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Development and validation of first order derivative UV-Spectrophotometric method for determination of Sitagliptin in bulk and in Formulation

Jain Pritam*, Chaudhari Amar, Desai Bhargav, Patel Shani, Patel Santsaran, Shimpi Hiren

Abstract

Objective: A simple, rapid, accurate and economical First order UV-derivative spectrophotometric method has been developed for estimation of sitagliptin from bulk and pharmaceutical formulation.

Materials and methods: The λ_{max} of sitagliptin in methanol and water was found to be 267 nm. The same spectrum was derivatised in to first order derivative; showed maximum amplitude of the trough at 275 nm. The drug follows linearity in the concentration range 10-60 µg/ml with correlation coefficient value 0.998.

Results: The proposed method was applied to pharmaceutical formulation and % amount of drug estimated 99.19 **%** was found in good agreement with the label claim. The accuracy of the method was checked by recovery experiment performed at three different levels i.e., 80%, 100% and 120 %. The % recovery was found to be in the range 98.54%– 99.98%. The low values of % R.S.D. are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intra-day, inter-day variations and repeatability. The % R.S.D. value less than 2 indicate that the method is precise. Ruggedness of the proposed method was studied with the help of two analysts.

Conclusion: The above method was a rapid and cost-effective quality-control tool for routine analysis of sitagliptin in bulk and in pharmaceutical dosage form.

*Corresponding author, Mailing address:
Jain Pritam
Department of Pharmaceutical Chemistry,
R. C. Patel Institute of Pharmaceutical Education and Research,
Shirpur Dist Dhule MS, India 425405
E mail : pritash79@yahoo.com

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Sitagliptin; First order derivative UV, quantitative determination, methanol.

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Introduction:

Sitagliptin chemically is (3R) -3-amino-1-[3-(trifluoromethyl)-6,8-dihydro-5h- [1,2,4] triazolo [3,4-c] pyrazin-7-yl]-4-(2,4,5-trifluorophenyl) butan-1-one (Fig. 1), an oral anti-diabetic agent that blocks Dipeptidyl peptidase-4 (DPP-4) activity. Sitagliptin

increased incretin levels (GLP-1 and GIP) which inhibit glucagon release, in turn decreases blood glucose, but more significantly increases insulin secretion [1-3].

Literature survey reveals that only LC-MS[4-7] methods were reported for the determination of sitagliptin in plasma and urine of humans, rats and dogs whereas two colorimetric methods were reported[8, 9]. So far, no assay procedure has been reported for the determination of this drug in its pharmaceutical formulations. Among the various methods available for the determination of drugs, spectrophotometry continues to be very popular, because of their simplicity, specificity and low cost. This study presents new spectrophotometric method for the determination of sitagliptin phosphate in bulk and pharmaceutical formulations. Accordingly, the objective of this study was to develop and validate the first order derivative method for the estimation of sitagliptin in bulk and pharmaceutical formulation as per ICH guidelines[10].

Experimental:

Materials:

Sitagliptin was a gift sample from Indoco Pharma, Mumbai. All chemicals and reagents used were of analytical grade and purchased from Qualigens Fine Chemicals, Mumbai, India.

Preparation of standard stock solution:

Accurately weighed 10 mg of Sitagliptin was transferred to 100 ml volumetric flask, dissolved in 20 ml distilled water by shaking manually for 10 min. The volume was adjusted with the same up to the mark to give final strength i.e. 100 µg/ml.

Selection of wavelength for analysis of Sitagliptin:

Appropriate volume 1 ml of standard stock solution of Sitagliptin was transferred into 10 ml volumetric flask, diluted to mark with distilled water to give concentration of 10 µg/ml. The resulting solution was scanned in UV range (200 nm – 400 nm). In zero order spectrum Sitagliptin showed absorbance maximum at 267 nm. The same spectrum was derivatized into first order using UV- probe software of the UV-spectrophotometer. The amplitude of the trough was found at 275 nm (Fig. 2).

Validation of the method:

The method was validated in terms of linearity, accuracy, precision, and ruggedness.

Linearity study:

Different aliquots of Sitagliptin in range 1-6 ml were transferred into series of 10 ml volumetric flasks and the volume was made up to the mark with distilled water to get concentrations 10, 20, 30, 40, 50 and 60 µg/ml, respectively. The solutions were scanned on spectrophotometer in the UV range 200 - 400 nm. The spectrum was derivatized into first order using UV-probe software of the spectrophotometer, amplitude of the trough was recorded at 275 nm. The calibration plot was constructed as concentration vs. amplitude (Fig. 3).

Accuracy:

To the preanalysed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80%, 100% and 120 %. The solutions were reanalyzed by proposed method.

Precision:

Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing the 20, 30 and 40 µg/ml of Sitagliptin solutions for three times in the same day. Inter-day precision was determined by analyzing the 20, 30 and 40 µg/ml of sitagliptin solutions daily for three days over the period of week.

Sensitivity:

The sensitivity of measurements of Sitagliptin by the use of the proposed method was estimated in terms of the Limit of Quanfication (LOQ) and Limit of Detection (LOD). The LOQ and LOD were calculated using equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where, 'N' is standard deviation of the peak

areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

Repeatability:

Repeatability was determined by analyzing 30 µg/ml concentration of Sitagliptin solution for six times.

Ruggedness:

Ruggedness of the proposed method is determined for 30 µg/ml concentration of Sitagliptin by analysis of aliquots from homogenous slot by two analysts using same operational and environmental conditions.

Determination of Sitagliptin in bulk:

Accurately weighed 10 mg of Sitagliptin was transferred into 100 ml volumetric flask containing 20 ml distilled water and volume was made up to the mark using same. Appropriate volume 0.6 ml of this solution was transferred to 10 ml volumetric flask and volume was adjusted to mark using distilled water. The resulting solution was scanned on spectrophotometer in the UV range 200 - 400 nm and amplitude of corresponding trough was measured at 275 nm. The concentrations of the drug were calculated from linear regression equations.

Application of proposed method for pharmaceutical formulation:

For analysis of commercial formulation 5 ml of sitagliptin eye drop solution was taken in 100 ml volumetric flask and the volume was made up to the mark with distilled water to give 100µg/ml concentration. From this 0.6 ml was taken and transferred to 10 ml volumetric flask and volume was made upto the mark with distilled water to give 6 µg/ml concentration. It was scanned on spectrophotometer in the UV range 200 - 400 nm. The spectrum was derivatised into first order derivative and amplitude of the trough was recorded at 275 nm. The concentrations of the drug were calculated from linear regression equation.

Results and Discussion:

Method Validation:

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

Linearity studies:

The linear regression data for the calibration curves showed good linear relationship over the concentration range 10-60 µg/ml for Sitagliptin. Linear regression equation was found to be Y = 0.0039 X + 0.0089 (r^2 = 0.998). The result is expressed in Table 1.

Accuracy:

The solutions were reanalyzed by proposed method; results of recovery studies are reported in Table 2 which showed that the % amount found was between 98.54% to 99.98% with %R.S.D. >2.

Precision:

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These result shows reproducibility of the assay. The % R.S.D. values found to be less than 2, so that indicate this method precise for the determination of both the drugs in formulation (Table 3).

Sensitivity:

The linearity equation was found to be Y = 0.0045 X + 0.0041. The LOQ and LOD for Sitagliptin were found to be 2.38 µg and 7.24 µg, respectively.

Repeatability:

Repeatability was determined by analyzing 30 µg/ml concentration of Sitagliptin solution for six times and the % amount found was between 98% to 102% with % R.S.D. less than 2 (Table 4).

Ruggedness:

Peak area was measured for same concentration solutions, six times. The results are in the acceptable range for both the drugs. The results are given in Table 5**.** The result showed that the % R.S.D. was less than 2%

Determination of Sitagliptin in bulk:

The concentrations of the drug were calculated from linear regression equations. The % amount found was between 99.17% to 100.43% (Table 6).

Application of proposed method for pharmaceutical formulation:

The spectrum was derivatised into first order derivative and amplitude of the trough was recorded at 262 nm. The concentrations of the drug were calculated from linear regression equation. The % amount found was between 97.36% to 101.31% (Table 7).

Conclusion:

This first order UV spectrophotometric derivative technique is quite simple, accurate, precise, reproducible and sensitive. The first order UV derivative method has been developed for quantification of sitagliptin in tablet formulation. The validation procedure confirms that this is an appropriate method for their quantification in the plant material and formulation. It is also used in routine quality control of the raw materials as well as formulations containing this entire compound.

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Table 1: Linearity study of Sitagliptin

Sr. no.	Concentration µg/ml	Absorbance* Mean ± S.D. (n=6)	% R.S.D.
1	10	0.049 ± 0.001	1.21
2	20	0.086 ± 0.002	1.63
3	30	0.135 ± 0.004	1.66
4	40	0.166 ± 0.006	1.83
5	50	0.211 ± 0.004	1.15
6	60	0.243 ± 0.006	1.37

* average of five estimations

Table 2: Recovery studies

Pre-analyzed sample solution (µg/ml)	Amount of drug added (µg/ml) (n=3)	Amount recovered* (µg/ml) (n=3)	% Recovery	% R.S.D.
30	0	29.91	98.54	1.38
	24	53.97	99.98	1.40
	30	59.92	98.68	1.44
	36	65.94	99.54	1.33

*average of three estimates

Table 3: Precision studies

Component	Concentration (µg/ml)	Intra-day precision* (n=3)		Inter-day Precision* (n=3)	
		Conc. found	% R.S.D.	Conc. found	% R.S.D.
Sitagliptin	20	19.95	1.47	19.99	1.43
	30	29.95	0.54	29.92	0.61
	40	39.99	1.24	39.94	1.13

*average of three estimates

Table 4: Repeatability studies

Component	Amount taken (µg/ml) (n=6)	Amount found* (%)	%R.S.D.
Sitagliptin	30	99.63 ± 0.38	0.64

*average of six estimations

Table 5: Ruggedness studies

Component	Amount taken (µg/ml) (n=3)	Amount Found (%) *	
		Analyst I ±S.D.	Analyst II ±S.D.
Sitagliptin	30	99.04 ± 1.3	98.90 ± 0.95

*average of six estimations

Table 6: Analysis of Sitagliptin in bulk

Concentration (µg/ml)	Amount found (µg)	Amount found (%)
30	29.94737	99.12
	29.97368	99.56
	29.97368	99.56
	29.71053	99.17
	29.97368	99.56
	30.02632	100.43
Mean ± S.D.	29.93 ± 0.102	98.90 ± 1.71
% R.S.D.	1.73	1.73

Table 7: Analysis of formulation

Conc. (µg/ml)	Amount found (µg)	Amount found (%)
30	29.89474	98.24
	29.97368	99.56
	29.84211	97.36
	29.97368	99.56
	29.94737	99.12
	30.07895	101.31
Mean ± S.D.	29.95 ± 0.08	99.19 ± 1.34
% R.S.D.	1.35	1.35

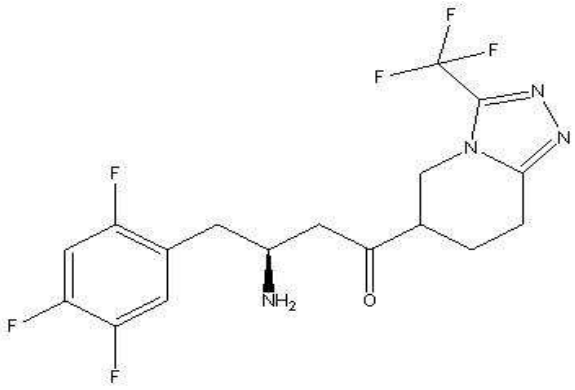

Figure 1: Chemical structure of sitagliptin

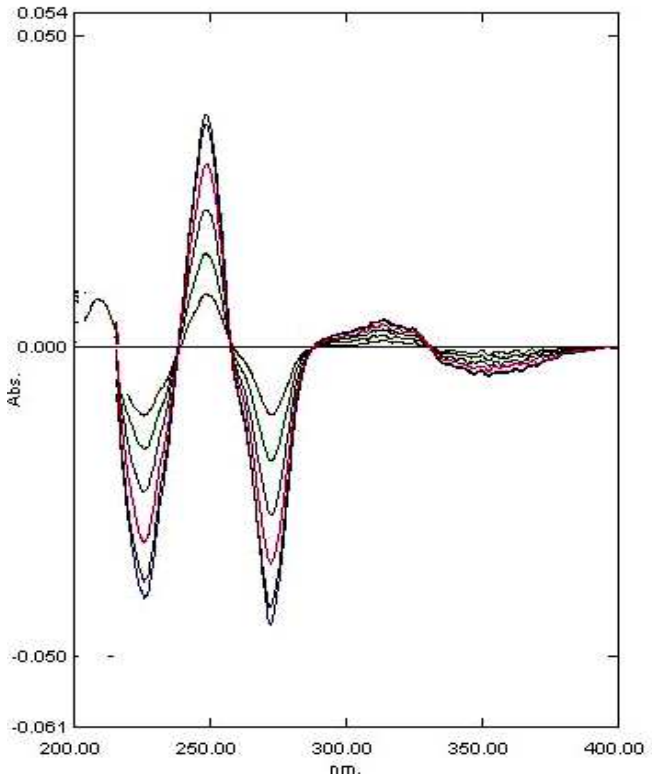

Figure 2: First Order Derivative Spectrum of Sitagliptin at 275 nm

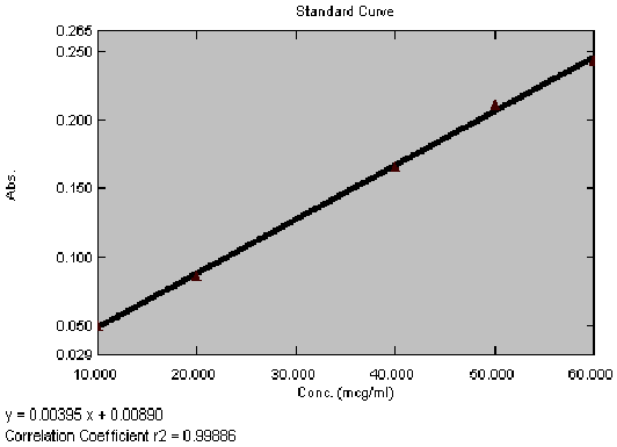

Figure 3: Calibration curve of Sitagliptin at 275 nm

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