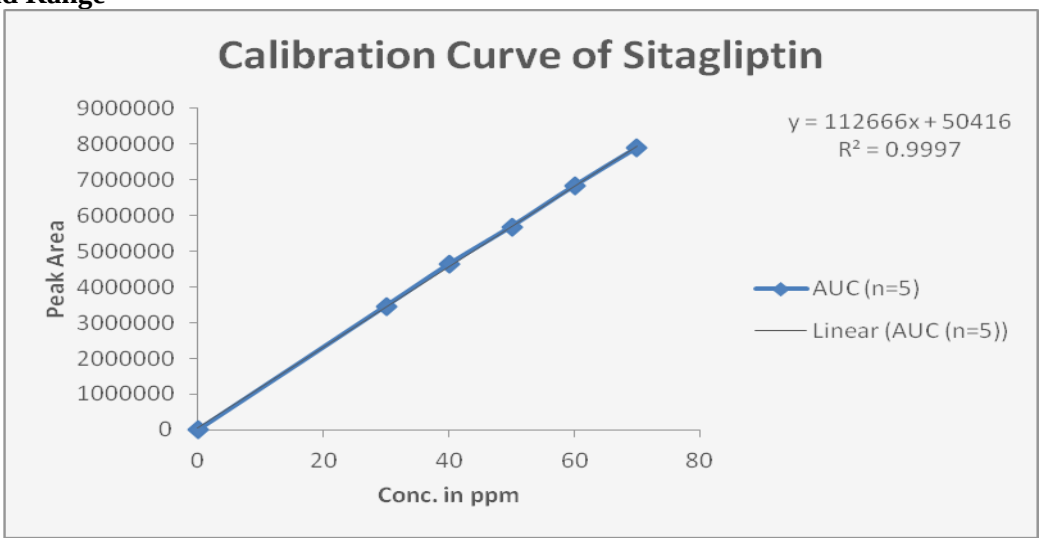


Fig-3: Calibration curve of Sitagliptin (API)

Table-6: Linearity Results of Sitagliptin

CONC.	AUC (n=5)
0	0
30	3465974
40	4626478
50	5682284
60	6815478
70	7878721

Method Robustness :

Table-7 : Result of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.56
Flow (0.9 ml/min)	0.87
Temperature (27^0C)	0.72
Temperature (23^0C)	0.53
Wavelength of Detection (257 nm)	0.61
Wavelength of detection (253 nm)	0.96

LOD & LOQ : The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.09 & 0.027 µg/ml respectively.

Estimation of Sitagliptin in Tablet Dosage Form

Table-8: Assay of SITAGLIPTIN Tablets

Brand Name of Tablets	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	% Purity
Januvia Tablets (100mg) (Merck & Co.)	100mg	99.895 (±0.056)	99.786 % (±0.584)

Stability Studies

Acid Degrdation :

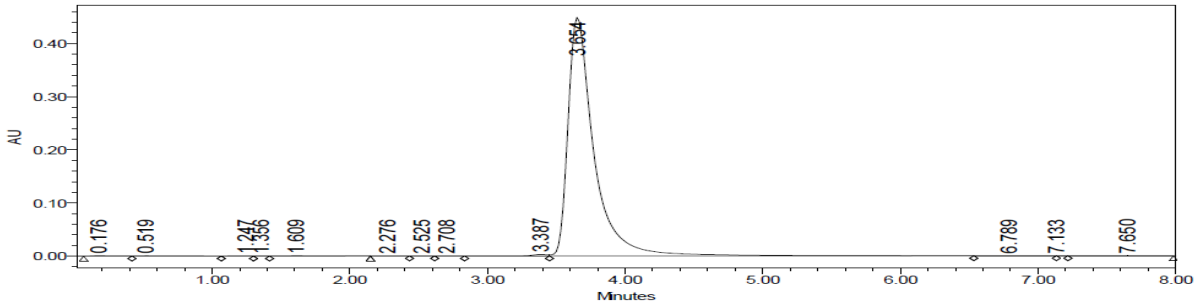


Fig-9: Acidic degradation

Basic Hydrolysis :

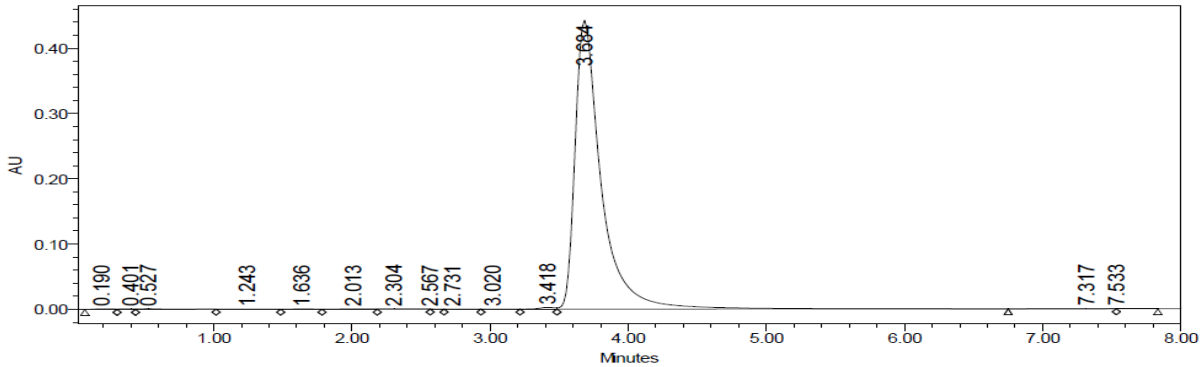


Fig.4: Basic degradation

Thermal Degradation :

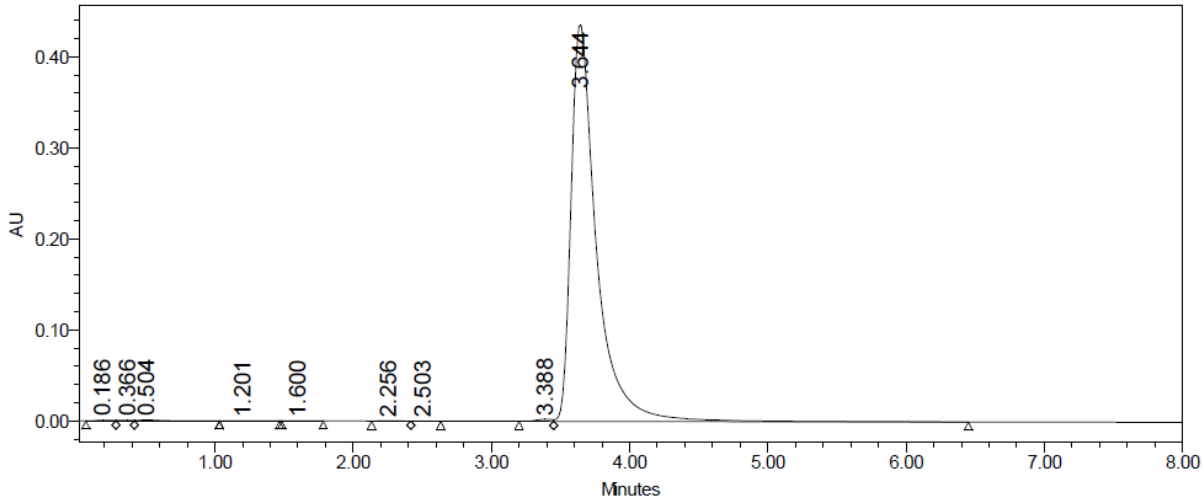


Fig-11: Thermal degradation studies

Photolytic Degradation :

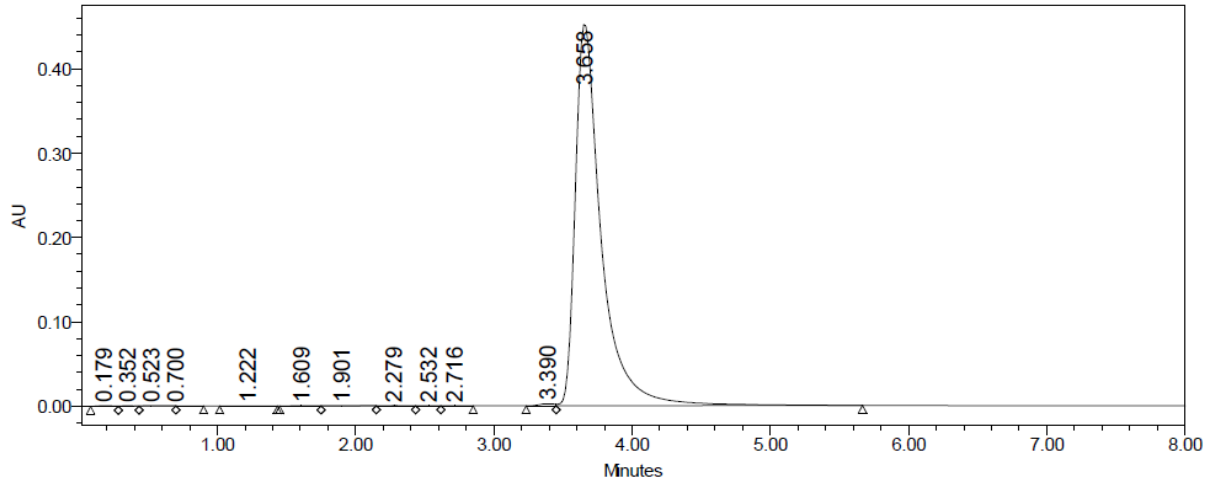


Fig.5: Photolytic degradation

Oxidation With (3%) H_2O_2 :

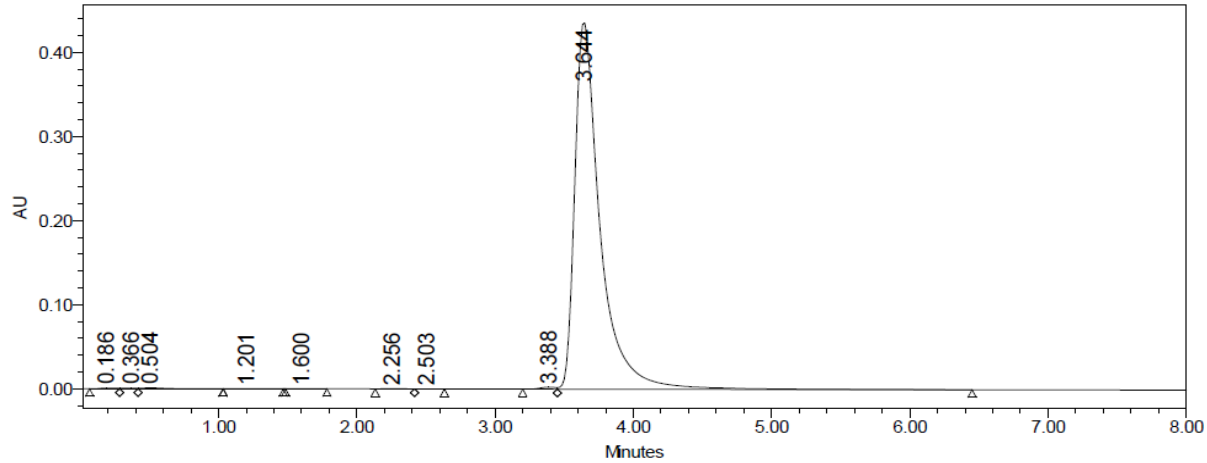


Fig.6: Peroxide degradation

Table-9: Results of forced degradation studies of Sitagliptin API.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	98.76	1.24	100.0
Basic Hydrolysis (0.I M NaOH)	24Hrs.	98.63	1.37	100.0
Thermal Degradation (50 ^{0}C)	24Hrs.	93.98	6.02	100.0
UV (254nm)	24Hrs.	98.84	1.16	100.0
3 % Hydrogen peroxide	24Hrs.	94.61	5.39	100.0

IV.CONCLUSION

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Sitagliptin API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is yet another suitable method for assay, purity which can help in the analysis of Sitagliptin in different formulations. Finally it was concluded that the method is simple, sensitive and has the ability to separate the drug from degradation products and excipients found in the dosage form.

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