

## Analytical Method for the Simultaneous Estimation of Sitagliptin and Simvastatin using RP-HPLC

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### Abstract

A novel, rapid, precise and accurate high performance liquid chromatographic method was developed and validated for the simultaneous determination of Sitagliptin phosphate and Simvastatin in bulk drug and pharmaceutical formulation. The components were separated on Ymc Cyano (150 mm × 4.6 mm I.D., 5 μm particle size) with a mobile phase composed of 20 mM ammonium formate and acetonitrile in the ratio of 50:50 v/v (Adjust the pH to 3.5 with 0.1% formic acid) at a flow rate of 1.2 mL/min. The response was measured at 218 nm. The peaks were detected at 5.33 minutes and 4.19 minutes for Sitagliptin phosphate and Simvastatin respectively. Calibration curves were found to be linear ( $r^2=0.999$  for both Sitagliptin phosphate and Simvastatin respectively) over the concentration range of 2.5-200 μg/mL for Sitagliptin phosphate and 1-80 μg/mL for Simvastatin. The method was validated for linearity, precision, accuracy, ruggedness and robustness. The proposed method can be applicable for simultaneous

quantitation of Sitagliptin phosphate and Simvastatin in tablet dosage form. Validation results assured that the recommended method was specific, rapid, reliable and reproducible. Good percent recoveries and low % RSD reveals the suitability of the present method for analysis of Sitagliptin phosphate and Simvastatin in quality control laboratories.

**Keywords:** Sitagliptin; Simvastatin; RP-HPLC; Estimation.

### Introduction

Sitagliptin phosphate (Fig. 1) is an oral dipeptidyl peptidase-4 (DPP-4) reversible inhibitor [1]. Chemically Sitagliptin is (3R)-3-amino-1-[3-(trifluoromethyl)-5,6 dihydro [1,2,4] triazolo [4,3-a] pyrazin-7(8H)-yl]-4-(2,4,5-trifluorophenyl)-1-butanone. It acts as DPP-4 inhibitor which exerts its action by slowing the inactivation of the incretin

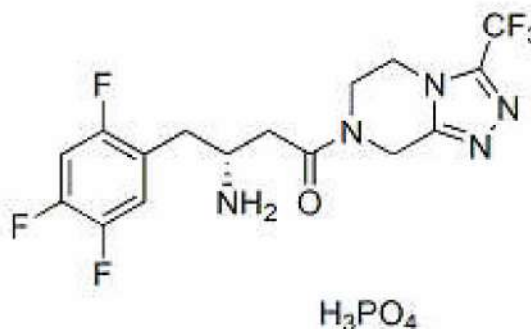


Fig. 1: Chemical structure of Sitagliptin phosphate

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hormones glucagon-like peptide-1 and glucose-dependent insulin tropic polypeptide and leads to increased the levels active incretin and insulin, and decreased the levels of glucagon [2]. Sitagliptin phosphate, which can be used as mono therapy or in combination with other antidiabetic drugs, especially for patients with early-stage type 2 diabetes and more severe hyperglycemia.

Simvastatin (Fig. 2) is a lipid regulating drug, it is a competitive inhibitor of 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMG-CoA reductase) [3]. Chemically Simvastatin is 2, 2-dimethyl-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2(tetrahydro 4-hydroxy-6-oxo-2H-pyran-2-yl)-ethyl]-naphthalenylester. It leads to reduced cholesterol synthesis in the liver and lower intracellular cholesterol concentrations. Simvastatin also tend to reduce triglycerides and to increase High Density Lipoprotein (HDL)-cholesterol concentrations. Simvastatin used for cardiovascular risk reduction [4].

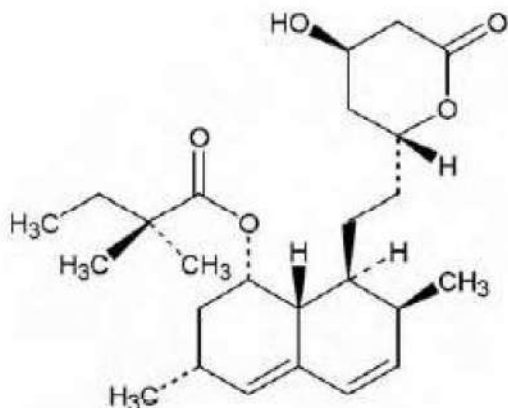


Fig. 2: Chemical structure of Simvastatin

A survey of literature found that few HPLC methods [5-8] were established for the simultaneous determination of Sitagliptin phosphate and Simvastatin in combined pharmaceutical dosage forms. Therefore, an attempt has been made to develop a simple, rapid, accurate and precise RP-HPLC method for the simultaneous estimation of Sitagliptin and Simvastatin in tablet dosage form and validated in accordance with ICH guidelines [9].

## Materials and Methods

### Instrumentation

To develop a high performance liquid chromatographic method for simultaneous

estimation of Sitagliptin phosphate and Simvastatin using Waters 2695 HPLC system on Ymc Cyano (150 mm × 4.6 mm I.D., 5 μm particle size) column was used. The instrument is equipped with pump-515, auto sampler-2707 and UV detector-2998. A 20 μL rheodyne injector port was used for injecting the samples. Data was analyzed by using Empower 2 software. A Shimadzu balance was used for weighing the materials and Systronics-361 pH meter was used for pH measurements.

### Chemicals and solvents

The reference samples of Sitagliptin phosphate and Simvastatin were obtained as gift samples from Sun Pharmaceutical Industries Ltd, Mumbai, India. Commercially formulation of tablets claimed to contain 100 mg of Sitagliptin phosphate and 40 mg of Simvastatin were purchased from local market. Ammonium formate, acetonitrile and formic acid were procured from S.D. Fine Chemicals, Mumbai, India.

### Chromatographic conditions

Twenty (20) mM ammonium formate and acetonitrile in the ratio of 50:50 v/v (pH was adjusted to 3.5 with 0.1% formic acid) was optimized as it is most suitable mobile phase for ideal chromatographic separation for the analysis of Sitagliptin phosphate and Simvastatin. The solvent mixture was sonicated for 10 minutes and filtered through 0.45 μm membrane filter. The flow rate was maintained at 1.2 mL/min. Injection volume was 20 μL and the column was maintained at ambient temperature. To equilibrate the column the mobile phase should be pumped through the column for at least 30 minutes before the injection of the drug solution. The response was detected at 218 nm. The run time was set as 8 minutes.

### Preparation of mobile phase and diluent

Mix equal portions of 20 mM ammonium formate (pH was adjusted to 3.5 with 0.1% formic acid) and acetonitrile, then the solution was degassed and filtered through 0.45 μm filter under vacuum. This solution was used as both mobile phase and diluent.

### Preparation of standard solution

Weigh 10 mg of Sitagliptin phosphate and 4 mg of Simvastatin working standards accurately and transferred into a 100 mL clean dry volumetric flask, add about 10 mL of diluents and final volume made upto the mark with the diluent. Pipette out 2.5 mL

of the above stock solution into 100 mL volumetric flask and diluted upto the mark with diluent.

#### *Preparation of sample solution*

Twenty tablets were weighed and crushed them to fine powder. The powder equivalent to 100 mg of Sitagliptin phosphate and 40 mg of Simvastatin were accurately weighed, taken into a 100 mL volumetric flask. Add 50 mL of diluent and the contents of flask were sonicated for 20 min, then solution was filtered through a 0.45  $\mu$ m membrane filter and volume made upto the mark with diluent. Transfer 2 mL of the this solution was to 100 mL volumetric flask and volume was made upto the mark with diluent to get the concentration containing 20  $\mu$ g/mL of Sitagliptin phosphate and 8  $\mu$ g/mL of Simvastatin.

#### *Procedure*

The column was maintained at room temperature. The run time adjusted for this analysis was 8 minutes. Before injecting the drug solutions, the equilibration of column was done by pumping the mobile phase through the column for at least 30 minutes. 20  $\mu$ L of the standard and sample solutions were injected for six times into the HPLC instrument at a flow rate of 1.2 mL/min and all the chromatograms were recorded. From these chromatograms, the average peak area was calculated.

#### **Method Validation**

##### *Linearity*

Prepare different aliquots of standard solutions of in the concentration range of 2.5-200  $\mu$ g/mL for Sitagliptin phosphate and 1-80  $\mu$ g/mL for Simvastatin. Then the solutions were injected into the HPLC system keeping the injection volume constant. The detection was monitored at 218 nm and the chromatograms were recorded. Calibration curves were drawn by taking concentration on X-axis and peak area on Y-axis. The regressions of the plots were computed by least square regression method.

##### *Precision*

Precision for Sitagliptin phosphate and Simvastatin was performed in terms of system precision, method precision and intermediate precision. For this the sample was repeatedly injected for six times. Measure the peak areas and %RSD was calculated.

##### *Accuracy*

Accuracy of Sitagliptin phosphate and Simvastatin and was assessed by spiked standard method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at 50%, 100% and 150% level. The solutions were analyzed in triplicate at each level as per the proposed method. The % recovery was calculated.

##### *System suitability*

System suitability was performed by injecting six replicates of the standard solution. The system suitability parameters like retention time, theoretical plates and tailing factor were determined by six replicate analyses of Sitagliptin phosphate and Simvastatin and compared with standard values.

##### *Limit of detection and limit of quantitation*

The limit of detection (LOD) and limit of quantitation (LOQ) of the developed method were estimated from signal-to-noise ratio. These values can be determined by injecting progressively low concentrations of the standard solutions of Sitagliptin phosphate and Simvastatin.

##### *Robustness*

Robustness was studied by making small changes in method parameters like mobile phase flow rate, buffer pH and composition of the mobile phase and %RSD was calculated.

#### **Results and Discussion**

The HPLC method was optimized with a view to develop an simple, precise, accurate and reproducible method for simultaneous determination of Sitagliptin phosphate and Simvastatin in tablet dosage form using Ymc Cyano (150 mm  $\times$  4.6 mm I.D., 5  $\mu$ m particle size) in isocratic mode with mobile phase composition of 20 mM ammonium formate and acetonitrile in the ratio of 50:50 v/v (pH was adjusted to 3.5 with 0.1% formic acid) resulted in peak with maximum separation, good shape and resolution. The flow of mobile phase was maintained at 1.2 mL/min. The peaks were detected at 5.33 minutes for Sitagliptin phosphate and 4.19 minutes for Simvastatin with good resolution. Total run time was 8 minutes. The

drug components were measured with UV detector at 218 nm. The results of optimized chromatographic conditions were represented in Table 1.

Linearity was obtained in the concentration range of 2.5-200 µg/mL for Sitagliptin phosphate and 1-80 µg/mL for Simvastatin. The correlation coefficient ( $r^2$ ) was found to be 0.999 for both Sitagliptin phosphate and Simvastatin respectively. The regression equation for calibration curve of Sitagliptin phosphate over its peak area was found to be  $y=10693x+20551$ , where  $x$  is the concentration of Sitagliptin phosphate (µg/mL) and  $y$  is the corresponding peak area. The regression equation for the calibration curve of Simvastatin over its peak area was found to be  $y=20453x-7302$ , where  $x$  is the concentration of Simvastatin (µg/mL) and  $y$  is the corresponding peak area. The results were shown that the method has a good correlation between peak area and concentration of drugs in the range of linearity. The results for linearity were given in

Table 2 and Table 3 and the calibration curves were represented in Fig. 3 and Fig. 4.

The %RSD for system precision, method precision and intermediate precision for Sitagliptin phosphate were found to be 0.16, 0.59 and 0.5 respectively (limit %RSD < 2.0%). The %RSD for system precision, method precision and intermediate precision for Simvastatin were found to be 0.23, 0.64 and 0.44 respectively (limit %RSD < 2.0%) and hence the method is precise. The precision data of Sitagliptin phosphate and Simvastatin were furnished in Table 4 to Table 7.

The % mean recovery of the drugs Sitagliptin phosphate and Simvastatin were found to be 98.86% and 98.90% respectively and the high percentage recovery of Sitagliptin phosphate and Simvastatin indicates that the proposed method is highly accurate. The results of accuracy studies of Sitagliptin phosphate and Simvastatin were tabulated in Table 8 and Table 9.

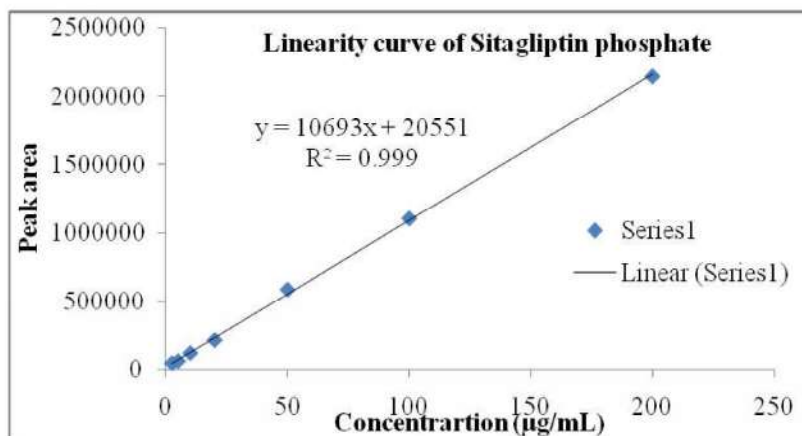


Fig. 3: Linearity plot of Sitagliptin phosphate

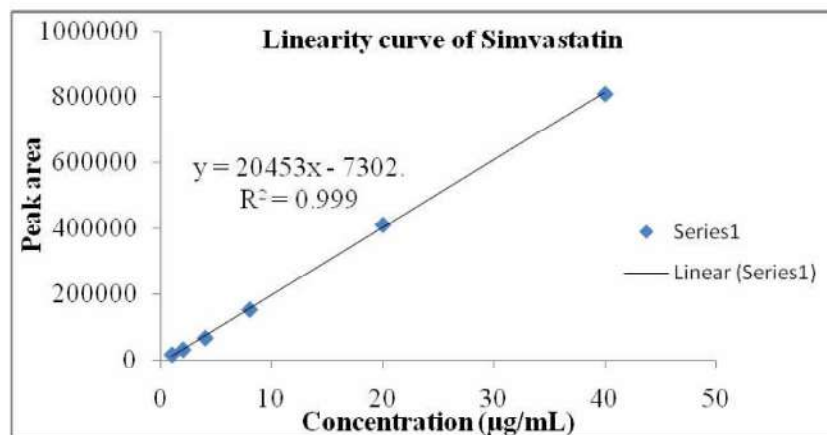


Fig. 4: Linearity plot of Simvastatin

The number of theoretical plates calculated for Sitagliptin phosphate and Simvastatin was 8457 and 7548 respectively. The tailing factor for Sitagliptin phosphate and Simvastatin was 1.1 and 0.98 respectively, which indicates efficient performance of the column. The limit of detection (LOD) and limit of quantitation (LOQ) for Sitagliptin phosphate were found to be 0.30 µg/mL and 0.90 µg/mL; 0.15 µg/mL and 0.47 µg/mL for Simvastatin respectively, which indicate the sensitivity of the method. The system suitability parameters and validation parameters were summarized in Table 10.

The robustness studies revealed that no significant effect was observed by making small

changes in method parameters. Therefore the proposed method is robust for the quantification of the drugs. In all deliberately varied conditions, the %RSD for replicate injections of Sitagliptin phosphate and Simvastatin were found to be within the limits of criteria.

Absence of extra peaks under optimized conditions, showing no interference from common tablet excipients and impurities. The two analyte peaks were eluted and separated with a good resolution. The standard chromatogram of Sitagliptin phosphate and Simvastatin was shown in Fig. 5.

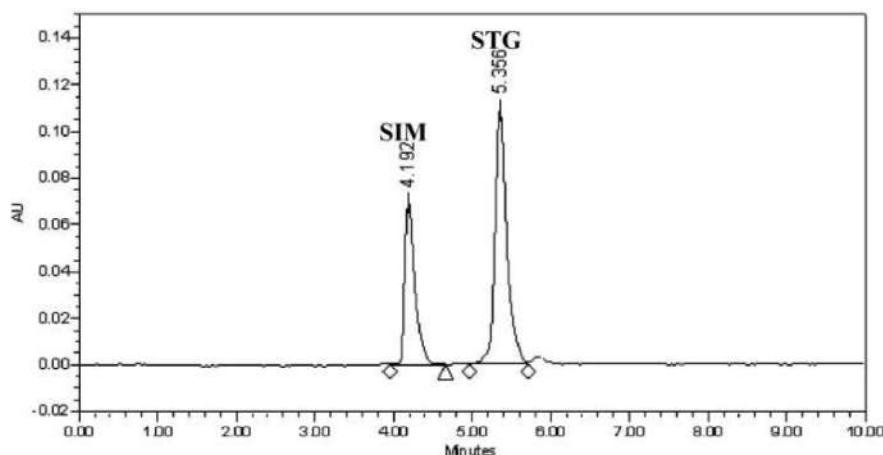


Fig. 5: Chromatogram of Sitagliptin phosphate and Simvastatin in standard solution

Table 1: Optimized chromatographic conditions

Parameter	Condition
Mobile phase	20 mM ammonium formate:acetonitrile (50:50, v/v)
Diluent	20 mM ammonium formate:acetonitrile (50:50, v/v)
Column	Ymc Cyano (150 mm × 4.6 mm, 5 µm)
Column temperature	Ambient
Wave length	218 nm
Injection volume	20 µL
Flow rate	1.2 mL/min.
Run time	8 min.

Table 2: Linearity results of Sitagliptin phosphate

Concentration (µg/mL)	Mean peak area
2.5	47283
5	63145
10	121665
20	217141
50	588277
100	1103688
200	2146382

Table 3: Linearity results of Simvastatin

Concentration (µg/mL)	Mean peak area
1	17822
2	32780
4	68132
8	153243
20	410620
40	807564
80	1711993

Table 4: System precision data

Injection number	Peak area of Sitagliptin phosphate	Peak area of Simvastatin
1	217141	153243
2	217154	153215
3	216899	152364
4	216587	152878
5	217541	153345
6	217458	152859
Mean	217130	152984
%RSD	0.16	0.23

**Table 5:** Method precision data

Sample number	%Assay	
	Sitagliptin phosphate	Simvastatin
1	98.98	100.01
2	99.36	98.23
3	99.84	99.47
4	100.2	99.65
5	98.54	99.87
6	99.54	99.71
Mean	99.41	99.49
%RSD	0.59	0.64

**Table 6:** Intermediate precision study of Sitagliptin phosphate

Preparation number	%Assay	Mean	%RSD
1	99.64	99.7	0.5
2	99.65		
3	100.21		
4	100.54		
5	99.45		
6	99.65		

**Table 7:** Intermediate precision study of Simvastatin

Preparation number	%Assay	Mean	%RSD
1	99.10	99.2	0.44
2	98.99		
3	99.23		
4	100.01		
5	99.21		
6	98.69		

**Table 8:** Recovery study of Sitagliptin phosphate

Level	Amount of Sitagliptin phosphate added ( $\mu\text{g}$ )	Amount of Sitagliptin phosphate recovered ( $\mu\text{g}$ )	%Recovery	%RSD
50%	10	9.5	99.5	1.02
	10	9.89	98.9	
	10	9.75	97.5	
100%	20	20.1	99.5	1.51
	20	19.45	97.25	
	20	19.76	98.8	
150%	30	29.65	98.8	0.6
	30	29.87	99.5	
	30	30.01	100.0	
	Mean % Recovery			98.86
	Overall %RSD			1.04

**Table 9:** Recovery study of Simvastatin

Level	Amount of Simvastatin added ( $\mu\text{g}$ )	Amount of Simvastatin recovered ( $\mu\text{g}$ )	%Recovery	%RSD
50%	4	3.89	97.25	1.58
	4	3.89	97.25	
	4	4.0	100.0	

100%	8	7.97	99.6	0.76
	8	7.85	98.1	
	8	7.89	98.6	
150%	12	11.98	99.8	0.25
	12	12.0	100.0	
	12	11.95	99.5	
	Mean %Recovery			98.9
	Overall %RSD			0.86

**Table 10:** System suitability parameters of proposed method

Parameters	Sitagliptin phosphate	Simvastatin
Linearity ( $\mu\text{g/mL}$ )	2.5-200	1-80
Correlation coefficient	0.999	0.999
Retention time (min.)	5.33	4.19
Tailing factor	1.1	0.98
Theoretical plates (N)	8457	7548
LOD ( $\mu\text{g/mL}$ )	0.30	0.15
LOQ ( $\mu\text{g/mL}$ )	0.90	0.47

## Conclusion

The present HPLC method is simple, rapid, sensitive, precise and accurate for the simultaneous quantitation of Sitagliptin phosphate and Simvastatin and can be reliably adopted for routine quality control analysis of Sitagliptin phosphate and Simvastatin in bulk and pharmaceutical dosage form.

## References

- V.S. Deshmukh, V.M. Motghare, S.L. Padwal, S. Jaykare, J. Patil and H. Pise. New drugs in the management of diabetes mellitus. *International Journal of Basic and Clinical Pharmacology*. 2013; 2(1):4-11.
- B. Gallwitz. Sitagliptin: profile of novel DPP-4 inhibitor for the treatment of type 2 diabetes. *Drugs Today*. 2007;43(1):13-25.
- S. Ramkumar, R. Ajay and R. Sudhakshini. Statin therapy: review of safety and potential side effects. *Acta Cardiologica Sinica*. 2016;32(6):631-39.
- T.R. Pedersen and J.A. Tobert. Simvastatin: a review. *Expert Opinion on Pharmacotherapy*. 2004; 5(12):2583-2596.
- K.Y. Kavitha, G. Geetha, R. Hari Prasad, G. Subramanian and R. Venkatanarayana. Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of Sitagliptin and Simvastatin. *International Research Journal of Pharmacy*. 2012;3(12):123-27.
- S. Sharma, M. Nimita, B. Priya, J. Prabat and H. Sohail. Development of UV spectrophotometry

- and RP-HPLC method and its validation for simultaneous estimation of Sitagliptin phosphate and Simvastatin in marketed formulation. *International Journal of Pharmaceutical & Biological Archive*. 2012;3(3):673-78.
7. V. Anil Kumar, V. Aruna Kumari, S.V. Simhadri, A. Srinivasa Rao and T. Santosh Kumar. Validated RP-HPLC method for the simultaneous estimation of Sitagliptin and Simvastatin in dosage form. *International Journal of Chemical and Analytical Science*. 2012;3(11):1611-14.
  8. G. Manikanta, K.P. Channabasavaraj and G.S. Madhusudan Yadav. Reverse phase high performance liquid chromatography method for the simultaneous estimation of Sitagliptin and Simvastatin in bulk and tablet formulation. *Drug Development and Therapeutics*. 2014;5(2):153-57.
  9. ICH Harmonised Tripartite Guideline, Validation of analytical procedures: Text and methodology, Q2 (R1), International Conference on Harmonization, Geneva. 2005;1-13.
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