Research & Reviews

Research and Reviews: Journal of Pharmaceutical Analysis

Analytical Method Development and Validation of Sitagliptine Phosphate Monohydrate in Pure and Tablet Dosage Form by UV-Vis Spectroscopy

G Jeyabalan[1] and Narendra Nyola*[1,2]

[1]Department of Pharmaceutical Analysis, Alwar Pharmacy College, Alwar - 301030, Rajasthan, India.
[2]Department of Pharmaceutical Sciences, Shridhar University, Pilani - 333031 ,Rajasthan

Short Communication

Received: 25/09/2012
Revised: 12/10/2012
Accepted: 12/11/2012

***For Correspondence:**
Alwar Pharmacy College, Alwar - 301030, Rajasthan, India.
Email: narennyola2@gmail.com

Keywords: sitagliptine, validation, UV-Vis spectroscopy, method development

ABSTRACT

A simple, rapid and accurate spectrophotometric method was developed for the determination of sitagliptine in pure and tablet dosage form. The proposed method is based on the principle that sitagliptine exhibiting an absorption spectra of wavelength maxima 267 nm. This method has successfully used for the analysis of drug in marketed preparations in the range of 20-60 µg/ml with correlation coefficient of 0.991. The percentage recovery was found to be 99.62-100.48%. LOD and LOQ were found to be 6.03 and 18.28 µg/ml respectively. This method has been validated for linearity, accuracy and precision and found to be rapid, precise, accurate and economical and can be applied for routine estimation of sitagliptine in solid dosage form. The validation of method was carried out utilizing ICH-guidelines

INTRODUCTION

Sitagliptin phosphate monohydrate (SPM) chemically, (3*R*)-3-amino-1-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-(2,4,5-trifluorophenyl)butan-1-one phos-phate hydrate (Fig. 1) is oral hypoglycemic drug of the dipeptidyl peptidase-4(DPP-4) inhibitor class [1]. DPP-4 inhibitors represent a new therapeutic approach to the treatment of type 2 diabetes that functions to stimulate glucose-dependent insulin release and reduce glucagons levels. This is done through inhibition of the inactivation of in cretins, particularly glucagon-like peptide- 1 (GLP-1) and gastric inhibitory polypeptide (GIP), thereby improving glycemic control[2]. Several analytical methods based on UV [2,3,4], Spectroflourimetry [5] , RP-HPLC [6,7], LC-MS/MS [8,9,10] was reported for the determination of sitagliptin phosphate in plasma and urine of humans, rats and dogs.

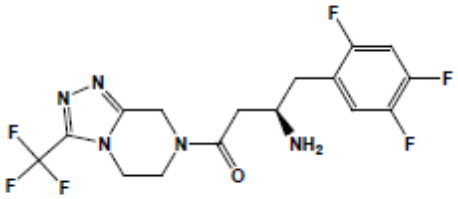

Figure 1: Structure of Sitagliptine

EXPERIMENTAL

Apparatus

A Shimadzu model 1800 double beam UV-Visible spectrophotometer with spectral width of 1 nm, wavelength accuracy of ± 0.1 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software (Ver.2.34).

Reagents and Materials

All chemicals and reagents were used of AR grade. Authentic of SPM was obtained as gift samples from MSD Pharmaceutical private Ltd. Maharashtra, India.

Selection of Detection Wavelength

Solution of drug in was scanned over the range of 200-400 nm. The absorbance maximum was found 267nm.

Preparation of Standard Stock Solutions

SPM was weighed (100 mg) and transferred to 100ml volumetric flasks and dissolved in 50 ml of methanol and make up the volume up to the mark with methanol and the final concentration of solution containing 1000 µg/ml.

Preparation of Working Solutions

Aliquot from the stock solutions of SPM was appropriately diluted with methanol to obtain working standard.

Method Validation

The developed method was validated for its linearity, accuracy, precision and specificity.

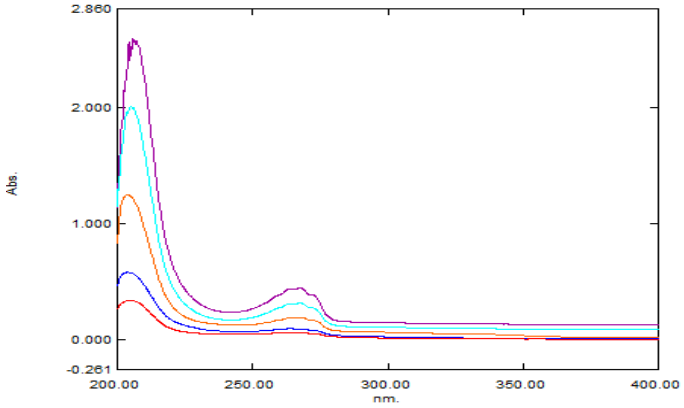

Figure 2: UV Spectra of Sitagliptine

Linearity

The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of SPM. The results are shown in table 1.

Table 1

Concentration	Absorbance
20	0.156
30	0.243
40	0.341
50	0.448
60	0.588

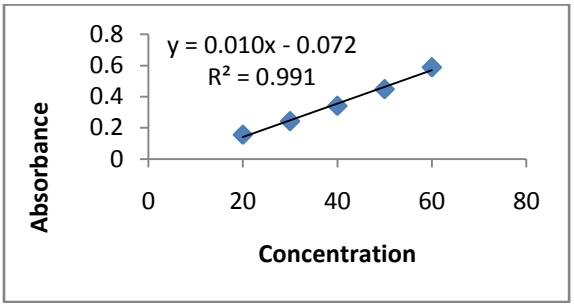

Figure 3: Calibration Curve of Sitagliptine

Accuracy

To ascertain the accuracy of the proposed method, recovery studies were carried out by standard addition method. The results are shown in table 2.

Table 2

% Recovery Study	Amount Present	Amount Added	Amount Recovered	% Recovery
80	30	24	54.26	100.48
100	30	30	60.12	100.20
120	30	36	65.75	99.62
			Mean	100.10
			SD	0.439
			% RSD	0.438

*Mean of three determinations in each level

Precision

The precision of the proposed method was determined by analyzing different concentrations (20-60µg/ml) at different time intervals on same day (Intra-day precision) and on three different days (Inter-day precision).

Specificity

Interference and non-interference of excipients and binders was confirmed by performing the specificity study. Specificity was performed by spiking placebo with standard drug.

LOD and LOQ

The LOD and LOQ were calculated from the equations, LOD =3.3 σ/S and LOQ = 10 σ/S, where σ is the standard deviation of the lowest standard concentration and S is the slope of the standard curve. The results are shown in table 4.

Estimation of Sitagliptine from Tablet.

Marketed preparation of SPM selected for the purpose of analysis. Twenty tablets were accurately weighed and powdered quantity equivalent to 100 mg of SPM was transferred in 100 ml volumetric flask and sonicated for 30 min. Then the volume was made upto the mark with methanol and the solution was filtered using Whatman filter paper no. 42 to obtain sample stock solution. 0.8 ml of filtrate was further diluted to 10 ml with same solvent and absorbance of sample was measured against blank. The amount of SPM was calculated from the calibration curve. The results of assay are shown in table 3.

Table 3: Assay of Dosage Form (Januvia)

Drug	Label Claim (mg/tablet)	Amount Estimated (mg/tablet)*	Percentage Label Claim (%)
Sitagliptine	50	49.4	100.4

*Mean of five reading

RESULTS AND DISCUSSION

As shown in fig. 1, SPM showed wavelength maxima at 267 nm in methanol. As shown in fig. 2,3 and table 1, the calibration curve was found to be linear in the range of 20-60 µg/ ml with regression equation of y = 0.010X -0.072; (r^2 = 0.991) which clearly indicates linearity of developed method. % Recoveries for SPM was found to be satisfied i.e. 99.62 to 100.48% as shown in table 2; clearly indicate that the developed method is accurate. Results of intra-day and inter-day precision is expressed in % RSD and found to be 0.330 and 0.280 respectively. As, % RSD is within the allowable limit of ≤ 2%. It clearly indicates that the developed method is precise. Assay result is in good agreements with the label claim. Hence, the proposed method can be successfully used for its analysis and quality control of marketed solid dosage preparation with good linearity, accuracy and precision.

CONCLUSION

From the above results it can be concluded that, the developed UV spectrophotometric method is simple, rapid, accurate, precise, specific and economical. Hence, this method can be applied for quantitative analysis of Sitagliptine in bulk and pharmaceutical formulation like tablet dosage form.

ACKNOWLEDGMENTS

The authors are thankful to MSD Pharmaceutical private Ltd. Maharastra, India for providing gift sample of SPM. The authors are very thankful to Principal and Management of Alwar Pharmacy College for providing necessary facilities to carry out research work.

Table 4: Summery of Method Validation Parameters

Parameter	Sitagliptine
Linearity range (µg/ml)	20- 60
Sandell's sensitivity	0.117
Correlation coefficient (r^2)	0.991
Slope (m)	0.010
Intercept (c)	0.072
Accuracy	
Precision (% RSD)	100.10
Repeatability	0.32
Inter-day (n=5)	0.74
Specificity	
LOD(µg)	Specific
LOQ(µg)	6.03
	18.28

REFERENCES

1. Herman G, Bergman A, Liu F, Stevens C, Wang A, Zeng W, Chen L, Snyder K, Hilliard D, Tanen M, Tanaka W, Meehan A, Lasseter K, Dilzer S, Blum R, Wagner J. Pharmacokinetics and pharmacodynamic effects of the oral DPP-4 inhibitor sitagliptin in middle-aged obese subjects. J Clin Pharmacol. 2006; 46 (8): 876-886.
2. Bala Sekaran C, Prameela Rani A. Development and Validation of spectrophotometric method for the determination of DPP-4 Inhibitor, Sitagliptin, in its pharmaceutical preparations. Int J Pharm Pharm Sci. 2010; 2(4): 138-142.
3. Parag Pathade, ImranMd, Vinod Bairagi,Yogesh Ahire. Development and Validation of Stability Indicating UV Spectrophotometric Method for the Estimation of Sitagliptin Phosphate in Bulk and Tablet Dosage Form. J Pharm Res. 2011; 4(3): 871-873.
4. Ghazala Khan, Dinesh SahuAgrawal Y P, NeetuSabarwal, AvnishJain,Gupta A K. Simultaneous Estimation of Metformin and Sitagliptin In Tablet Dosage Form. Asian J Biochem Pharma Res 2011; 1(2): 352-358.
5. Ramzia El-Bagary I, Ehab Elkady F, Bassam Ayoub M. Spectroflourometric and Spectrophotometric Methods for the Determination of Sitagliptin in Binary Mixture with Metformin and Ternary Mixture with Metformin and Sitagliptin Alkaline Degradation Product. Int J Biomed Sci.2011; 7(1): 62-69.
6. Ravi Pratap Pulla, Sastry B S, Rajendra Prasad Y, Appala Raju N. Simultaneous Estimation of Metformin HCl and Sitagliptin Phosphate in Tablet Dosage Forms by RP-HPLC. Res J Pharm Tech. 2011; 4(4): 646-649.
7. Shyamala M, Mohideen S, Satyanarayana T, Narasimha Raju Ch, Suresh Kumar P, SwethaK. Validated RP-HPLC for simultaneous estimation of Sitagliptin phosphate and Metform in hydrochloride in tablet dosage form. American J Pharm Tech Res. 2011; 1(2): 93-101.
8. Zeng W, Xu Y, Constanzer M, Woolf EJ. Determination of sitagliptinin human plasma using protein precipitation and tandem mass spectrometry. J Chromatogr B. 2010; 878(21):1817-1823.
9. Wei Zeng, Donald Musson G, Alison Fisher L, Li Chen, Michael Schwartz S, Eric Woolf J, Amy Qiu Wang. Determination of sitagliptin in human urine and hemodialysate using turbulent flow online extraction and tandem mass spectrometry. J Pharm Biomed Anal. 2008; 46(3): 534-542.
10. Ramakrishna Nirogi, Vishwottam Kandikere, Koteshwara Mudigonda, Prashanth Komarneni, Raghupathi Al et al. Rajeshkumar Boggavarapu. Sensitive liquid chromatography tandem mass spectrometry method for the quantification of sitagliptin, a DPP-4 inhibitor, in human plasma using liquid-liquid extraction. Biomed Chromatogr. 2008; 22(2): 214-222.