ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Development and Validation of RP-HPLC Method for Content Analysis and Dissolution Studies Of Sitagliptin Phosphate Monohydratein Pharmaceutical Dosage Forms

R Revathi*[1], Perala Kiranmayi[1], Inturi Venkatesh Chowdary[2], and S Saravanan[1]

[1]Department of Analysis, The Erode College of Pharmacy & Research Institute, Erode, Tamilnadu, India.
[2]Department of Pharmacology, Bapatla College of Pharmacy, Bapatla, Andhra Pradesh, India.

ABSTRACT

A simple and accurate reverse phase high performance liquid chromatography (RP-HPLC) method for the validative determination of assay and dissolution of Sitagliptin Phosphate Monohydrate 100mg was developed and validated as per USP 1225 and ICH guidelines Q2(R1).The suitable chromatographic conditions were optimizedat a flow rate of 1.0ml/min along with the detection wavelength of 257nm.The method shows the linearity in the range of 50-150mg/mL with correlation coefficient of 0.9993.The assay value was obtained between 98%-101%. Precision shows relative standard deviation not more than 2%.The satisfactory conditions for the validation of dissolution were 900mL of distilled water at 37°C±0.5°C, basket apparatus with 100rpm stirring speed. The cumulative percentage drug release was found to be higher than 95% within 45min under the validated conditions. The stability of drug was satisfied up to 24hr at room temperature and at refrigeration and does not show any variations in the percentage drug release. The method was simple and free of sophisticate expenditure as it involves the usage of most commonly available reagents, medium and buffer. Hence, it was concluded that the proposed method was cost effective and sensitive for regular dissolution and content analysis determination of Sitagliptin Phosphate Monohydrate.

Keywords: Sitagliptin Phosphate Monohydrate, Reverse phase-HPLC, Dissolution, Validation.

**Corresponding author*

ISSN: 0975-8585

INTRODUCTION

Sitagliptin Phosphate Monohydrate is chemically designated as (R)-4-oxo-4-[3(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.The chemical structure of Sitagliptin Phosphate Monohydrate is provided here.

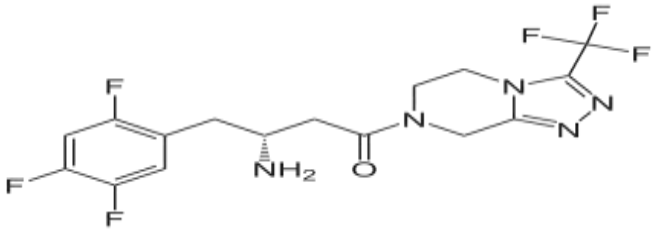

Figure 1: Structure of Sitagliptin Phosphate Monohydrate

Sitagliptin is a highly selective DPP-4 inhibitor, which is believed to exert its actions in patients with type 2 diabetes by slowing the inactivation of incretin hormones, thereby increasing the concentration and prolonging the action of these hormones. Incretin hormones, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulin tropic polypeptide (GIP), are released by the intestine throughout the day, and levels are increased in response to a meal. These hormones are rapidly inactivated by the enzyme, DPP-4. The benefit of this medicine is expected to be its lower side-effects of hypoglycaemia in the control of blood glucose values. The drug works to diminish the effects of a protein/enzyme (by the inhibition of this protein/enzyme) on the pancreas at the level of release of glucagon (diminishes its release) and at the level of insulin (increases its synthesis and release) until blood glucose levels are restored toward normal, in which case the protein/enzyme-enzyme inhibitor becomes less effective and the amounts of insulin released diminishes thus diminishing the "overshoot" of hypoglycaemia seen in other oral hypoglycaemic agents.

MATERIALS AND METHODS

Reagents

Sitagliptin Phosphate Monohydrate WS, Sitgaliptin Phosphate Monohydrate tablet placebo, Perchloric acid AR grade, Acetonitrile - AR grade, Hydrochloric acid, Sodium Hydroxide, Hydrogen Peroxide, Acetonitrile- HPLC grade.

Instruments

HPLC system configuration (Waters E 2998, 2985 Separation module) ,UV Spectroscopy-Shimadzu corp. 04608,Analytical weighing balance - Shimadzu AUW22OD,Digital pH meter, Ultra Sonicator (Make fast clean),Laboratory accessories.

ISSN: 0975-8585

For Content analysis

Standard preparation: Accurately weighed 64.2 mg of Sitagliptin Phosphate Monohydrate working standard was taken in 50 mL volumetric flask and was dissolved in the diluent and made up to the volume with the diluent. Further 5mL of this solution is added to 50mL volumetric flask. It was mixed and filtered through a 0.45 µm membrane filter.

Sample preparation: About 20 tablets of sitagliptin were weighed and triturated into fine powder. A quantity of powder equivalent to 50 mg of Sitagliptin was transferred into a 50 mL volumetric flask. About 25- 30 mL of diluent was added and sonicated for 30 minutes with intermediate shaking. Then made up to the volume with diluent and mixed. Further 5 mL of this solution was diluted to 50mL with diluent and mixed. The solution was filtered through 0.45µm membrane filter and was injected into the liquid chromatography and the areas for major peaks were recorded.

For Dissolution Studies

Standard preparation: About 5 mL was transferred from stock solution into a 50 mL volumetric flask. Further it was made up to the volume with dissolution medium and filtered the solution through 0.45 µm membrane filter.

Sample preparation: About 64.5 mg of working standard Sitagliptin Phosphate Monohydrate was transferred into a 50 mL volumetric flask. Then 30 mL of diluent was added and sonicated to dissolve, and made up to 50 mL with the diluent and was injected into liquid chromatograph and chromatograms were recorded.

RESULTS

For Content Analysis

System Suitability

This parameter has been performed before starting any validation parameter each time. The purpose for this parameter is the checking of a system, before or during analysis of unknowns, to ensure system performance.

Table-1: Results for System suitability

S.No.	System suitability results	
	USP Plate count	USP Tailing
1	11207	0.10
2	7693	0.18
3	8188	0.05
4	8075	0.03
5	7213	0.48
Mean	8475	0.16

ISSN: 0975-8585

Specificity

Specificity of the method was established through determination of purity peak of Sitagliptin Phosphate Monohydrate using a PDA detector. No peak observed at retention time for placebo and standard determinations.

Accuracy

The mean % recovery values of not less than 98.0 % and not more than 102.0 % of at each level indicated the good accuracy of the method.

Table-2: Results for Accuracy

S. No.	Test Concentration (%)	Peak response	Recovery Std (ppm)	Standard added(ppm)	Recovery (%)
1.	50%	204406	73.32	72.78	100.8
		2043692	73.31	72.89	100.8
		2043275	73.29	72.52	100.8
2.	100%	4021046	144.23	143.30	100.9
		4018635	144.15	143.30	100.9
		4018202	144.13	142.42	100.9
3.	150%	5869532	210.54	213.72	98.3
		5872220	210.64	214.14	98.3
		5867142	210.45	214.44	98.3
Average					99.2
Std deviation					1.26
%RSD					1.26

Precision:

The %RSD for system precision, intermediate precision and method precision were lower than 2.0% and were within the acceptable limits.

Table-3: Results for Precision

S.No.	Precision	Peak area	Retention time (Mean)	SD	%RSD
1	System precision	3584167	4.7	0.1	1.09
2	Intermediate precision	4082526	4.5	0.1	1.08
3	Method precision	3583015	4.6	0.1	1.05

Linearity

The linearity of response was determined at different concentrations from 50% to 150% of the target concentration and the Correlation Coefficient value 0.9993 indicated that the method was linear.

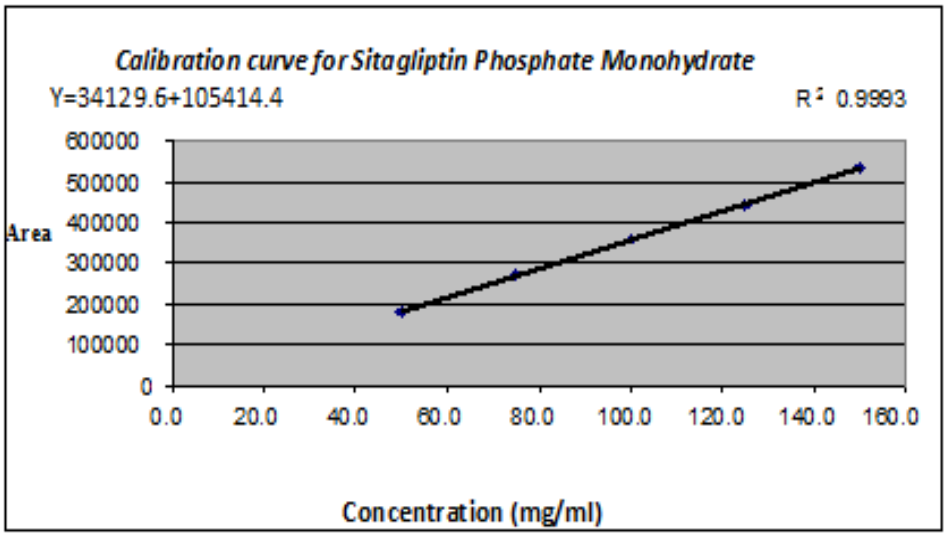

Figure 2: Calibration curve for Linearity

Solution stability

The %RSD for peak response of standard solution and test solution at 24 hours are 0.04%, 0.24%, 0.24%.The standard solution and test solution are stable up to 24 hours.

Robustness

The content of the drug was not adversely affected by the changes of flow rate and mobile phase concentration, as evident from the low value of RSD, indicating that the method is robust.

Table-4: Results for Robustness

S.No.	Parameters		%RSD	Tailing factor	Average area	Theoretical plate
1	Flow rate (mL/min)	0.8	0.05	1.7	4510589	7502
		1.2	0.02	1.7	4511264	7524
2	Mobile phase	58:42	0.07	1.7	4510389	7512
		62:38	0.06	1.7	4512712	7531

For Dissolution studies

Specificity

Specificity of the method was established through determination of purity peak of Sitagliptin Phosphate Monohydrate using a PDA detector. No peak observed at retention time for placebo and standard determinations.

ISSN: 0975-8585

Accuracy

The mean % recovery values of not less than 98.0 % and not more than 102.0 % of at each level indicated the good accuracy of the method.

Table-5: Results for Accuracy

S.No.	Percent test Concentration	Peak response	Recovery Std (ppm)	Standard added(ppm)	Recovery (%)
1	50%	2048491	73.48	73.14	100.8
		2041690	73.31	72.89	
		2044217	73.27	72.52	
2	100%	4020047	134.21	133.30	100.8
		4028655	134.15	133.30	
		4017202	134.13	132.42	
3	150%	5861512	211.54	211.72	98.3
		5870250	211.62	211.14	
		5866102	211.41	211.44	
Avg					99.92
Std dev					1.25
% RSD					1.24

Precision

The %RSD for system precision, intermediate precision and method precision were lower than 2.0% and were within the acceptable limits.

Table-6: Results for Precision

S.No.	Precision	Peak area	Retention time (Mean)	SD	%RSD
1	System precision	2230020	4.7	0.41	0.39
2	Intermediate precision	4200251	4.5	0.42	0.37
3	Method precision	2053681	4.6	0.47	0.33

Linearity

The linearity of response was determined at different concentrations from 50% to 150% of the target concentration and the Correlation Coefficient value 0.9993 indicated that the method was linear.

Solution stability

The %RSD for peak response of standard solution and test solution at 24 hours are 0.05%, 0.25%.The standard solution and test solution are stable up to 24 hours.

ISSN: 0975-8585

Robustness

The content of the drug was not adversely affected by the changes of flow rate and mobile phase concentration, as evident from the low value of RSD, indicating that the method is robust.

Table7: Results for Robustness

S .No.	Robustness parameters	Chromatographic Conditions	
		Lower	Higher
1	Bowl temperature	35^0C	39^0C
2	RPM (Buffer : Acetonitrile)	90	110
3	%RSD	0.65	0.81

CONCLUSION

The proposed analytical method is simple, accurate and reproducible. Sitagliptin Phosphate Monohydrate showed λ_{max} at 257 nm. The advantages lie in the simplicity of sample preparation and the cost economic reagents used. Hence, this method can be used for analysis of different solid dosage formulations in commercial quality control laboratories. Considering the efficiency of HPLC, an attempt has been made to develop simple, accurate, precise, rapid and economic methods for estimation of Sitagliptin Phosphate Monohydrate in a solid dosage form. Thus, the method described enables quantification of Sitagliptin Phosphate Monohydrate. Results from statistical analysis of the experimental results were indicative of satisfactoryprecision.

Hence, this HPLC method can be used for analysis of different formulations in quality control laboratories.

ACKNOWLEDGEMENT

The authors wish to thank respective guide and all the professors of the institution.

REFERENCES

[1] Arun M Kashid, Anup A Dhange, Vandana T. Am J PR 2012; 2(5): 806-810.
[2] Balasekaran C, Prameela Rani. IJPPS. 2010; 2(4): 138-142.
[3] Chandanam Sridhar, Manogna K. Res J Pharm Biol Chem Sci 2012; 3(4): 20.
[4] Chirag B Patel, Mitesh H. IJABC 2013; 3(1):47-51.
[5] Gurdeep R Chatwal, Sham K Anand. Instrumental Methods of Chemical Analysis, Himalaya Publishers; New Delhi; 2007; 5th edition: 2.624-2.639.
[6] Ghazala Khan, Dinesh Sahu. AJBPR 2011; 2(1): 2231-2560.
[7] Hitesh P Inamdar, Ashok A Mhaske. IJPSR 2012; 3(9):3267-3276.

[8] Hobart H Willard, Lynne l Merritt, John A Dean, Frank A. Instrumental Methods of Analysis. CBS Publishers; New Delhi; 2001; 7th edition: 2-5.
[9] ICH R1 Guidelines on validation of Analytical procedure, text and methodology (2005).
[10] ICH Q4B Annex 7 Guidelines on Dissolution Test General Chapter (2007).
[11] Jing jing Liu, Xueheng Cheng TRSC 2012; 15: 1-30.
[12] Ramzia I, El-Bagary. IJBS 2011; 7(1): 62-68.
[13] Sheetal Sharma, Nimita Manocha, Priya Bhandari. IJPBA 2012; 3(3): 673-678.