Development and Validation of New Spectrophotometric Methods for the Determination of Sitagliptin

Gunuputi Sushma, Duggirala Sri Harsha and Mukthinuthalapati Mathrusri Annapurna*

GITAM Institute of Pharmacy, GITAM (Deemed to be) University, Visakhapatnam, India

*Corresponding Author: Mukthinuthalapati Mathrusri Annapurna, GITAM Institute of Pharmacy, GITAM (Deemed to be) University, Visakhapatnam, India.

Received: February 27, 2020

Published: February 28, 2020

© All rights are reserved by Mukthinuthalapati Mathrusri Annapurna., et al.

Abstract

Sitagliptin is a novel oral hypoglycemic drug of dipeptidyl peptidase-4 inhibitor class. Dipeptidyl peptidase-4 inhibitors represent a new therapeutic approach for 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 incretins, particularly glucagon-like peptide-1 and gastric inhibitory polypeptide), thereby improving glycemic control. Five new spectrophotometric methods have been established for the determination of Sitagliptin tablets. SHIMADZU Model No. UV – 1800 double beam spectrophotometer with quartz cells was used for the proposed study Sitagliptin has shown absorption maxima at 267 nm in reagents such as phosphate buffers (pH 5.0 and 8.0), acetate buffer (pH 4.7), 0.1N NaOH and borate buffer (pH 9.0) Sitagliptin obeys Beer-Lambert's law over the concentration range 5 - 100 µg/ml for all the above mentioned methods and all the methods were validated as per ICH guidelines. The methods are simple, precise, accurate and economical and can be used for the quantification of Sitagliptin tablets.

Keywords: Sitagliptin; Spectroscopy; Phosphate Buffer; Borate Buffer; Acetate Buffer; Sodium Hydroxide; Validation

Introduction

Sitagliptin (Figure 1) is an antidiabetic agent which is an oral dipeptidyl peptidase-4 inhibitor which exerts its actions in type 2 diabetes patients by slowing the inactivation of incretin hormones. Sitagliptin ($C_{16}H_{15}F_6N_5O$; Mol. Wt. 407.314 g/mol) is chemically (R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro [1,2,4] triazolo [4,3-a] pyrazin-7(8H)-yl]-1-(2,4,5-Trifluoro phenyl) butan-2 amine. It stimulates insulin secretion when hyperglycemia is present and inhibits glucagon secretion [1-4]. Various spectrophotometric methods [5-19] have been developed for the assay of Sitagliptin in literature and the present methods were compared with the previously published methods. The authors have chosen five different reagents for the assay of Sitagliptin in pharmaceutical dosage forms present study and validated [20].

Figure 1: Chemical structure of Sitagliptin.

Materials and Methods

Double beam spectrophotometer (SHIMADZU Model No. UV - 1800) with quartz cells was used for the present study. All the solutions were scanned at 200 - 400 nm range. Sitagliptin is available as film coated tablets (100 mg) with brand name JANUVIA (MSD Pharma), ISTAVEL (Sun Pharma), ZITA (Glenmark). Sitagliptin was obtained as a gift sample from Sun Pharma, India. Reagents such as phosphate buffers with pH 5.0 and 8.0, acetate buffer (pH 4.7), 0.1N NaOH and borate buffer (pH 9.0) and were prepared as per IP 2010.

Procedure

25 mg of Sitagliptin was accurately weighed and transferred in to a 25 ml volumetric flask and dissolved in methanol (1000 µg/ml) and a series of dilutions were prepared with respective buffers as per the requirement. The reagents were prepared as given below.

Preparation of phosphate buffer (pH 5.0)

6.8 grams of potassium dihydrogen phosphate was dissolved in water and the pH was adjusted to 5.0 with 10M potassium hydroxide in a 1000 ml volumetric flask.

Preparation of phosphate buffer (pH 8.0)

50 ml of 0.2M potassium dihydrogen phosphate was placed in a 200 ml volumetric flask and 46.1 ml of 0.2M sodium hydroxide was added and the volume was made up to 200 ml.

Preparation of acetate buffer (pH 4.7)

8.4 grams of sodium acetate and 3.35 ml of acetic acid was dissolved in sufficient water to make 1000 ml in a 1000 ml volumetric flask.

Preparation of 0.1 N sodium hydroxide

4 grams of sodium hydroxide was dissolved in sufficient water and made up to 1000 ml in 1000 ml volumetric flask.

Preparation of borate buffer (pH 9.0)

6.20 grams of boric acid was dissolved in 500 ml of water and the pH was adjusted with 1M sodium hydroxide (about 41.5 ml) and diluted with water to produce 1000 ml in a 1000 ml volumetric flask.

Method validation

Linearity

A series of Sitagliptin solutions 1-100 µg/ml were prepared using different buffer solutions - phosphate buffer pH 5 (Method I), phosphate buffer pH 8 (Method II), acetate buffer pH 4.7 (Method III), NaOH (Method IV), and borate buffer pH 9 (Method V) and scanned against their reagent blank at range of 200–400 nm. Sitagliptin has shown its λ_{max} at 267 nm in all methods. A calibration curve was drawn by taking the concentration on the X-axis and their respective absorbance on the Y-axis for all the methods.

Precision and accuracy

Precision was studied by measuring the absorbance of 6 solutions of the same concentration (n = 6) and thereby mean, standard deviation and relative standard deviation were calculated. Accuracy was studied by spiking the formulation solution of a fixed concentration with pure drug solution (50%, 100% and 150%) by standard addition method and there by percentage recovery and relative standard deviation were calculated.

Assay of sitagliptin tablets

20 tablets of Sitagliptin of two different brands were procured from the local pharmacy store and extracted with methanol and further dilutions were made with respective buffers and assay was carried out for all the methods.

Results and Discussion

New spectrophotometric methods were developed for the determination of Sitagliptin in different reagents such as phosphate buffer (pH 5.0) (Method I), phosphate buffer (pH 8.0) (Method II), acetate buffer (pH 4.7) (Method III), NaOH (Method IV), and borate buffer (pH 9.0) (Method V) respectively. The present methods were compared with the previously published methods and summarized in table 1.

The absorption spectrum of Sitagliptin has shown (λ_{max} 267 nm) in all the above mentioned methods (Figure 2). Sitagliptin obeys

Method	Reagent	λ _{max} (nm)	Linearity (µg/mL)	Ref
Spectrophotometry	Distilled water	267	2 - 10	[5]
Spectrophotometry	Distilled water	267	10 - 60	[6]
Spectrophotometry	Distilled water	267	20 - 70	[7]
Spectrophotometry	Distilled water	267	20 - 160	[8]
Spectrophotometry	Double distilled water	267	10 - 100	[9]
Spectrophotometry	Distilled water			[10]
Spectrophotometry	Methanol	267	20 - 60	[11]
Spectrophotometry	Water and Methanol (70:30)	266	20 - 100	[12]
Spectrophotometry	Methanol	272.5	35 - 85	[13]
Spectrophotometry	Quinalizarin in DMSO	580	15 - 65	[14]
Spectrophotometry	Acetyl acetone and Formaldehyde	430	5 - 25	[15]
Spectrophotometry	Acid phthalate buffer (pH 2.4) and Methyl Orange	633	30 - 120	[16]
		415	20 - 80	
Spectrophotometry	2,4-DNP with 0.1 N H ₂ SO ₄ and Methanol	400	2 - 10	[17]
Spectrophotometry	0.1 N HCl	267	5 - 40	[18]
Spectrophotometry	0.1 N HCl	267	2 - 30	[19]
Spectrophotometry	0.1 N HCl	267	20 - 100	[20]
Spectrophotometry	NaOH Phosphate buffer (pH 5.0) Phosphate buffer (pH 8.0) Borate buffer (pH 9.0) Acetate buffer (pH 4.7)	267	5 -100	Present methods

Table 1: Review of Previously published articles.

Beer-Lambert’s law over the concentration range 5 - 100 µg/ml for all the methods (Table 2). Calibration curves (Figure 3) were drawn by taking the concentration on the x-axis and the corresponding absorbance on the y-axis. The linear regression equations are found to be y = 0.004x + 0.0061 (0.9989), y = 0.0041x + 0.0042 (0.9994), y = 0.0051x + 0.0014 (0.9996), y = 0.0047x + 0.0008 (0.9996), y = 0.004x + 0.0036 (0.9993) for phosphate buffer (pH 5.0 and pH 8.0), acetate buffer (pH 4.7), NaOH and borate buffer (pH 9.0) respectively. The percentage of purity of Sitagliptin was found to be 99.69-99.93 (Table 3) in the marketed formulations and there is no interference of excipients. The percentage RSD in precision and accuracy was found to be <2 in all the above methods indicating that the methods are accurate and precise (Table 4). The Optical characteristics of Sitagliptin were shown in table 4.

Figure 2: Absorption spectra of Sitagliptin (60 µg/ml).

Conc. (µg/ml)	Absorbance				
	Method I	Method II	Method III	Method IV	Method V
5	0.025	0.023	0.024	0.025	0.025
10	0.047	0.048	0.051	0.044	0.039
20	0.090	0.089	0.104	0.090	0.084
40	0.170	0.170	0.210	0.189	0.166
60	0.244	0.252	0.308	0.285	0.246
80	0.333	0.334	0.409	0.388	0.320
100	0.399	0.407	0.503	0.484	0.394

Table 2: Linearity of Sitagliptin.

Figure 3: Calibration curves of Sitagliptin in different methods.

Brand	Method I		Method II		Method III		Method IV		Method V	
	Observed amount (mg)	% Recovery	Observed amount (mg)	% Recovery	Observed amount (mg)	% Recovery	Observed Amount (mg)	% Recovery	Observed Amount (mg)	% Recovery
I	99.87	99.87	99.88	99.88	99.82	99.82	99.80	99.80	99.81	99.89
II	99.81	99.81	99.86	99.86	99.93	99.93	99.79	99.79	99.69	99.73

Table 3: Assay of Sitagliptin (Label claim: 100 mg).

*Mean of three replicates

Parameters		Method I	Method II	Method III	Method IV	Method V
Linearity (µg/ml)		1 - 100	1 - 100	1 - 100	1 - 100	1 - 100
λ _{max} (nm)		267	267	267	267	267
Molar extinction coefficient (litre/ mole/ cm ⁻¹)		1.914×10 ³	3.625×10 ³	2.077×10 ³	1.792×10 ³	1.588×10 ³
Sandell's sensitivity (µg/cm ² /0.001 absorbance unit)		0.212	0.224	0.196	0.227	0.256
Slope		0.004	0.0041	0.0051	0.0047	0.004
Intercept		0.0061	0.0042	0.0014	0.0008	0.0036
Correlation coefficient		0.9989	0.9994	0.9996	0.9996	0.9993
Precision (%RSD)	Intraday	0.86 - 0.91	0.78 - 0.99	0.76 - 0.92	0.62 - 0.79	0.43 - 0.94
	Interday	1.09 - 1.16	0.81 - 0.86	0.84 - 0.91	0.29 - 0.65	0.78 - 0.79
Accuracy (% RSD)		0.91 - 1.31	0.15 - 0.38	0.22 - 0.69	0.68 - 0.98	0.36 - 0.52

Table 4: Optical characteristics of Sitagliptin.

Conclusion

The new proposed spectrophotometric methods were validated and applied for the pharmaceutical formulations i.e. Tablets. The percentage relative standard deviation was found to be less than 2.0 in all the five methods indicating that the methods are precise and accurate. The proposed methods are economical and the methods can be successfully applied for the determination of Sitagliptin in pharmaceutical dosage forms. There is no interference of excipients.

Acknowledgement

The authors are grateful to M/s GITAM (Deemed to be University), Visakhapatnam for providing the research facilities and to SUN Pharma for providing the gift samples of Sitagliptin.

Bibliography

1. Ahren B. "What mediates the benefits associated with dipeptidyl peptidase-IV inhibition?" *Diabetologia* 48 (2005): 605-607.

2. Ahren B., et al. "Beta-cell expression of a dominant-negative HNF-1alpha compromises the ability of inhibition of dipeptidyl peptidase-4 to elicit a long-term augmentation of insulin secretion in mice". *European Journal of Pharmacology* 521 (2005): 164-168.

3. Aschner P., et al. "Effect of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy on glycemic control in patients with type 2 diabetes". *Diabetes Care* 29 (2006c): 2632-2637.

4. Deacon CF, et al. "Dipeptidyl peptidase IV inhibition as an approach to the treatment and prevention of type 2 diabetes: a historical perspective". *Biochemical and Biophysical Research Communications* 294 (2002): 1-4.

5. Ravisankar P., et al. "A simple validated UV Spectrophotometric method for quantitative analysis of Sitagliptin phosphate in pharmaceutical dosage form". *Journal of Chemical and Pharmaceutical Sciences* 7.3 (2014): 254-258.

6. Jain Pritam., et al. "Development and validation of first order derivative UV-Spectrophotometric method for determination of Sitagliptin in bulk and in Formulation". *International Journal of Drug Development and Research* 3.4 (2011): 194-199.

7. Maste Meenaxi M., et al. "Spectrophotometric method for estimation of Sitagliptin phosphate in bulk and tablet dosage form". *International Journal of Pharmaceutical Formulation and Analysis* 2.2 (2011): 47-50.

8. Vinit Chavhan and Atul Kadam "UV Spectrophotometric method development and validation for estimation of Sitagliptin phosphate in bulk and tablet dosage Formby absorption ratio and area under the curve method". *Journal of Biological and Scientific opinion* 1.4 (2013): 317-322.

9. Parag Pathade., et al. "Development and validation of stability indicating UV spectrophotometric method for the estimation of Sitagliptin phosphate in bulk and tablet dosage form". *Journal of Pharmacy Research* 4.3 (2011): 871-873.

10. Jeyabalan G and Narendra N. "Analytical method development and validation of Sitagliptine phosphate monohydrate in pure and tablet dosage form by derivative spectroscopy". *Journal of Applied Pharmaceutical Science* 3.1 (2013): 95-98.

11. Jalkote RN and Kalshetti MS. "UV Spectrophotometric method development and validation for determination of Sitagliptin in API and in pharmaceutical dosage form". *World Journal of Pharmacy and Pharmaceutical Sciences* 8.5 (2019): 916-925.

12. Patil Sachin., et al. "Validated UV spectrophotometric method for estimation of Sitagliptin phosphate in tablet dosage form". *Research Journal of Pharmacy and Technology* 3.3 (2010): 798-800.

13. Saad Antakli., et al. "Spectrophotometric determination of Sitagliptin in tablets formulations". *Asian Journal of Chemistry* 27.1 (2015): 375-378.

14. Bala Sekaran C and Prameela Rani A. "Development and validation of spectrophotometric method for the determination of DPP-4 inhibitor, Sitagliptin, in its pharmaceutical preparations". *Eclética Química* 35.3 (2010): 45-53.

15. Deepanjali Mohanty., et al. "Extractive spectrophotometric estimation of Sitagliptin phosphate". *International Journal of ChemTech Research* 10.4 (2017): 255-263.

16. Disha NS and Gurupadhyaya BM. "Spectrophotometric determination of Sitagliptin Phosphate in bulk and pharmaceutical formulations". *International Journal of Innovative Science, Engineering and Technology* 2.7 (2015): 702-709.

17. Tarkase KN., et al. "Development and validation of UV spectrophotometric method for estimation of Sitagliptin phosphate". *Der Pharmacia Lettre* 5.3 (2013):315-318.

18. Namratha Sunkara., et al. "UV spectrophotometric method development and validation of Sitagliptin in bulk and pharmaceutical dosage form". *International Journal of Pharma and Chemical Research* 3.3 (2017): 577-580.

19. Anudeepa J., et al. "Development of UV-Spectrophotometric method for Sitagliptin in bulk and pharmaceutical formulation". *International Journal of Science, Engineering and Technology* 3.5 (2015): 1352-1354.

20. ICH Q2 [R1] validation of analytical procedures: Text and Methodology: November (2005).

Assets from publication with us

- Prompt Acknowledgement after receiving the article
- Thorough Double blinded peer review
- Rapid Publication
- Issue of Publication Certificate
- High visibility of your Published work

Website: www.actascientific.com/
Submit Article: www.actascientific.com/submission.php
Email us: editor@actascientific.com
Contact us: +91 9182824667