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RESEARCH ARTICLE

Nallagatla. Vijaya Bhaskar Rao et al , The *Experiment, December. 2012 , Vol. .5(5). 315-323*

A NOVEL RP-HPLC METHOD FOR THE QUANTIFICATION OF SITAGLIPTIN IN FORMULATIONS

ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Sitagliptin tablet dosage form. Isocratic elution at a flow rate of 1ml/min was employed on a Kromasil C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted Methanol: Acetonitrile: Water 70:10:20 v/v/v. The UV detection wavelength was 285nm and 20µl sample was injected. The retention time for Sitagliptin was 4.583min. The % RSD for precision of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Sitagliptin tablet dosage form and bulk drug.

KEY WORDS Sitagliptin, RP-HPLC, UV detection, recovery, precise, 285nm.

INTRODUCTION

Sitagliptin is a new oral antihypoglycemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs.[1] It is indicated as treatment for patients with type 2 diabetes mellitus.[2] 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 improvement in glycaemic control observed with Sitagliptin may be mediated by enhancing the levels of active incretin hormones. Incretin hormones, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), are released by the intestine throughout the day, and levels are increased in response to a meal.

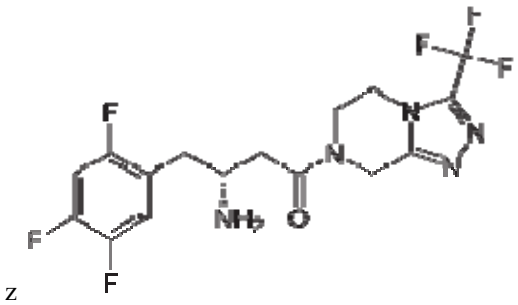

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Figure 1: Structure of Sitagliptin

The drug works to competitively inhibit a protein/enzyme, dipeptidyl peptidase 4 (DPP-4), [3] that results in an increased amount of active in cretins (GLP-1 and GIP), reduced amount of release of glucagon (diminishes its release) and increased release of insulin. When blood glucose concentrations are normal or elevated, GLP-1 and GIP increase insulin synthesis and release from pancreatic beta cells. It can be used as monotherapy in patients who are inadequately controlled by diet and exercise alone and for whom metformin is inappropriate due to oral therapy in combination with metformin or a sulphonylureum derivate or a peroxisomproliferator-activated receptor gamma (PPARγ) agonist or as triple oral therapy in combination with a sulphonylurea and metformin or a PPARγ agonist and metformin. It is also indicated as add-on to insulin (with or without metformin).

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EXPERIMENTAL

Materials

Working standard of Sitagliptin was obtained from well reputed research laboratories. HPLC grade water, Acetonitrile, Methanol was purchased from E. Merck (Mumbai, India).

Apparatus

A Series HPLC system PEAK LC 7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Kromasil C18 250x4.6mm, 5µm, Electronic balance-DENVER (SI234), manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used. UV 2301 Spectrophotometer was used to determine the wavelength of maximum absorbance.

Determination of wavelength of maximum absorbance

The standard solutions of Sitagliptin were scanned in the range of 200 - 400 nm against mobile phase as a blank. Sitagliptin showed maximum absorbance at 285nm. So the wavelength selected for the determination of Sitagliptin was 285nm.

Chromatographic equipment and conditions

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of Sitagliptin an isocratic peak HPLC instrument with Kromasil C18 column (250 mm x 4.6mm, 5µm) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC–7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

The mobile phase consisted of Methanol: Acetonitrile: Water 70:10:20 v/v/v. Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1 ml/min. Detection was performed at 285nm with 8min runtime.

Standard and sample solutions

A 10 mg amount of Sitagliptin reference substance was accurately weighed and dissolved in 10 ml mobile phase in a 10 ml volumetric flask to obtain 1000 µg/ml concentrated solution. Required concentrations were prepared by serial dilution of this solution.

A composite of 20 [Januvia-50mg] tablets ware prepared by grinding them to a fine, uniform size powder. 10 mg of Sitagliptin tablets sample powder was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 25 ml mobile phase were added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 120 µg/ml.

Method validation

Method validation was performed following ICH specifications for system suitability specificity, range of linearity, LOD, LOQ, accuracy, precision and robustness.

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RESULTS AND DISCUSSION

System Suitability

Having optimized the efficiency of a chromatographic separation, the quality of the chromatograph was monitored by applying the following system suitability tests: Tailing factor and Theoretical plates. The system suitability method acceptance criteria set in each validation run were: Tailing factor <2.0 and Theoretical plates >2500. In all cases, the % Relative standard deviation (R.S.D) for the analytic peak area for consecutive injections was <2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2.

API Conc.	30 ppm
Mobile Phase	Methanol: Acetonitrile: Water 70:10:20 v/v/v
Wavelength	285nm
Column	C_{18} Column
P^H	5.2
Retention Time	4.583min
Run Time	8min
Area	214451
Th. Plates	9141
Tailing Factor	1.16
Pump Pressure	8.2 MPa

Table.1: System suitability parameters of Sitagliptin.

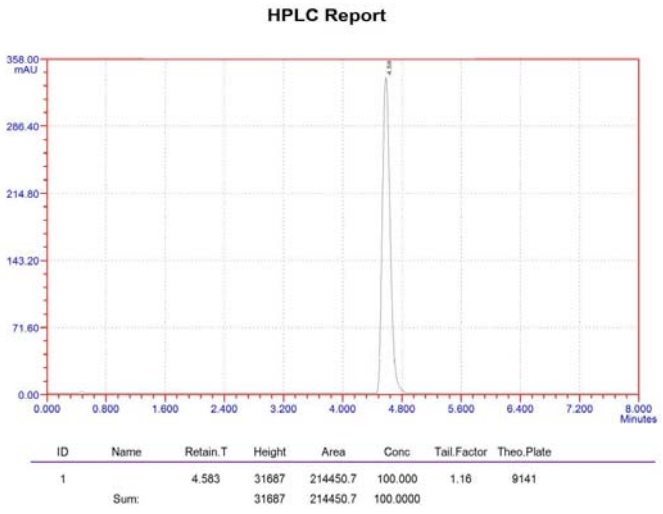

Figure.2: Standard chromatogram of Sitagliptin.

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Range of linearity

Standard curves were constructed daily, for three consecutive days, using six standard concentrations in a range of 10, 20, 30, 40, 50 and 60 for Sitagliptin. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 6987.118 + 5018.179x (r=0.99967). Linearity values can show in Table.2.

S.No	Conc. (µg/ml)	Area
1	10	80684
2	20	146817
3	30	214451
4	40	279975
5	50	356882
6	60	423613
	Slope Intercept CC	6987.118 5018.179 0.99967

Table.2: Linearity results of Sitagliptin.

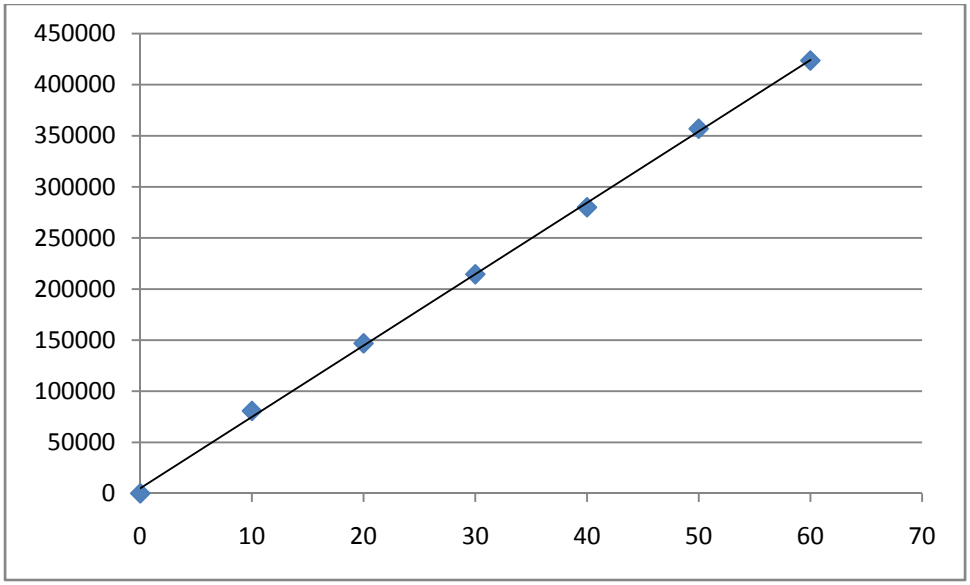

Figure 3: Calibration curve of Sitagliptin.

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Precision

To study precision, six replicate standard solutions of Sitagliptin (30ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table.3 and Table.4.

Sample Preparation No. (API Conc. 30 µg/ml)	Area
1	214451
2	219736
3	218542
4	216397
5	215160
6	218364
RSD	0.96

Table 3: Intraday Precision Results for Sitagliptin.

Sample Preparation No. (API Conc. 30 µg/ml)	Area
1	222015
2	219170
3	219398
4	220173
5	216514
6	219175
RSD	**0.811**

Table 4: Inter day Precision results of Sitagliptin.

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.2ppm dilution Peak was not clearly observed, based on which 0.2ppm is considered as Limit of Detection and Limit of Quantification is 0.065ppm.

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Parameter	Measured Value
Limit of Quantification	0.02ppm
Limit of Detection	0.065ppm

Table.5: LOD and LOQ results of Sitagliptin.

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. The robustness study was performed by slight modification in composition of the mobile phase, pH of mobile phase and wavelength of the detector. Sitagliptin at standard concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above. Results were shown in Table.6.

S.NO	Parameter	Change	Area	% of Change
1	Standard	No change in mobile phase preparation.	214451	……
2	MP	Methanol: Acetonitrile: Water 75:05:20 65:15:20	 213230 211210	 0.57 1.57
3	PH	5.3 5.1	212387 217598	0.97 1.46
4	WL	280nm 290nm	211917 217990	1.19 1.37

Table.6: Robustness results of Sitagliptin.

Ruggedness:

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different. Ruggedness also expressed in terms of percentage relative standard deviation.

Sample	Conc. (ppm)	Injection No.	Peaks Area	R.S.D (Acceptance criteria < 2.0%)
Sitagliptin	30 ug/ml	1	217508	**1.53**
		2	219321	
		3	218432	
		4	212657	
		5	216322	
		6	211076	

Table.7: Ruggedness results of Sitagliptin

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Recovery

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. Recovery test was performed at 3 different concentrations i.e. 90ppm, 120ppm, 150ppm. The percent recovery was calculated and results are presented in Table. Satisfactory recoveries ranging from 98.07 to 101.14 were obtained by the proposed method. This indicates that the proposed method was accurate. Results are given in Table.8.

% Recovery	Sitagliptin					
	Target Conc. (ppm)	Spiked conc. (ppm)	Final Conc. (ppm)	Area	Conc. Obtained	% of Recovery
50%	20	10	30	213790	29.9	99.66
	20	10	30	212651	29.74	99.13
	20	10	30	215729	30.17	100.56
100%	20	20	40	275923	39.42	98.55
	20	20	40	279838	39.98	99.95
	20	20	40	278062	39.72	99.3
150%	20	30	50	356542	49.95	99.9
	20	30	50	353315	49.5	99
	20	30	50	355530	49.81	99.62

Table.8: Recovery results of Sitagliptin.

Formulation	Dosage	Conc.	Amount found	% Assay
Januvia	50mg	30ppm	29.77	99.23

Table.9: Formulation Analysis.

Degradation studies:

Forced degradation studies of Sitagliptin drug was carried out under conditions acid, alkali, peroxide, heat, Sun light, uv light, aqueous etc. After exposing sample was tested immediately and 48 hours incubation. It can be concluded that the method separates the drugs from their degradation products. It may be employed for analysis of stability samples of Sitagliptin. Degradation studies are given in Table.10.

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Condition after 48 hours	Observation
3% Peroxide	Sitagliptin degraded in to four compounds.
0.1 N Basic	Sitagliptin degraded in to two compounds
0.1 N Acidic	Sitagliptin degraded in to three compounds
Sun light	Sitagliptin degraded in to two compounds
UV light	Sitagliptin degraded in to three compounds
Aqueous (HPLC)	Sitagliptin degraded in to two compounds
Thermal (heat)	Standard peak was spited into two peaks

Table.10: Degradation studies.

Stability studies:

Stability test was conducted by injecting the sample solution in different time intervals after preparation. The sample has shown the stable up to 24 hours after preparation. Stability studies are given in Table.11.

S.No.	Time (hours)	Area	% of assay
1	0	212025	98.86
2	2	212109	98.90
3	4	211646	98.69
4	6	211724	98.72
5	12	212287	98.99
6	18	209663	97.76
7	24	209843	97.85
8	36	190063	88.62
9	48	177842	82.92

Table.11: Stability studies.

CONCLUSION

In the proposed study, stability-indicating HPLC method was developed for the simultaneous determination of Sitagliptin and validated as per ICH guidelines. Statistical analysis proved that method was accurate, precise, and repeatable. The proposed method for the assay of Sitagliptin in tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

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BRC Sekhar Reddy, Dr. Nallagatla. Vijaya Bhaskar Rao

Micro Labs Limited-API, # 43-45 KIADB Industrial Area Phase-IV, Bommasandra, Bangalore- 560 105.

Email id: brcsekharreddy@gmail.com