International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (2): 1274-1279
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved
UGC Approved Journal

A New RP-HPLC Method Development and Validation of Sitagliptin in Bulk and Pharmaceutical Dosage Form

T.R. Mohapatra*, M. Pati, S.K. Parida and P. Nanda
Department of Pharmaceutical Chemistry, IMT, Pharmacy college, Puri

Received: 10 Jan 2019 / Accepted: 9 Mar 2018 / Published online: 1 Apr 2019
***Corresponding Author Email:** tusharmpharm@rediffmail.com

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. The chromatographic method was standardized using Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. column with UV detection at 266 nm and Methanol: Phosphate buffer(pH-4.2) with35:65 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 0-14µg/ml. The recovery was in the range of 98% to 102% and limit of detection was found to be 0.001 µg/ml and quantification was found to be 0.003 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.

*****

INTRODUCTION:
Sitagliptin previously identified as MK-0431 and marketed as the phosphate salt[1] under the trade name Januvia) is an oral antihyperglycemic (antidiabetic drug)[2] of the dipeptidyl peptidase-4 (DPP-4) inhibitor class[3]. It was developed, and is marketed, by Merck & Co. This enzyme-inhibiting drug is used either alone or in combination with other oral antihyperglycemic agents (such as metformin or a thiazolidinedione) for treatment of diabetes mellitus[4] type 2. Sitagliptin works to competitively inhibit the enzyme dipeptidyl peptidase 4 (DPP-4)[5]. This enzyme breaks down the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal. By preventing GLP-1 and GIP activation[6], they are able to increase the secretion of insulin and suppress the release of glucagon by the alpha cells of the pancreas[7-10]. This drives blood glucose levels towards normal. As the blood glucose level approaches normal, the amounts of insulin released and glucagon suppressed diminishes, thus tending to prevent an "overshoot" and subsequent low blood sugar (hypoglycemia) which is seen with some other oral hypoglycemic agents[11-13].
It is official in IP-2007[14], USP-2010[15], and BP-2009[16]. The Chemical Structure of Hydrochlorothiazide is shown in Fig-01The 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.

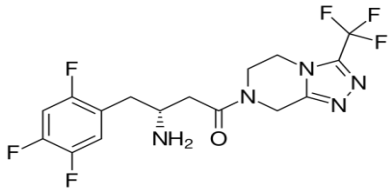

Fig 1: Chemical Structure of Sitagliptin

The present research manuscript describes a novel, simple, economical, accurate, specific, robust, rugged and rapid HPLC method developed in selected solvent system (Mobile Phase) and validated in accordance with International Conference on Harmonization (ICH) Guidelines Q2 (R1)[17], for the simultaneous estimation of sitagliptinin bulk drug and in its combined tablet dosage forms.

EXPERIMENTAL

Materials and Methods:

Pharmaceutical grade working standard vilazodone were obtained from Syncorp Pvt. Laboratories, Hyderabad, India. All chemicals and reagents were HPLC grade and were purchased from S D Fine-Chem Limited and Loba Chemie Pvt Ltd, Mumbai, India.

HPLC Instrumentation & Conditions:

The analysis was performed using HPLC Hitachi with UV detector and data handling system Lachrome software, UV-Visible double beam spectrophotometer (ELICO SL-159), analytical balance 0.1mg Sensitivity (SHIMADZU), pH meter (Labindia), Ultra Sonicator. The column used is Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d. (as Stationary phase) with the flow rate 1.0ml/min (isocratic).

UV-spectrophotometer analysis:

Samples were 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 266nm. The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450.

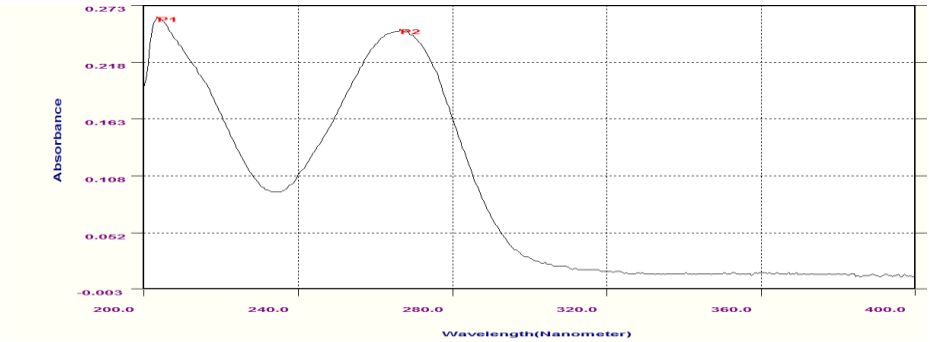

Fig 2: UV spectrum

Optimized Chromatographic Conditions:

Column: Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm.
Mobile Phase: Methanol: Phosphate buffer(pH-4.2) in the ratio (35:65 v/v)
Flow Rate: 1.0ml/minute
Wavelength: 266 nm
Injection volume:20µl
Run time:10 mins.
Column temperature: Ambient
Sampler cooler: Ambient

MOBILE PHASE PREPARATION

Mobile phase was prepared by taking Methanol: Phosphate buffer(pH-4.2) in the ratio (35:65 v/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 AND STANDARD PREPARATION FOR THEANALYSIS

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.

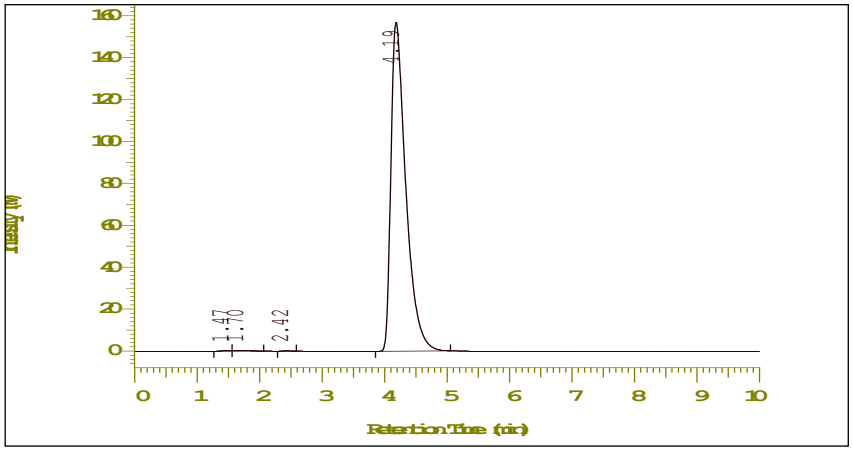

Fig-3: Optimized chromatographic conditions

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 10µg/ml. From that rate recuperation esteems were ascertained. The outcomes were appeared in Table-1.

Table-1: Accuracy Reading

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	8	8.157	595625	101.962	Mean= 101.387%
S_2 : 80 %	8	8.099	591457	101.237	S.D. = 0.516599
S_3 : 80 %	8	8.077	589875	100.962	% R.S.D.= 0.509532
S_4 : 100 %	10	10.077	734587	100.77	Mean= 100.43%
S_5 : 100 %	10	9.948	725268	99.48	S.D. = 0.833727
S_6 : 100 %	10	10.104	736524	101.04	% R.S.D.= 0.830157
S_7 : 120 %	12	11.989	872949	99.908	Mean= 100.6997%
S_8 : 120 %	12	12.190	887456	101.583	S.D. = 0.841254
S_9 : 120 %	12	12.073	878975	100.608	% R.S.D.= 0.835409

Precision:

Repeatability

The precision of each method was ascertained separately from the peak areas and 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-2.

Table-2: Repeatability Results of Precision

HPLC Injection Replicates of Sitagliptin	Retention Time	Peak Area
Replicate – 1	4.19	652542
Replicate – 2	4.19	653345
Replicate – 3	4.21	652841
Replicate – 4	4.19	653687
Replicate – 5	4.22	653874
Replicate -6	4.21	653427
Average	4.201667	653286
Standard Deviation	0.013292	506.2545
% RSD	0.316341	0.077494

Intraday & Inter day: The intra and 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.

Table-3: Results of Intraday & 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
8	8.06	1.08	7.86	1.05
10	10.26	0.97	10.13	0.94
12	12.51	0.92	11.09	0.96

Linearity and Range

Linearity range was found to be 0-14µg/ml for Sitagliptin.The correlation coefficient was found to be 0.999, the slope was found to be 72353 and intercept was found to be 5437 for Sitagliptin.

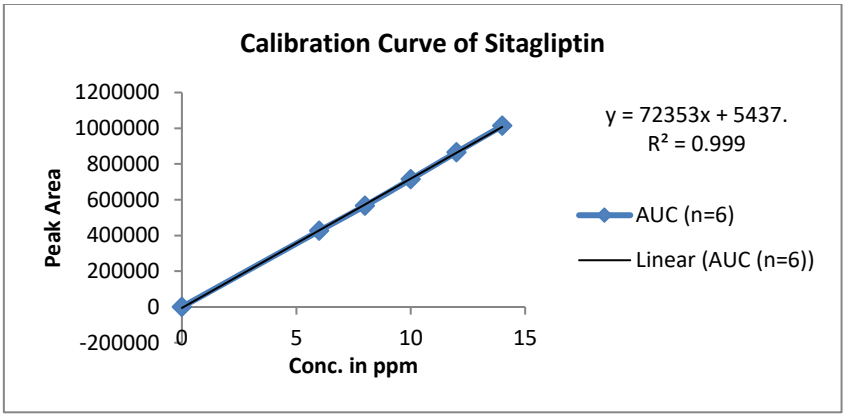

Fig-4: Calibration curve of Sitagliptin (API)

Table-4: Linearity Results of Sitagliptin

CONC.	AUC (n=5)
0	0
6	425874
8	565872
10	714542
12	865632
14	1013121

LOD & LOQ: The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.001 & 0.003 µg/ml respectively.

STABILITY STUDIES

ACID DEGRDATION

A precisely measured 10 mg of unadulterated medication was exchanged to a clean and dry round base jar. 30 ml of 0.1 N HCl was added to it and it was refluxed in a water shower at 600C for 4 hours. Permitted 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 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 HCl (0.1N) and observed its degradation profile. The typical chromatogram shown below is the degradation profile of Sitagliptinin 0.1N HCl.

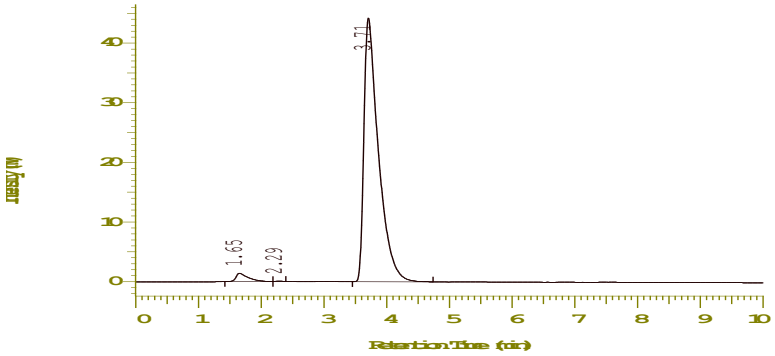

Fig-5: Acidic degradation

BASIC HYDROLYSIS:

A precisely measured 10 mg of unadulterated medication was exchanged to a clean and dry round base carafe. 30 ml of 0.1N NaOH was added to it. & it was refluxed in a water bath at 60^{0}C 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 Sitagliptinin 0.1N NaOH.

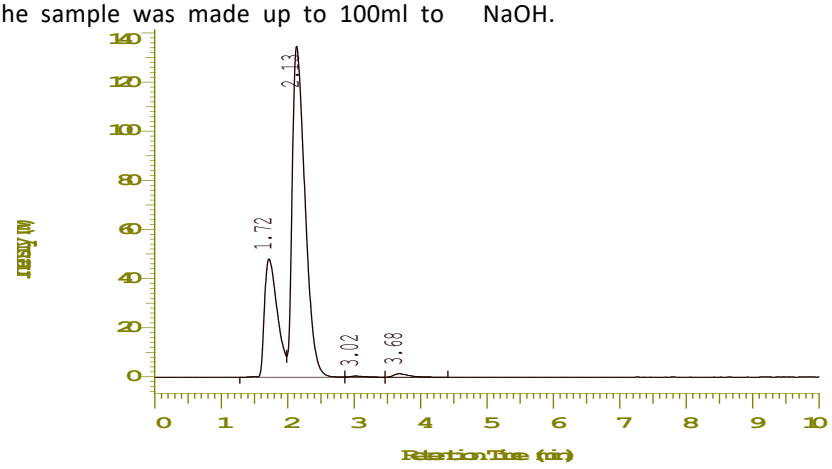

Fig-6: Basic degradation

OXIDATIVE HYDROLYSIS (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

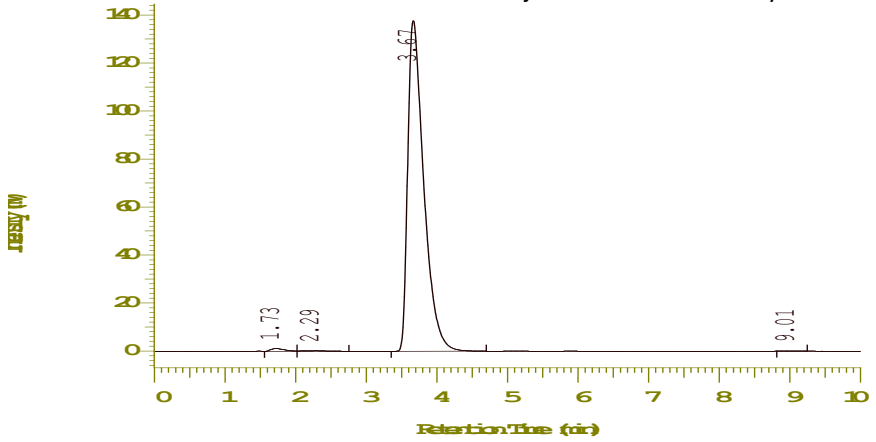

Fig-7: Peroxide degradation

Table-5: Results of forced degradation studies of Sitagliptin API.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	74.86	25.14	100
Basic Hydrolysis (0.1N NaOH)	24Hrs.	15.05	84.95	100
3% Hydrogen peroxide	24Hrs.	93.28	6.72	100

CONCLUSION

A delicate and specific, sensitive RP-HPLC strategy has been created and approved for the investigation of Sitagliptin API. Facilitate the proposed RP-HPLC strategy has amazing affectability, exactness and reproducibility.

REFERENCE

1. Herman GA, Stevens C, van Dyck K, Bergman A, Yi B, De Smet M, Snyder K, Hilliard D, Tanen M, Tanaka W, Wang AQ, Zeng W, Musson D, Winchell G, Davies MJ, Ramael S, Gottesdiener KM, Wagner JA (December 2005). "Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: results from two randomized, double-blind, placebo-controlled studies with single oral doses". Clin PharmacolTher. 78 (6): 675–88. doi: 10.1016/j.clpt.2005.09.002. PMID 16338283.
2. National Prescribing Service (August 2010). "Sitagliptin for Type 2 Diabetes". Archived from the original on 18 July 2010. Retrieved 27 August 2010.
3. "Januvia Side Effects & Drug Interactions". RxList.com. 2007. Archived from the original on 2007-11-20. Retrieved 2007-11-28.
4. Zhao Q, Hong D, Zheng D, Xiao Y, Wu B (2014). "Risk of diarrhea in patients with type 2 diabetes mellitus treated with sitagliptin: a meta-analysis of 30 randomized clinical trials". Drug Des DevelTher. 8: 2283–94. doi:10.2147/DDDT.S70945. PMC 4234286. PMID 25419118.
5. International Journal of Current Pharmaceutical Research ISSN- 0975-7066 Vol 3, Issue 4, 2011
6. Journal of Applied Pharmaceutical Science 01 (08); 2011: 239-243
7. International Journal of Pharm Tech Research CODEN(USA): IJPRIF ISSN : 0974-4304 Vol.1, No.2, pp 121-124, April-June 2009
8. Eurasian J. Anal. Chem. 4(2): 161-167, 2009
9. Maryadele J O Neil. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals, 14th ed. Whitehouse Station, N.J: Merck and Co. Inc; 2006; 386.
10. Sweetman SC. The Martindale: The Complete Drug Reference. 35th ed. London, UK: Pharmaceutical Press; 2007; 219.
11. Maryadele J O Neil. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals, 14th ed. Whitehouse Station, N.J: Merck and Co. Inc; 2006; 1184.
12. Sweetman SC. The Martindale: The Complete Drug Reference. 35th ed. London, UK: Pharmaceutical Press; 2007; 759.
13. Chaudhari SK. Quintessence of Medical Pharmacology. 1st ed. New Delhi: New Central Book Agency (P) Ltd; 1997; 484.
14. The Indian Pharmacopoeia, Vol. II, Government of India. Delhi: The Controller of Publications; 2010; 1090.
15. The United States Pharmacopoeia, USP 32, NF 27, Vol. 2. Rockville, MD: The United States Pharmacopoeial Convention, Inc; 2009; 1939.
16. British Pharmacopoeia, Vol. I, Stationary Office. London: Medicines and Healthcare Products Regulatory Agency; 2010; 510.
17. The International Conference on Harmonization. Validation of Analytical Procedure: Text and Methodology, ICH Q2 (R1); 2005.