Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 1, 2013

Research Article

STABILITY-INDICATING RPHPLC METHOD FOR ANALYSIS OF SITAGLIPTIN IN THE BULK DRUG AND IT'S PHARMACEUTICAL DOSAGE FORM

V.DEEPTHI*[1], POORNIMA.Y, G.DEVALA RAO, T.SANDEEP REDDY

Department of Pharmaceutical Analysis, K.V.S.R. Siddhartha College of pharmaceutical sciences, Vijayawada-520010, India.
Email: deepthi759@gmail.com

Received: 09 Nov 2012, Revised and Accepted: 11 Dec 2012

ABSTRACT

A novel stability-indicating RP-HPLC method has been develop and validated for quantitative analysis of Sitagliptin in the bulk drug and in its pharmaceutical dosage forms using Hypersil–BDS- C18 column (250x4.6mmi.d, 5µ particle size) with 10mM Phosphate buffer (PH-3.5): ACN 60:40%v/v as isocratic mobile phase enabled separation of the drug from its degradation products. UV detection was performed at 260 nm. The method was validated for linearity, accuracy (recovery), precision, sensitivity, ruggedness and robustness. The linearity of the method was excellent over the range 10–60µg/ml (correlation coefficient 0.999). The limits of detection and quantification were 0.21 and 0.640µg/ml, respectively. Recovery of Sitagliptin from the pharmaceutical dosage form ranged from 99.99 to 100.05%.

Sitagliptin was subjected to stress conditions (Hydrolysis (acid, base), oxidation, thermal and photo degradation) and the stressed samples were analysed by use of the method. Degradation was observed in acid, base, and 30% H2O2. The drug was stable under the other stress conditions investigated. The degradation products were well resolved from main peak. The forced degradation studies prove the stability indicating power of the method.

Keywords: Sitagliptin, Dosage form, RP-HPLC, Forced degradation, Method validation.

INTRODUCTION

Sitagliptin[1,2] is a new oral hypoglycemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. This enzyme-inhibiting drug is to be used either alone or in combination with metformin or a thiazolidinedione for control of type 2 diabetes mellitus. The drug works to competitively inhibit a protein/enzyme, dipeptidyl peptidase 4 (DPP-4), that results in an increased amount of active incretins (GLP-1 and GIP), reduced amount of release of glucagon (diminishes its release) and increased release of insulin.

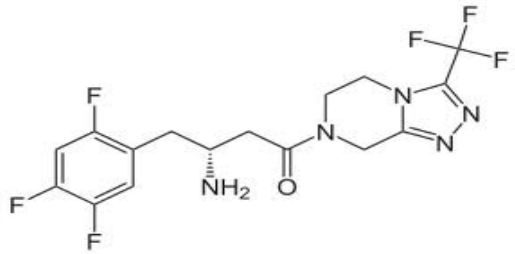

Fig. 1: structural formula of Sitagliptin

Stability testing forms an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, and light, which enables storage conditions, retest periods, and shelf life to be recommended. The two main aspects of study of the stability of a drug product that play an important role in shelf life determinations are assay of the active drug and the degradation products generated during stability studies. Assay of a drug product in a stability test sample must be performed with stability-indicating method, as recommended by the International Conference on Harmonization (ICH).

A literature survey revealed that very few methods[3,4,5,6,7,8] were developed and none of the reported procedures enables analysis of the Sitagliptin in pharmaceutical dosage forms in the presence of their degradation products. This manuscript describes the development and validation, in accordance with ICH guidelines, of arapid, economical, precise, and accurate stability-indicating isocratic RP-HPLC method for analysis of Sitagliptinin the presence of its degradation products.

MATERIALS AND METHODS

Chemicals and solutions

An analytically pure sample of Sitagliptin (purity 99.7%) was procured as gift sample from Granules pharmacy (Hyderabad, India) and Tablet formulation [JANUVIA (Brand name), Granules pharmacy, Hyderabad, India] was procured from a localpharmacy with labeled amount 50mg. Acetonitrile and Methanol (HPLC grade) were obtained from Merck Fine Chemicals(Mumbai, India), Potassium Di-hydrogen phosphate (AR grade) from Loba chemicals (Mumbai, India), sodium-hydroxide (NaOH), hydrochloric acid (HCl), and hydrogen peroxide(H_2O_2) were from Qualigens Fine Chemicals (Glaxo, Mumbai, India).The 0.45-µm Nylon pump filter was obtained from Advanced Microdevices (AmbalaCantt., India). Doubledistilled water was used throughout the experiment. Other chemicals used were of analyticalor HPLC grade.

Preparation of standard solutions

Accurately weigh and transfer 10 mg of Sitagliptin Workingstandard into a 100 ml volumetric flask, add about 50 ml of Methanol (Diluent)and sonicate to dissolve it completely and make volume up to themark with the same solvent (100µg/ml, Stock solution). Furtherpipette 1 ml of the above stock solution into a 10ml volumetric flaskand dilute up to the mark with Mobile phase (diluent), it was 10µg/ml. Standardcalibration solutions (10–60µg/ml) for assessment of linearitywere prepared from this stock solution by dilution with suitablediluent.

Preparation of sample solutions

The commercially available Tablet contains Sitagliptin (100mg). 20 tablets were weighed individually and finely powdered. A powder blend equivalent to 10mg of SIT was transferred to a 10mL volumetric flask containing 10 ml of the Methanol(diluent) and sonicated for 5min. and then final solution was made with diluent to get the solution of 100µg/ml From this solution, 1 ml was taken in 100ml standard volumetric flask and diluted to 100 ml with Mobile phase(diluent) togive a solution of 10µg/ml From this stock solution, various dilutions of solution were prepared and analysed.

Chromatography

The liquid chromatographic system consisted of following components: Shimadzu HPLC model containing LC-20AT (VP

series)pump, variable wavelength programmable UV/ VIS detector SPD-20A (VP series) and Hamilton syringe (705NR,50µl). Chromatographic analysis was performed using Empower -2 software on a Hypersil–BDS-C18 column with 250 x 4.6 mmi.d, 5µm particle size. The optimized mobile phase was consisted of 10mM Phosphate buffer (PH-3.5): ACN with 60:40%v/v .The flow rate was 1.2ml / min. Detection wavelength 260 nm was selected by scanning drug over a wide range of wavelength 200 nm to 400 nm in spectrophotometer. The 20µl sample was injected and the total run time was 6 min. Chromatogram showed peak of Sitagliptinat retention time of 2.157 ± 0.02 min.

Forced degradation study

To study the effect of acid, approximately 100 mg Sitagliptinwas accurately weighed and dissolved in 25 ml of 1M hydrochloric acid (HCl) and refluxed for 70°C for approximately 2 hr in water bath. The solution was then left to reach ambient temperature, and neutralized to pH 3.5 by addition of 1M sodium hydroxide(NaOH) then diluted to 50 ml with diluent (Mobile Phase) from this solution target concentration was prepared and injected.

To study the effect of alkali, approximately 100 mg Sitagliptin was accurately weighed and dissolved in 25 ml of 1M sodium hydroxide(NaOH) and refluxed for 70°C for approximately 2 hr in water bath. The solution was then left to reach ambient temperature, and neutralized to pH 7 by addition of 1M hydrochloric-acid (HCl) then diluted to 50 ml with diluent (Mobile Phase) from this solution target concentration was prepared and injected.

To study the effect of oxidising conditions, approximately 50 mg Sitagliptin was accurately weighed and dissolved in 25 ml of30% H2O2 and refluxed for 40°C for approximately 2 hr in water-bath. The solution was then left to reach ambient temperature, and diluted to 50 ml with diluent (Mobile Phase) from this solution target concentration was prepared and injected.

To study the effect of photolytic effect, approximately 50mgSitagliptin was accurately weighed and exposed to UV light for 24 hours. Then the drug substance was made to 100ug/ml with diluent (mobile phase) and refluxed for 70°C for approximately 2hr inwater bath. The solution was then left to reach ambient temperature, from this solution target concentration was prepared and injected.

To study the effect of temperature, approximately 50 mg Sitagliptin was stored at 80°C for 48 hr, then dissolved in few ml of diluent and sonicate for 5min then diluted to 50 ml with diluent (Mobile Phase) from this solution target concentration was prepared and injected.

Method validation

The method was validated according to International Conference on Harmonization [9,10] guidelines for validation of analytical procedures.

RESULTS AND DISCUSSION

System suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (R_t), number of theoretical plates (N), tailing factor (T), and peak asymmetry (A_f) were evaluated for five replicate injections of the drug at a concentration of 30µg / ml. The results given in **Table 1** were with-in acceptable limits. A typical chromatogram of Sitagliptin is presented in fig 2.

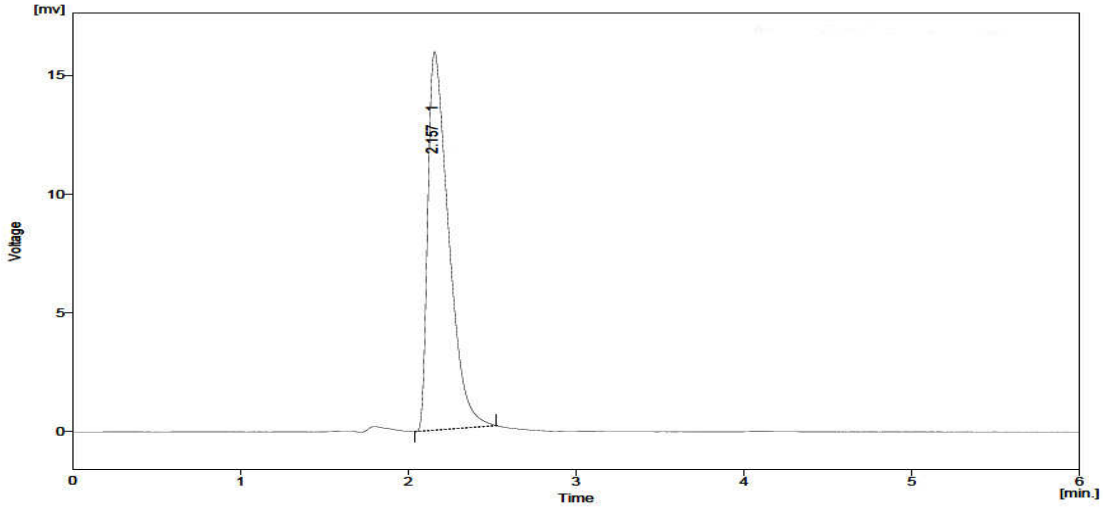

Fig. 2: Chromatogram of Sitagliptin formulation at 260 nm

Table 1: Results from system suitability studies

Property	Values*	Required limits
Retention time (R_t)	2.157RSD ≤ 1%	
Theoretical plates (N)	4988.17	$N > 2000$
Tailing factor (T)	0.88	$T \leq 2$
Asymmetric factor (A_f)	0.795$A_f \leq 1.5$	

* Mean ± S.D. from six determinations

Table 2: Calibration data of Sitagliptin by RPHPLC method

Sr. No	Concentration (µg/ml)	Retention time(min)	Peak Area(mV.s)
1	10	2.207	38.882
2	20	2.213	68.076
3	30	2.217	101.059
4	40	2.203	130.749
5	50	2.21	156.696
6	60	2.227	190.673

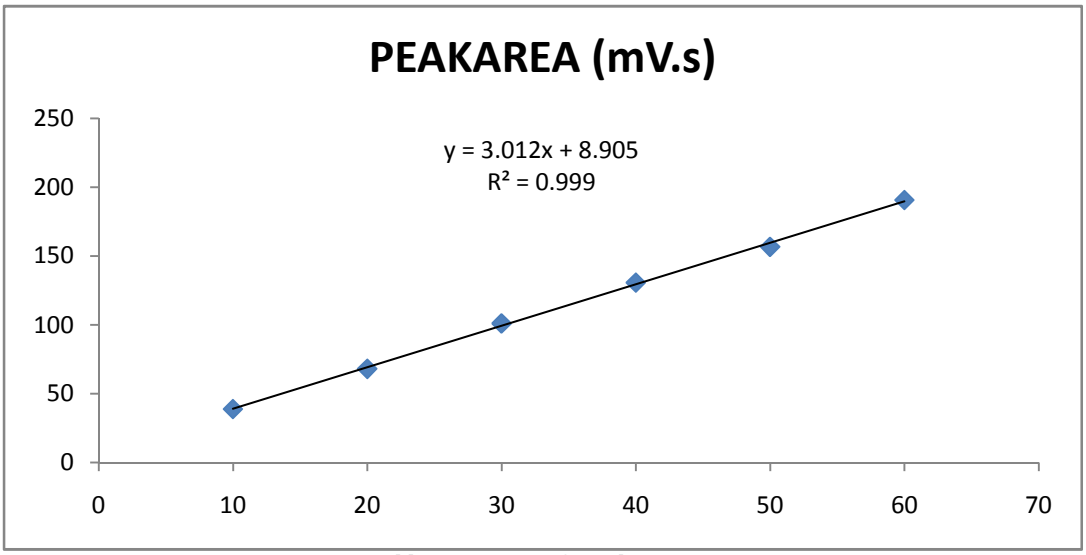

Fig. 3: Calibration curve of Sitagliptin at 260 nm

Linearity and range

Appropriate aliquots of standard Sitagliptin stock solutions (100µg / ml) were taken in different 10 ml volumetric flask andresultant solution was diluted up to the mark with Mobile phase(diluent) to obtain final concentration of 10-60µg / ml. The solutions were preparedin triplicate. Calibration curve were constructed by plotting the concentration of Sitagliptin versus corresponding mean peakarea. The results show that an excellent correlation exists betweenpeak area and concentration of drugs within the concentration rangeand the results given in **Table 2** and **Fig: 3.**

Precision

The intra-day precision was determined by analyzing standard solution of concentration 30µg/ml for 6 times on the same daywhile inter-day precision was determined by analyzing corresponding standards daily for 6 day over a period of one week.The values of percentage relative standard deviation (% RSD) forintra-and inter-day variation are given in **Table 3.**

Accuracy

Accuracy of the method was checked by recovery study usingstandard addition method known amount of standard Sitagliptinwas added into pre analysed sample and subjected it to theproposed high performance liquid chromatographic method. Thesestudies were carried out at three levels i.e,(80, 100 and 120%). Therecovery studies were carried out and the % recovery and standard-deviation of the % recovery were calculated and presented in **Table4.**

Sensitivity

The sensitivity of measurement of Sitagliptin by use of theproposed method was estimated in terms of the limit of quantitation(LOQ) and the limit of detection (LOD). The LOQ and LOD werecalculated by the use of the equations LOD = 3 x N / B and LOQ = 10x N / B where N is the standard deviation of intercept of calibrationplot and B is the average of the slope of the correspondingcalibration plot. The limit of detection (LOD) was 0.21µg / ml and the limit of quantitation (LOQ) was 0.640µg / ml.

Table 3: Precision results for Sitagliptin

S. No.	Concentration(µg/ml)	Intraday precision(Area)	Interday precision(Area)
1	30	101.059	102.892
2	30	101.112	102.908
3	30	102.008	103.112
4	30	100.992	103.008
5	30	102.102	102.578
6	30	101.928	102.765
Mean		101.533	102.877
Std.Dev		0.529	0.187
%RSD		0.521	0.182

Table 4: Results from recovery studies

Brand used	Label claim (mg/mL)	Initial amount (µg/mL)	Amount added (µg/mL)	Total amount (µg/mL)	Amount Recovered (µg/mL)	Recovery± SD*(%)	%RSD
		30	24	54	53.998	99.993 ± 0.23	0.230
JANUVIA	100	30	30	60	60.015	100.05 ± 0.31	0.309
		30	36	66	66.008	100.02 ± 0.18	0.179

*Average of six determinations

Table 5: Ruggedness studies of Sitagliptin by RPHPLC method

Brand used	Label claim (mg/mL)	Analyst-I amount found(mg/mL)	Recovery± SD*(%)	Analyst-II amount found (mg/mL)	Recovery± SD*(%)
JANUVIA	100	99.84	99.20±0.27	99.94	99.70 ± 0.39

*Average of six determinations

Ruggedness and robustness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst. The results of ruggedness testing are reported in the **Table 5.**

Robustness is a measure of capacity of a method to remain unaffected by small but deliberate variations in the method conditions, and is indications of the reliability of the method. Amethod is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and chromatographic response was evaluated. The organic composition in the mobile phase was varied from 55% to 65%, and the variation in mobile phase flow rate by 1.1 ml / min (0.5 and 0.8ml/min) had no significant effect on the retention time and chromatographic response of the 30µg/ml solution, indicating that the method was robust. The results are shown in **Table 6.**

Table 6: Robustness studies of Sitagliptin by HPLC method

Condition	Modification	Mean area ± SD* [mV.s]	RSD (%)	Mean R_t± SD(min)
	55:45	101.823 ± 180.68	0.214	2.21 ± 0.019
Mobile phase	60:40	102.928 ± 160.693	0.316	2.157 ± 0.015
Composition (v /v)	65:35	101.958 ± 127.68	0.485	2.050 ± 0.022
	3.2	102.582 ± 163.466	0.382	2.187 ± 0.014
Mobile phase pH	3.5	103.002 ± 140.263	0.298	2.167 ± 0.015
	3.8	101.363 ± 125.362	0.356	2.20 ± 0.022
Flow Rate of	1.1	102.268 ± 136.825	0.269	2.168 ± 0.02
Mobile Phase	1.2	101.865 ± 140.252	0.396	2.185 ± 0.025
	1.3	102.118 ± 138.568	0.459	2.21 ±0.02

*Average of six determinations

Table 7: Characteristic parameters ofSitagliptin for the RPHPLC method

Parameters	RPHPLC
Calibration range (µg / ml)	10-60
Detection wavelength	260 nm
Mobile phase	60 : 40
(10mM Phosphate buffer : ACN)	(v / v, pH 3.5)
Retention time	2.157 ± 0.02min
Regression equation	Y = 3.0129 x + 8.9053
Slope (b)	3.0129
Intercept (a)	8.9053
Correlation coefficient(r2)	0.999
Intraday Precision (% RSD*)	0.182
Interday Precision (% RSD*)	0.521
Limit of detection (µg / ml)	0.21
Limit of quantitation (µg / ml)	0.640

*Y = bx + a, where x is the concentration of compound in µg/ ml and ; Y is the peak area ; *Average of six determinations

Forced degradation study

When establishing the stability-indicating properties of analytical methods, the intermediate degradation products should not interfere with any stage of drug analysis. The System suitability parameters of forced degradation studies are summarised in **Table 8.** The results from forced degradation studies are given **Table 9.**Chromatograms obtained from after degradation under different stress conditions are shown in **Fig: 4 – 8,** respectively. No peaks co-eluted with the drug peak, suggesting the method enabled specific analysis of Sitagliptin in the presence of its degradation products.

Table 8: System suitability parameters of Sitagliptin by degradation studies

S. No.	System suitability parameters	Sitaglitpin	Degradation	Products A B
1	Retention time(minutes)	2.167	1.893	2.050
2	Theoretical plates	5.396	5784.123	0.960
3	Resolution	9.747	0.000	1.741
4	Asymmetry	1.435	1.550	0.969
5	Tailing Factor	1.287	1.032	0.997

Table 9: Results from analysis of samples by the forced degradation study, showing percentage degradation of Sitagliptin

Sr. No. Parameters DegradationproductsTotal % degradation (Stress condition/duration) (R_t) A B C
1 Acidic/1 M HCl/70°C/2hr 2.910 - - 37.129
2 alkali/1 M NaOH/70°C/2hr 1.813- -8.873
3 Oxidation/30% H2O2/40°C/2hr 2.877- -26.554
4 Photolytic /70°C/2hr -- -No degradation
5 Thermal/80°C/48hr1.6231.803 2.017 40.123

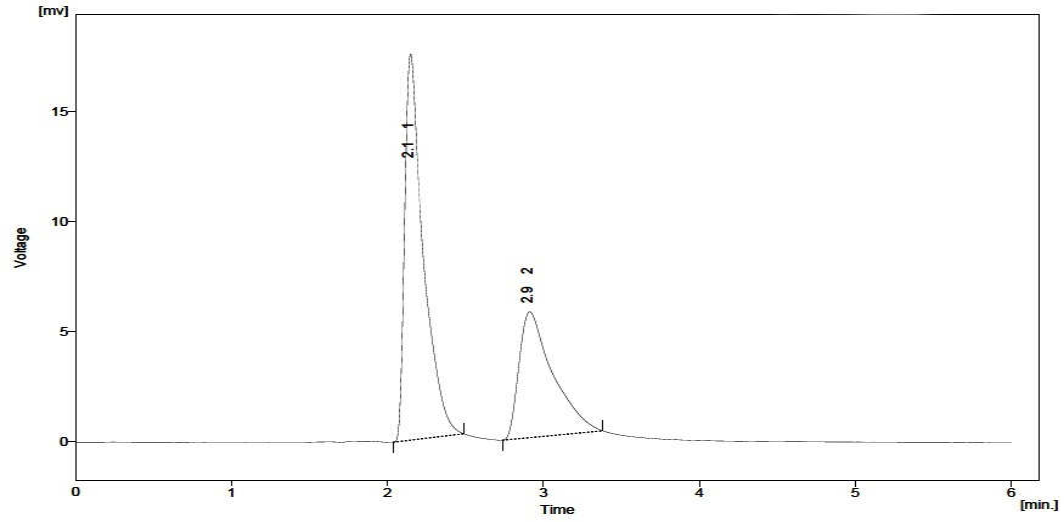

Fig. 4: Typical chromatogram obtained after degradation of Sitaglitpin under acidic conditions

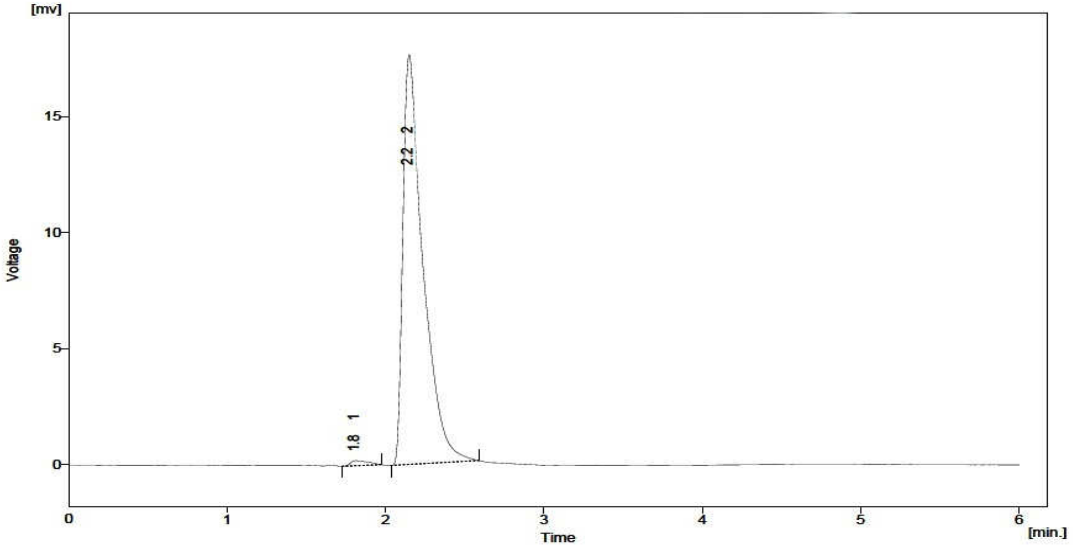

Fig. 5: Typical chromatogram obtained after degradation of Sitagliptin under alkali conditions

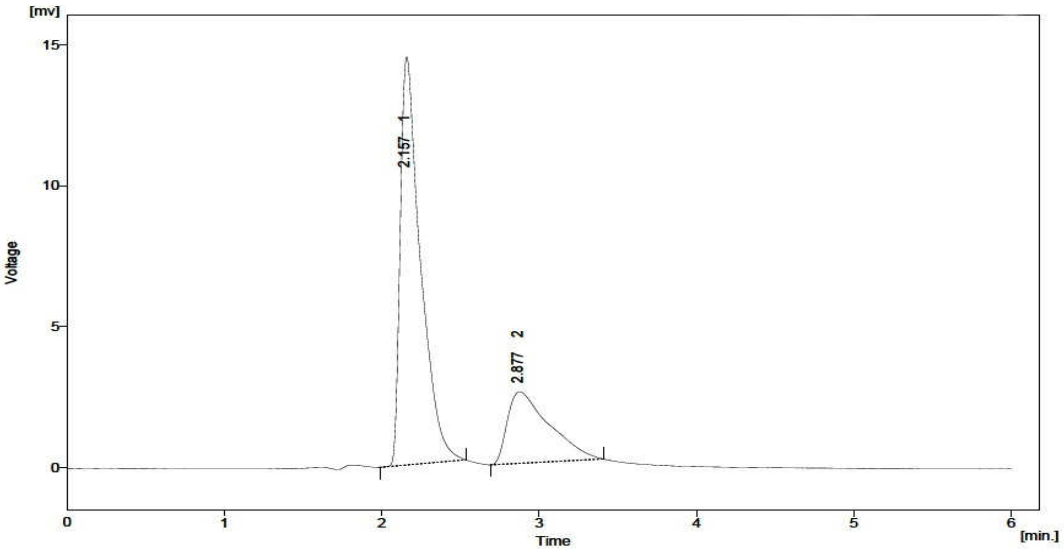

Fig. 6: Typical chromatogram obtained after degradation of Sitagliptin under oxidising conditions

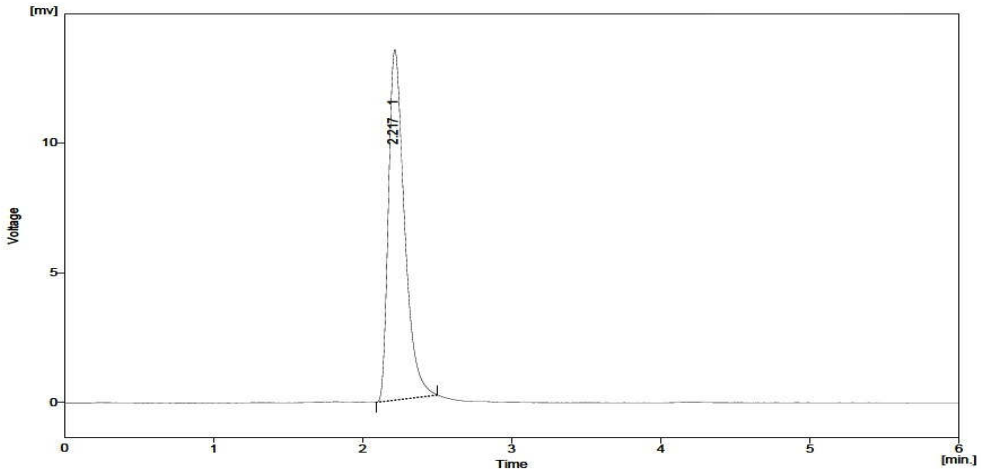

Fig. 7: Typical chromatogram obtained after degradation of Sitagliptin under photolytic conditions

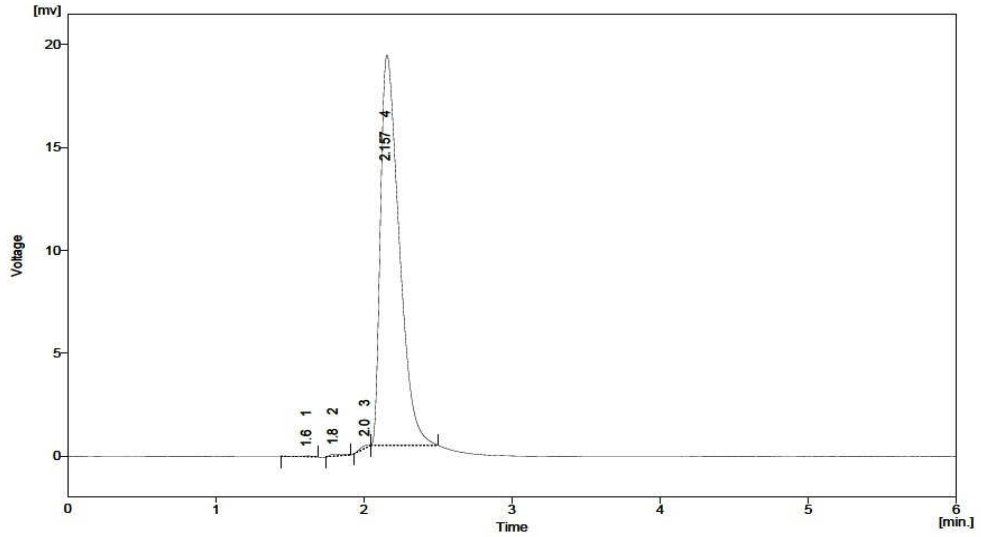

Fig. 8: Typical chromatogram obtained after thermal degradation of Sitagliptin

CONCLUSION

The method developed for quantitative analysis of Sitagliptinis rapid, precise, accurate, and selective. The method was completely validated as per ICH guidelines and satisfactory results were obtained for all the characteristics tested. The method is stability-indicating and can be used to assess the stability of Sitagliptinin bulk and pharmaceutical dosage forms. The method can be conveniently used for assay of Sitagliptin in the bulk drug and in pharmaceutical dosage forms. The method can be conveniently used in quality control laboratory.

ACKNOWLEDGEMENT

We would like thank to Granule chemicals pvt ltd, Hyderabad for providing Pure sample of Sitagliptin and also to the Principal Dr. Devala Rao. Garikapati, K.V.S.R. Siddhartha college of pharmaceutical sciences, Vijayawada for providing facilities to carry work.

REFERENCES

1. Available on www.drugbank.ca
2. Available on www.sitgapltin.in
3. Balosekaran.C, Prameela.Rani. A Development and validation of spectrophotometric method for the determination of DPP-4 Inhibitor, Sitagliptin, in its pharmaceutical dosage forms. International Journal of Pharmacy and Pharmaceutical sciences.vol.2,Issue 4,2010.
4. Monika.N, Ravipratap.P, HarshiniShabad. New extractive method development of Sitagliptin Phosphate in API and its Dosage forms by spectrophotometry. International Journal of Pharmacy and Biological sciences.2278-3008vol-1,Issue 6,July-August 2012;Pg:37-40.
5. Swati Kupkar, ShailajaYadhav.Simultaneous estimation of Sitagliptin and Metformin hydrochloride in Bulk and Dosage forms by U.V-Spectrophotometry. International Journal of Pharmacy Research 2012;5(1),580-582.
6. Sheetal Sharma, NimitaManocha, PriyaBhandari. Development of U.V.Spectrophotometry and RP-HPLC method and its validation for simultaneous estimation of Sitagliptin phosphate and simvastatin in marketed formulation. International Journal of Pharmaceutical and Biological Archieves 2012; 3(3):pg:673-678
7. Mohamed Salim, Nehed EI-Enany, FathallahBelah. Simultaneous determination of Sitagliptin and Metformin in pharmaceutical preparations by Capillary Zone electrophoresis and its application to Human plasma analysis. Analytical chemistry Insights 2012;731-46.
8. Phaneemdra.D,V.Venkatesh. Simultaneous estimation of Simvastatin and Sitagliptin by different Analytical methods. International Journals of Advances in Pharmaceutical Analysis.vol-2, Issue 1,2012;pg:589
9. ICH, Q2A Text on validation of analytical procedures, Oct, 1994.
10. ICH, Q3B Validation of analytical procedures: methodology,Nov, 1996.