WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 16, 1093-1110. Research Article ISSN 2277–7105

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTITATIVE ESTIMATION OF SITAGLIPTIN PHOSPHATE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

M. Phanisri*[1], M. Jhansi Lakshmi[1], D. Rama Brahma Reddy[2] and H. Padmalatha[2]

Gyana Jyothi College of Pharmacy, Uppal, Hyderabad -500089, Telangana, India.

Article Received on
17 October 2017,
Revised on 06 Nov. 2017,
Accepted on 27 Nov. 2017
DOI: 10.20959/wjpr201716-10282

***Corresponding Author**
M. Phanisri
Gyana Jyothi College of Pharmacy, Uppal,
Hyderabad -500089,
Telangana, India.

ABSTRACT

A simple and rapid reversed phase-high performance liquid chromatographic method was developed for simultaneous determination of Sitagliptin Phosphate In Bulk And Pharmaceutical Dosage forms. The elution was done with a mobile phase of Methanol, Acetonitrile & 0.1% ortho phosphoric acid in the ratio of 40:55:05 & adjust to pH 4.1 by using triethyl amine on Zodiac C_{18} column (250 × 4.6 mm, 5µ). The wavelength of detector was set at 265 nm. The reliability and analytical performance of the proposed HPLC procedure were statistically validated according to the respect of linearity, ranges, precision, accuracy, repeatability, reproducibility, detection and quantification limits. Linear ranges were established between 60-210 µg/mL for the drug. The LOD and LOQ for Sitagliptin was found to be 0.05, 0.16 respectively. The described High Performance Liquid Chromatography method was successfully employed for the analysis of pharmaceutical formulations.

KEYWORDS: Sitagliptin, RP- HPLC, validation, UV Detection, Zodiac C_{18} column.

INTRODUCTION

Sitagliptin phosphate is an oral dipeptidyl peptidase-4 (DPP-4) inhibitor, which improves glycaemic control by inhibiting DPP-4 inactivation of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). This increases active incretin and insulin levels and decreases glucagon levels and post-glucose-load glucose excursion.

Category: Dipeptidyl peptidase-4 (DPP-4) inhibitor.

Therapeutic Category: Type-II Diabetes mellitus.

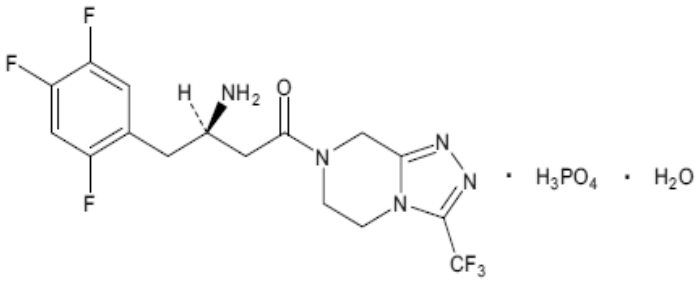


Table 1: Data for available formulations.

Drug name	Brand name	Company	Available strength
Sitagliptin phosphate	Januvia	Merck Sharp & Dohme Co.	Tablet: 25mg, 50mg, 100mg.
Combination:			
Sitagliptin phosphate + Metformin hydrochloride	Janumet	Merck Sharp & Dohme Co.	Tablet: 50mg + 500mg, 50mg + 1000mg.
Sitagliptin phosphate + Simvastatin	Juvisync	Merck Sharp & Dohme Co.	Tablet: 100mg + 20mg.

High Performance Liquid Chromatography is the most widely used of all the analytical separation techniques. The reason for the popularity of the method is its sensitivity, its suitability for separating non-volatile or even thermally fragile ones, its ready adaptability to quantitative determinations and above all its wide spread applicability to substances that are of primary interest to industry, to many fields of science, biomedical applications.

Reverse phase chromatography is a bonded phase chromatographic technique that uses water as base solvent. Separation is based on solvent strength and selectivity. Separation is also affected by column temperature and p^H. In general, the more polar compounds elute faster than the less polar compounds.

Method validation is an integral part of the method development; it is the process by which a method is tested by the developer or user for reliability, accuracy and preciseness of its intended purpose and demonstrating that analytical procedures are suitable for their intended use that they support the identity, quality, purity, and potency of the drug substances and drug products. The search for the reliable range of a method and continuous application of this knowledge is called validation. Simply, method validation is the process of proving that an analytical method is acceptable for its intended purpose.

MATERIALS AND METHODS

Drug samples

Sitagliptin phosphate working standard was received as gift sample from Mylan Laboratories Ltd., Hyderabad, India.

Formulation used

Januvia tablets containing 100mg of sitagliptin phosphate were procured from local pharmacy.

Reagents and Chemicals used

- Sitagliptin phosphate WS (Potency-99.9%).
- Acetonitrile (HPLC grade).
- Methanol (HPLC grade).
- Milliq water.
- Ortho phosphoric acid.
- Triethyl amine.

Instruments used

- Schimadzu LC20-AD HPLC system with Rheodyne universal injector 7725 and LC20-AD UV-Visible detector module equipped with SPINCHROM software was used.
- Ultra Sonicator (Make fast clean).
- Water bath shaker (Make: Lab tech; Model: HHE-32 cm).
- Thermo Orion pH meter.
- Analytical balance (Mettler).

Development and Optimization of Chromatographic Condition

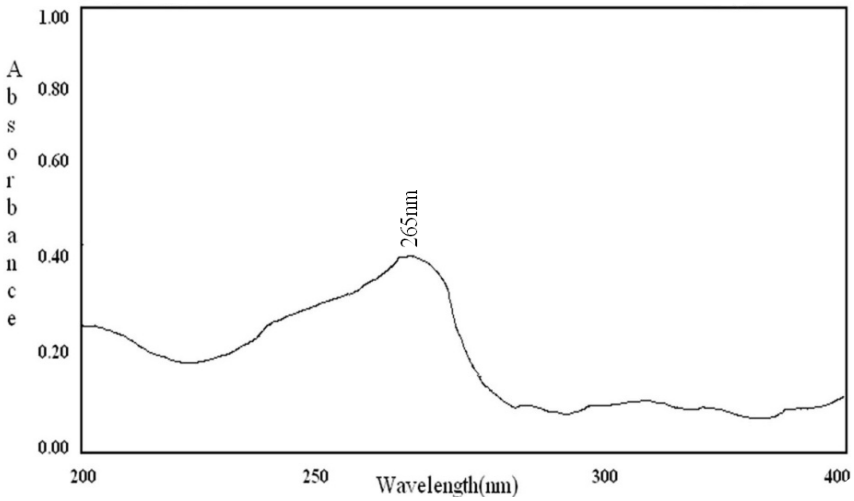
Solubility

According to literature review, Sitagliptin phosphate is freely soluble in methanol and acetonitrile. Therefore the solubility of the drug was checked with different dilutions of buffer methanol and acetonitrile. Finally methanol and acetonitrile was chosen as solvent for present work.

Selection of wavelength (λmax)

By scanning the sample solution of Sitagliptin phosphate by using UV method at a wavelength range of about 200nm to 400 nm against mobile phase as a blank. The wave

length selected was 265 nm because it shows maximum absorbance and was chosen for further studies.



UV spectrum for Sitagliptin phosphate.

Selection of mode of separation

Appropriate selection of mode of separation depends upon the characteristic nature of the sample (ionic or neutral) molecular weight and solubility. The nature of Sitagliptin phosphate is polar hence reverse phase mode was proposed for initial chromatographic conditions.

Optimized chromatographic conditions

Stationary Phase	:	Zodiac C_{18} column (250 × 4.6 mm, 5µ).
Pump mode	:	Isocratic.
Flow rate	:	1.0 ml/min.
Column temperature	:	Ambient.
Selected wave length	:	265 nm.
Mobile phase ratio	:	Methanol, Acetonitrile & 0.1% Ortho phosphoric acid in the ratio of 40:55:05 & adjust to pH 4.1 by using triethyl amine.
Diluents	:	Mobile Phase.
Injection Volume	:	20 µl.
Run Time	:	10 minutes.

Quantitative Estimation of Tablet Formulation by Proposed Method:

Preparation of mobile phase

Prepare 40ml of methanol, 55 ml of acetonitrile and 5ml of 0.1% ortho phosphoric acid was mixed and degassed in ultrasonic water bath for 5 minutes, and adjust to pH 4.1 by using triethyl amine. This was filtered through 0.45µ membrane filter.

Preparation of working standard solution

Accurately weighed 10 mg of sitagliptin phosphate working standard was taken in 10 ml volumetric flask, dissolved and diluted to volume with mobile phase and mixed.

Preparation of sample preparation

Exactly 20 tablets were weighed and grinded to fine powder. A quantity of powder equivalent to 10 mg of sitagliptin phosphate was transferred into a 10 ml volumetric flask and dissolved in 7 ml of diluent. The solution was sonicated for 15 min and shaken for 30 min. Then diluted to volume with diluent and mixed. Pipette out 1mL of the above stock solution into a 10mL volumetric flask and diluted up to the mark with diluent. Mix well and filter through 0.45µ membrane filter. The filtrate was injected for the further analysis.

Procedure

Separately inject the standard preparation and the sample preparation in to the liquid chromatography and record the area for major peaks. The % assay results were tabulated in Table 11.

Calculation

The % assay of the sample was determined by using the following formula

$$\frac{\text{Sample area X Standard weight X Sample dilution X Purity of working standard X Average weight X 100}}{\text{Standard area X Standard dilution X Sample weight X Label claim}}$$

VALIDATION PARAMETERS

1. System suitability

From the chromatogram obtained for the standard preparation, the column efficiency was determined. The theoretical plates obtained should be not less than 2500 and the tailing factor should be not more than 2.0 and the relative standard deviation of replicate injection should be not more than 2.0%.

2. Accuracy

A study of Accuracy was conducted by recovery studies. Recovery studies were performed by spiking the previously analyzed sample of Sitagliptin phosphate with the known amounts of pure drug at different concentration levels. The spiked levels were 50%, 100% and150%. The % recovery was calculated three times at each level and the average % recovery was calculated.

3. Precision

a). System precision

Standard preparation

Accurately weighed 10 mg of sitagliptin phosphate working standard was taken in 10 ml volumetric flask, dissolved and diluted to volume with mobile phase and mixed.

Procedure

Separately inject the standard solution for 5 times in to the liquid chromatography and confirm the system suitability and then record the response of area in record of analysis for system precision.

b). Intraday precision

Sample Preparation

Weigh and grind 20 tablets to fine powder. Transfer a quantity of powder equivalent to 10 mg of Sitagliptin phosphate into a 10 ml volumetric flask; add 7 ml of mobile phase. Sonicate for 15 minutes and shake for 30 minutes. Dilute to volume with mobile phase and mix. 10ml of this solution is taken into 10 ml of volumetric flask and the volume is made up to the mark to get a final concentration of 120 ppm of Sitagliptin phosphate. Filter through 0.45 µ membrane filter by using the filtrate as the sample preparation.

Procedure

Separately inject the standard solution for 5 times in to the liquid chromatography and confirm the system suitability and then record the response of area. Prepare the sample preparation for 6 times and inject all the solutions in duplicate and run the sample injections for 30 minutes. Record all the areas in record of analysis for repeatability.

c). Interday precision

Evaluates the reliability of the method in different environments. The objective is to ensure that the method provides the same results when same sample are analyzed on different days on different instruments and by different analysts.

4. Linearity

A Series of solutions are prepared using Sitagliptin phosphate working standard at concentration levels from 60 ppm to 210 ppm of target concentration (60, 90, 120, 150, 180

and 210 ppm). Peak area response of solutions at Level 1 and Level 6 was measured six times.

5. Limit of detection and Limit of quantitation

Limit of detection and Limit of quantitation represents the concentration of analyte that would yield Signal to Noise ratio of 3:1 and 10:1. The limit of quantitation is approximately twice than that of limit of detection**.**

6. Robustness

For demonstrating the robustness of the developed method, experimental conditions were purposely altered and evaluated. The method must be robust enough to withstand such slight changes and allow routine analysis of the sample. Following optimized conditions were slightly varied.

a). Effect of variation in mobile phase composition

A study was conducted to determine the effect of variation in Organic phase composition in mobile phase. Standard solution prepared as per the test method was injected into the HPLC system.

b). Effect of variation of flow rate

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system and the chromatograms were recorded using flow rates, 0.8ml/min and 1.2ml/min.

c). Effect of variation of pH

A study was conducted to determine the effect of variation in pH. Standard solution prepared as per the test method, was injected into the HPLC system at 4.0 and 4.2 pH.

RESULTS AND DISCUSSION

Quantitative Estimation of Tablet Formulation by Proposed Method

Percentage assay of Sitagliptin phosphate tablets were carried out by proposed method.

The % purity of Sitagliptin phosphate was found to be 98.50 %.

Table 2: Quantitative estimation of tablet formulation.

S.No	Brand name	Standard area	Sample area	Labelled claim (mg)	Amount found(mg)	% Purity
1.	Januvia	539793	533644	100	98.50	98.50

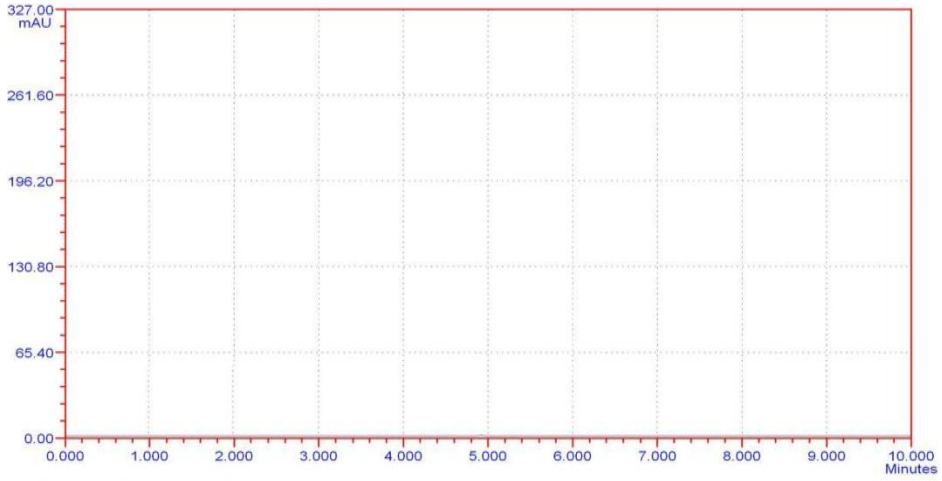


Fig 1: Chromatogram for Sitagliptin phosphate blank.

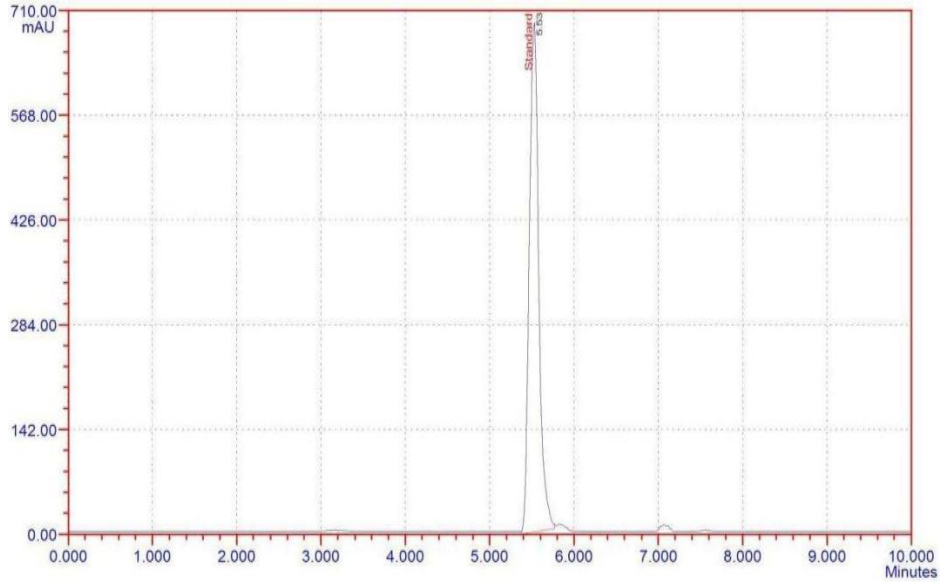


Fig 2: Chromatogram for Sitagliptin phosphate WS.

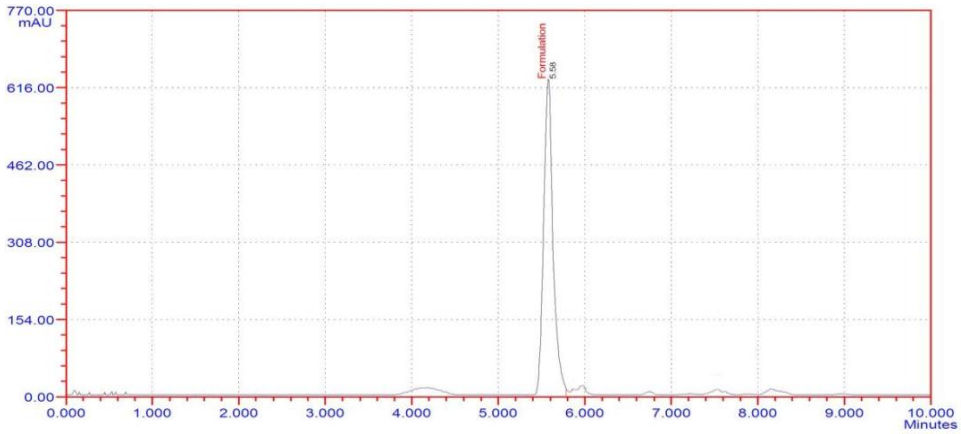


Fig 3: Chromatogram for Sitagliptin phosphate tablet formulation.

Validation Results

1.System suitability

The % RSD values is NMT than 2.0% for the retention times of principal peak from 5 replicate injections of Standard solution indicate that the system was acceptable. The number of theoretical plates (N) for the Sitagliptin phosphate peak is NLT 2500.The Tailing factor (T) for the Sitagliptin phosphate is NMT 2.0.

Table 3: Data for system suitability.

S.NO	System suitability results	
	USP Plate count	USP Tailing
1.	9564	1.28
2.	9942	1.34
3.	9375	0.56
4.	9515	0.66
5.	9383	0.78
Mean	9556	0.92

2. Accuracy (Recovery studies)

The mean % recovery values of not less than 98.0% and not more than 102.0% of Sitagliptin phosphate at each level indicates the good accuracy of the method. The results were tabulated in Table 6.

Table 4: Data for accuracy recovery studies

Level	Target in ppm	Amount of Sitagliptin spiked (ppm)	Total in ppm	Amount of Sitagliptin recovered (ppm)	% Recovery
50 %	60	30	90	88.71	98.57
	60	30	90	89.84	99.82
	60	30	90	88.94	98.82
100%	60	60	120	119.57	99.64
	60	60	120	120.62	100.51
	60	60	120	121.02	100.85
150%	60	90	150	152.81	101.87
	60	90	150	151.33	100.89
	60	90	150	152.37	101.58

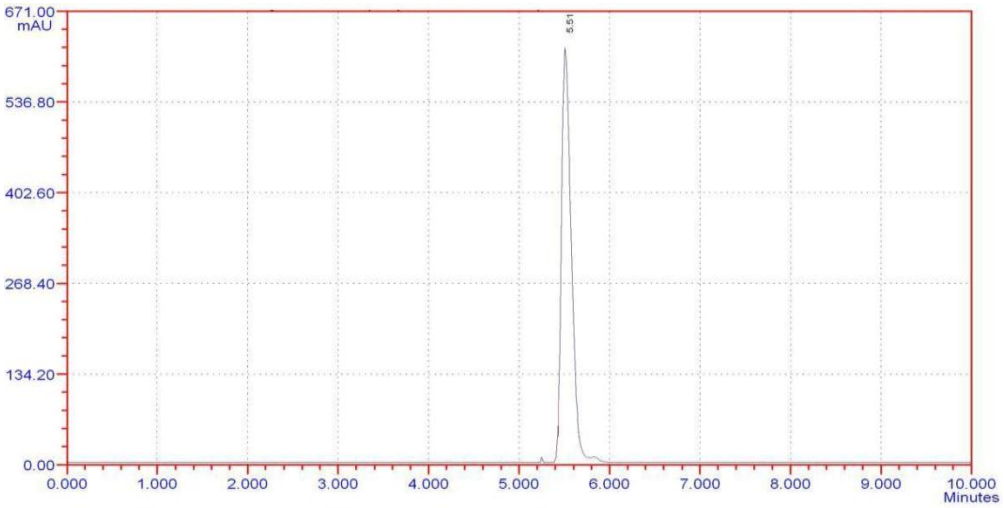


Fig 4: Chromatogram for accuracy (50% level).

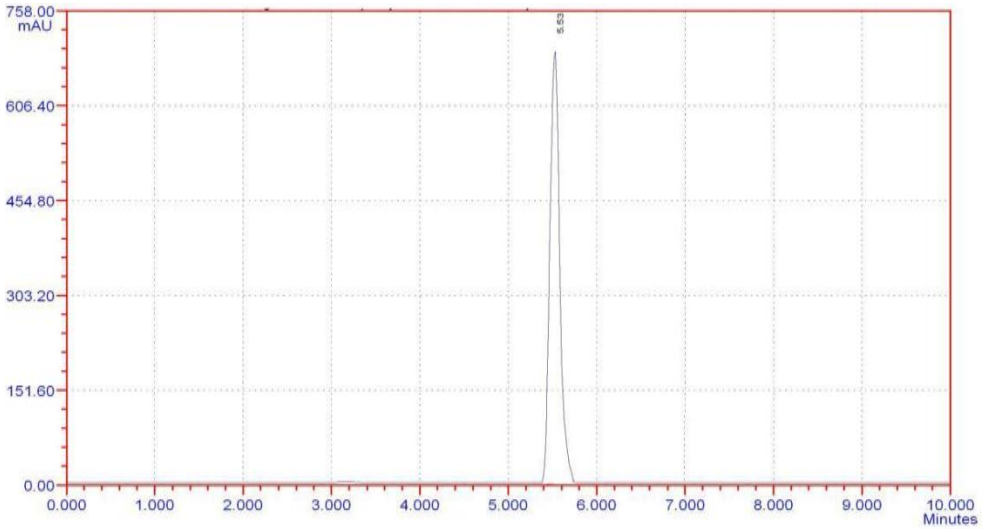


Fig 5: Chromatogram for accuracy (100% level).

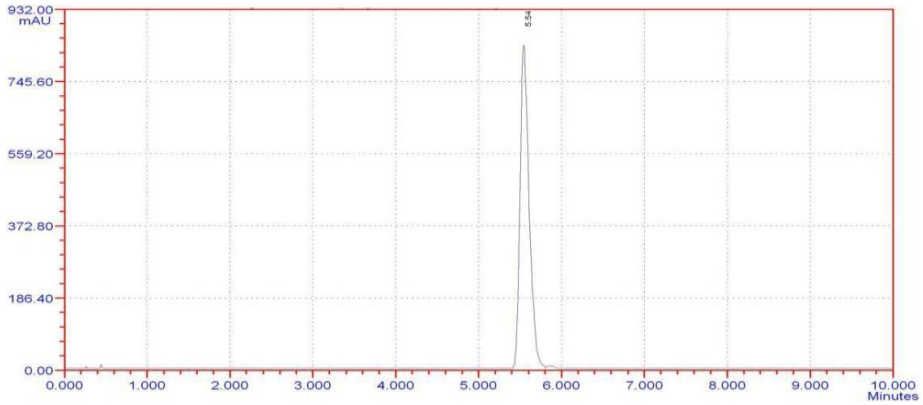


Fig 6: Chromatogram for accuracy (150% level).

3. Precision

a). System precision

Standard preparation was injected 5times. The responses for Sitagliptin phosphate peak area were shown in Table 5.

Table 5: Data for system precision.

S.NO	Retention time	Peak area
1.	5.53	539793
2.	5.54	540808
3.	5.56	537399
4.	5.55	531240
5.	5.57	530875
Mean	5.54	536023
SD	0.02	4700
% RSD	0.39	0.88

b). Intraday precision

The % assay values of Sitagliptin phosphate calculated from the responses of intraday precision was NLT 95% and NMT 102%. The results of the analysis were shown in Table 6.

Table 6: Data for intraday precision.

Sample preparation	Observed values	
	Area	% Assay
1.	536063	98.8 %
2.	535151	99.5 %
3.	530278	97.4 %
4.	532011	96.8 %
5.	539975	97.4 %
6.	526693	99.8 %
Mean	533362	98.2 %
SD	4691	0.01
% RSD	0.88	1.27

c). Interday precision

The % assay values of Sitagliptin phosphate calculated from the responses of intraday precision was NLT 95% and NMT 105%. The results of the analysis are shown in Table 7.

Table 7: Data for interday precision.

Day	Observed values	
	Area	% Assay
1.	531868	97.1 %
2.	539113	98.4 %
3.	529707	97.8 %
4.	526726	99.7 %
5.	525325	98.9 %
6.	527193	97.4 %
Mean	529989	98.2 %
SD	5041	0.01
% RSD	0.95	1.0

4. Linearity

The linearity of response for Sitagliptin phosphate was determined at different concentrations (60-210ppm) as shown in Table 8. The Correlation Coefficient value 0.9996 indicates that the method was linear over a concentration range of 60- 210ppm for Sitagliptin phosphate as shown in Table 8.

Table 8: Data for linear graph.

S.NO	Concentration (ppm)	Peak area
1.	60	288728
2.	90	417581
3.	120	539793
4.	150	684954
5.	180	824260
6.	210	962211

Table 9: Results for regression analysis.

S.NO	Drug name	Linear dynamic range (ppm)	Correlation coefficient	Slope	Intercept
1.	Sitagliptin phosphate	60-210	0.9996	4548.2	4780.5

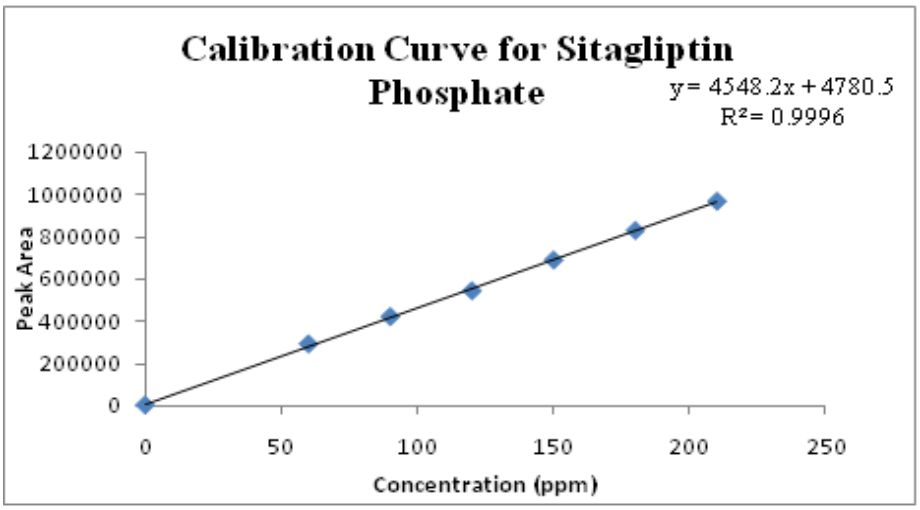


Fig 7: Linearity curve for Sitagliptin phosphate.

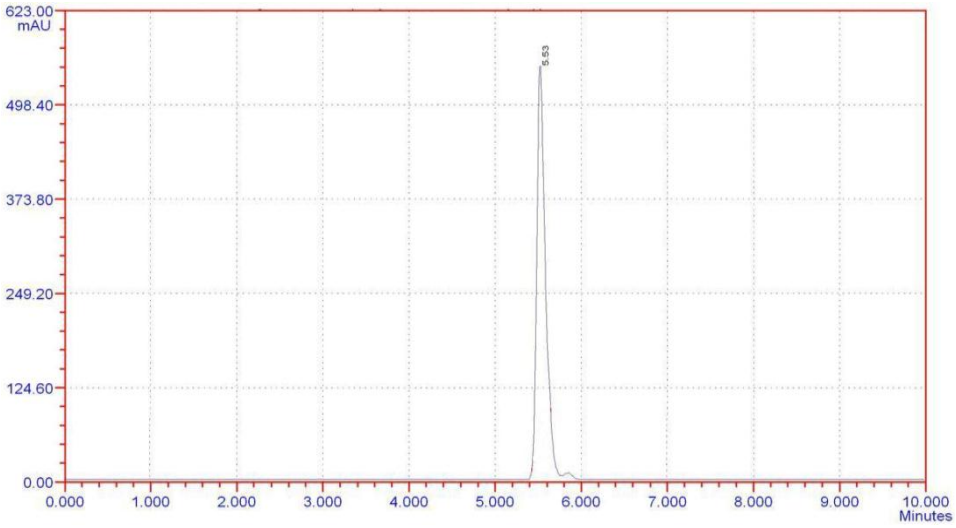


Fig 8: Chromatogram for linearity 60 ppm.

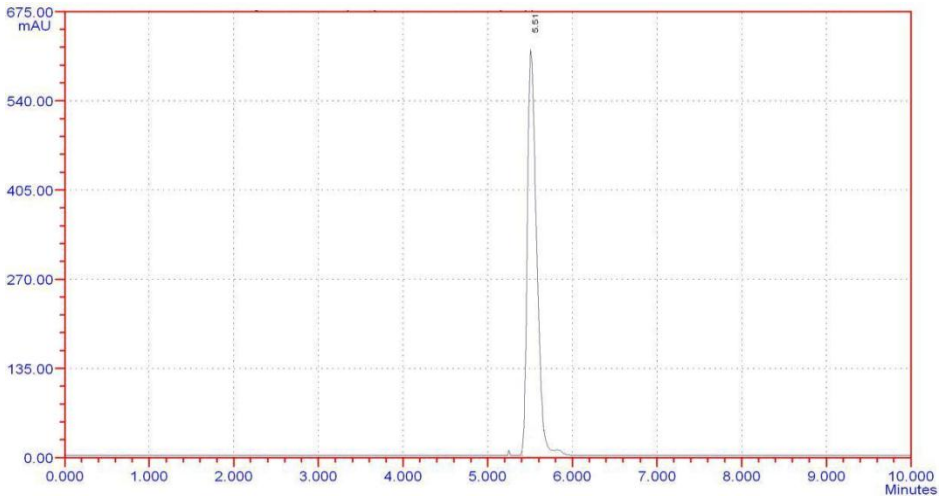


Fig 9: Chromatogram for linearity 90 ppm.

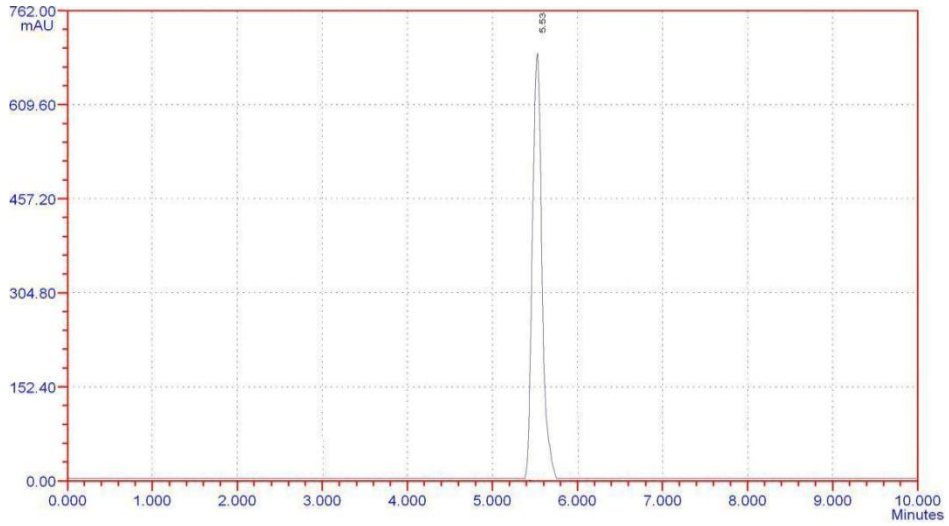


Fig 10: Chromatogram for linearity 120ppm.

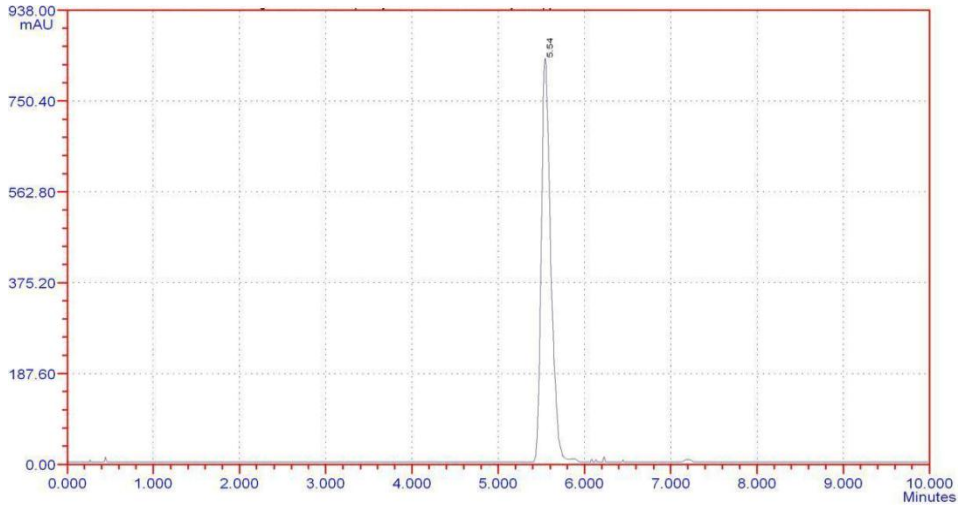


Fig 11: Chromatogram for linearity 150 ppm.

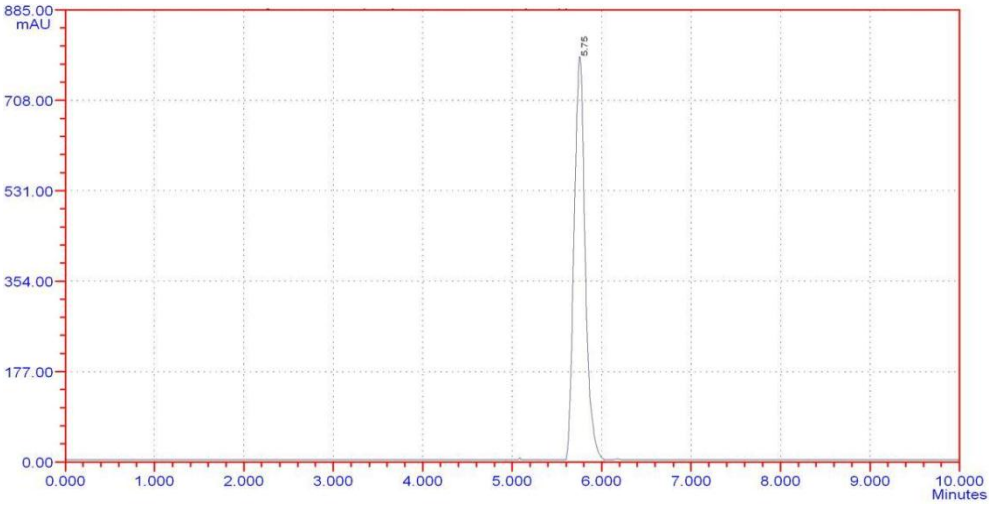


Fig 12: Chromatogram for linearity 180 ppm.

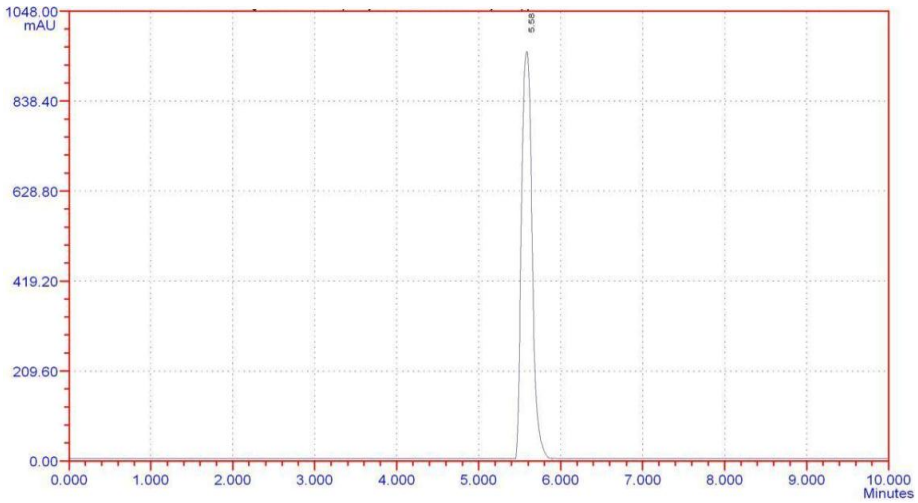


Fig 13: Chromatogram for linearity 210 ppm.

5. Limit of detection and Limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) represent the concentration of Sitagliptin phosphate stock solution in order to obtain signal-to-noise ratio of 3:1 for LOD and 10:1 for LOQ were determined.

Table 10: LOD and LOQ values for Sitagliptin phosphate.

Sample	LOD	LOQ
Sitagliptin phosphate	0.05µg/ml	0.16µg/ml

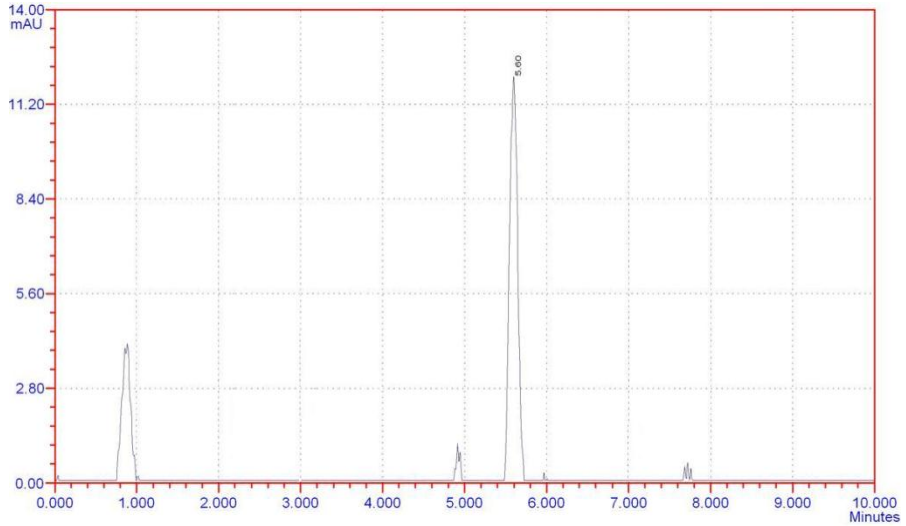


Fig 14: Chromatogram for LOD.

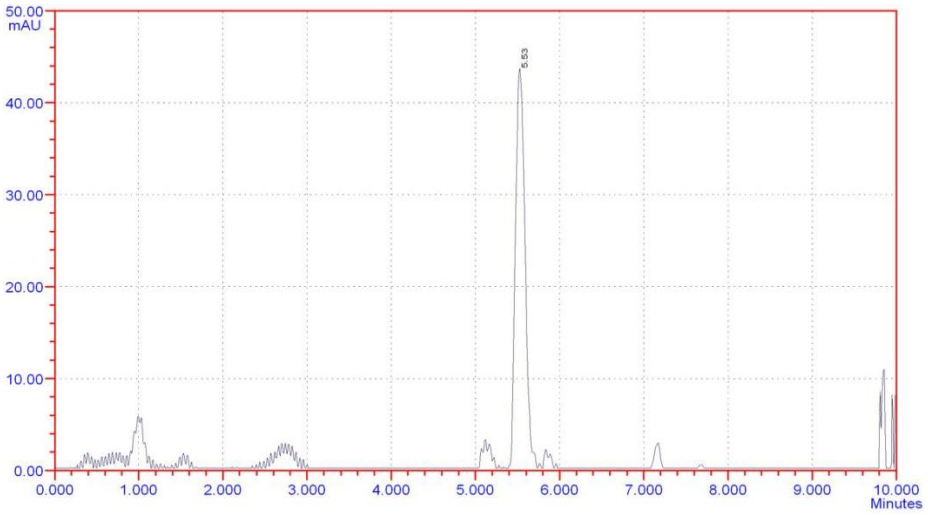


Fig 15: Chromatogram for LOQ.

6. Robustness

For demonstrating the robustness of the developed method, experimental conditions were purposely altered and evaluated. The method must be robust enough to with stand such a slight changes and allow routine analysis of the sample.

Table 11: Data for robustness.

Condition	Mean area	% difference
Unaltered	539793	……
Flow rate at 0.8 ml/min	537519	0.42
Flow rate at 1.2 ml/min	538041	0.32
Mobile phase MEOH : ACN : 0.1% OP		
38% 57% 05%	530367	1.75
42% 53% 05%	541617	0.34
pH of mobile phase 4.0	538983	0.15
pH of mobile phase 4.2	542948	0.58

CONCLUSION

From the reported literature, there were few methods established for the determination of Sitagliptin Phosphate in individual and in combination with other drug.

It was concluded that there was no method reported for the above selected dosage form, which promote to carry out the present work. The scope and objective of the present work is to develop and validate a new simple RP-HPLC method for quantitative estimation of Sitagliptin Phosphate in bulk and pharmaceutical dosage form.

In RP-HPLC method development, the mobile phase selected after optimization was mixed with methanol, acetonitrile and 0.1% ortho phosphoric acid in the ratio of 40:55:05 to pH 4.1 adjusted with triethyl amine was found to be ideal. The chromatographic condition was set at a flow rate of 1.0ml/min with the UV detection at 265 nm.

Sitagliptin Phosphate showed linearity in the range of 60-210ppm respectively. The correlation coefficient was found to be 0.9996 respectively for Sitagliptin Phosphate which indicates excellent correlation between response factor Vs concentration of standard solutions.

Precision of the developed method was studied. The %RSD values for precision was found to be within the acceptable limit, which revealed that the developed method was precise. The developed method was found to be robust. The %RSD value for percentage recovery of Sitagliptin Phosphate was found to be within the acceptance criteria.

Hence, the chromatographic method developed for Sitagliptin Phosphate was said to be rapid, simple, specific, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis.

REFERENCE

1. A. K. Gupta, Dinesh Sahu, Y. P. Agrawal, Avnish Jain. 2011. Simultaneous estimation of Metformin and Sitagliptin in tablet dosage form. Asian Journal of Biochemical and Pharmaceutical Research, 2(1): 352-358.
2. Amruta B. Loni, Minal R. Ghante, S. D. Sawant. 2012. Simultaneous UV spectrophotometric method for estimation of Sitagliptin phosphate and Metformin hydrochloride in bulk and tablet dosage form. Der Pharma Chemica, 4(3): 854-859.
3. Ankur Kothari and Sheetal Sharma. 2012. Development and validation of spectrophotometric method for simultaneous estimation of sitagliptin phosphate and simvastatin in tablet dosage form. International Journal of Pharmacy, 2(3): 609-612.
4. Anonymous. November 2005, ICH Harmonized Tripartite Guidelines, Text on Validation of Analytical procedures: Text and Methodology. Q2(R1).Geneva, 1-8.
5. Anonymus. www.ich.org.
6. Anonymus. United State Pharmacopoeia 26. 2003. National Formulary. The United states Pharmacopoeial Convention: MD, 21: 1223.

7. Beckett AH, Stenlake JB. 2007. *Practical Pharmaceutical Chemistry*. *Part-II*. 4th edn., CBS Publishers and Distributors: New Delhi, 85, 86, 92.
8. Chellu S. N. Malleswararao, Mulukutla V. Suryanarayana. 2012. Simultaneous determination of Sitagliptin phosphate monohydrate and Metformin hydrochloride in tablets by a validated UPLC method. Scientia Pharmaceutica, 80: 139–152.
9. Douglas A Skoog, Donald M West, James F Holler, Stanley R Crouch. 2007. *Fundamentals of Analytical Chemistry*. 8th edn., Thomson Asia Pvt. Ltd: Singapore, 4, 921, 975.
10. D. Phaneemdra, V. Venkatesh, Ramarao N. 2012.Simultaneous estimation of Simvastatin and Sitagliptin by using different analytical methods. International Journal of Advances in Pharmaceutical Analysis, 2(1): 19-23.
11. Gurdeep R Chatual, Shan K Anand. 2006. Instrumental Methods of Chemical Analysis. 5th edn., Himalya publishing House; Mumbai; 1.2, 2.108, 2.168, 2.147.
12. Hobart H Willard, Lynne L Merritt, Jr., John A Dean, Frank A Settle, Jr. 1986. *Instrumental Methods of Analysis*. 7th edn., CBS Publishers and Distributors: New Delhi, 1, 592, 622-628.
13. Jeffery GH, Bassett J, Mondham J, Denney RC. 1989. *Vogel's Text Book of Quantitative Chemical Analysis.* 5th edn. Longmen Singapore Publishers Pvt Ltd: Singapore, 5: 216-217.
14. Lloyd R Slyder, Joseph J Kirkland, Joseph L Glajch. 1997. *Practical HPLC Method Development*. 2nd edn. John Wiley and Sons, Inc, USA, 1-14.
15. Parag Pathade, Md Imran, Vinod Bairagi. 2011. 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): 871-873.
16. Sandy Lindsay. 1998. HPLC by open Learning. John Wiley and Sons: New York; 30-45.
17. Sethi PD. 1997. Quantitative Analysis of Drugs in Pharmaceutical Formulations. 3rd edn., CBS Publishers and Distributors: New Delhi, 6-9.
18. Sharma. B.K., 2007, High Performance Liquid Chromatography, Instrumental Methods of Chemical Analysis, GOEL publishing house, Meerut, 6th edition, C-290-291.
19. Sheetal Sharma, Ankur Kothari, M.S. Harsoliya. 2012. Simultaneous UV Spectrophotometric method for estimation of Sitagliptin Phosphate and Simvastatin in Tablet dosage Form. Journal of Pharmacy Research, 5(1): 444-446.
20. www.frorumsci.coil/HPLC/HPLC_overview_handouts 2002.pdf.
21. http://medical-dictionary.thefreedictionary.com/sitagliptin phosphate.