CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

Available online at: http://www.iajps.com *Research Article*

METHOD DEVELOPMENT AND VALIDATION OF SITAGLIPTIN AND SIMVASTATINE IN TABLET DOSAGE FORM BY RP-HPLC

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Article Received: August 2022 **Accepted:** September 2022 **Published:** October 2022

Abstract:

A simple and selective LC method is described for the determination of Sitagliptin and Simvastatin in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 80 volumes of methanol and 20 volumes of water with detection of 241 nm. Linearity was observed in the range 60-140µg /ml for Sitagliptin (r^2 =0.997) and 61-155µg /ml for Simvastatin (r^2 =0.997) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. From the above experimental results and parameters, it was concluded that, this newly developed method for the simultaneous estimation Sitagliptin and Simvastatin was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

Keywords: *Sitagliptin, Simvastatin, RP-HPLC, Simultaneous estimation.*

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Please cite this article in press Sai Kiran et al, ***Method Development And Validation Of Sitagliptin And Simvastatine In Tablet Dosage Form By Rp-Hplc .,*** *Indo Am. J. P. Sci, 2022; 09(10).*

INTRODUCTION:

Sitagliptin is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus. It is not used to treat type 1 diabetes or patients with a history of pancreatitis. [1] Inhibition of DPP-4 by sitagliptin slows DPP-4 mediated inactivation of incretins like GLP-1 and GIP. [2] Incretins are released throughout the day and upregulated in response to meals as part of glucose homeostasis. [3] Reduced inhibition of incretins increase insulin synthesis and decrease glucagon release in a manner dependant on glucose concentrations. [4] These effects lead to an overall increase in blood glucose control which is demonstrated by reduced glycosylated hemoglobin (HbA1c). [5] IUPAC name of Sitagliptin is (3R)-3-amino-1-[3-(trifluoromethyl)-5H,6H,7H,8H-[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4 (2,4,5-trifluorophenyl)butan-1-one. Molecular Formula is $C_{13}H_{15}F_6N_5O$. Molecular Weight is 407.3.

Simvastatin is indicated for the treatment of hyperlipidemia to reduce elevated total cholesterol (total-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein B (Apo B), and triglycerides (TG), and to increase high-density lipoprotein cholesterol (HDL-C). [6,7] This includes the treatment of primary hyperlipidemia (Fredrickson type IIa, heterozygous familial and nonfamilial), mixed dyslipidemia (Fredrickson type IIb), hypertriglyceridemia (Fredrickson type IV hyperlipidemia), primary dysbetalipoproteinemia (Fredrickson type III hyperlipidemia), homozygous familial hypercholesterolemia (HoFH) as an adjunct to other lipid-lowering treatments, as well as adolescent patients with Heterozygous Familial Hypercholesterolemia (HeFH). Simvastatin is a prodrug in which the 6-membered lactone ring of simvastatin is hydrolyzed in vivo to generate the beta,delta-dihydroxy acid, an active metabolite structurally similar to HMG-CoA (hydroxymethylglutaryl CoA). Once hydrolyzed, simvastatin competes with HMG-CoA for HMG-CoA reductase, a hepatic microsomal enzyme, which catalyzes the conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol biosynthesis.2 Simvastatin acts primarily in the liver, where decreased hepatic cholesterol concentrations stimulate the upregulation of hepatic low density lipoprotein (LDL) receptors which increases hepatic uptake of LDL. Simvastatin also inhibits hepatic synthesis of very low-density lipoprotein (VLDL). The overall effect is a decrease in plasma LDL and VLDL. [8] IUPAC name of Simvastatin is 4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate. Molecular formula is $C_{25}H_{35}O_5$. Molecular Weight is 418.27. Simvastatin is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF), which should be purged with an inert gas. The solubility of Simvastatin in ethanol is approximately 15 mg/ml and approximately 30 mg/ml in DMSO and DMF. Simvastatin is sparingly soluble in aqueous buffers.

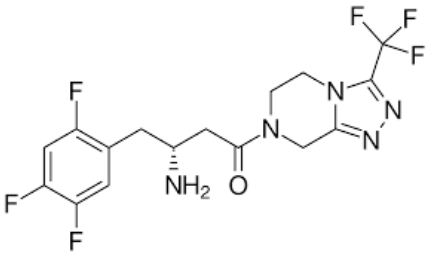

Figure 1: Structure of Sitagliptin

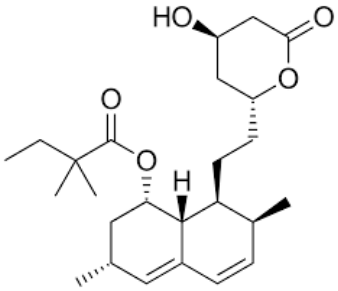

Figure 2: Structure of Simvastatin

Literature survey shows that a number of methods have been reported for estimation of Sitagliptin And Simvastatin individually or in combination with other drugs Those are UV [9],HPLC [10-13], LC-MS/MS[14]. However, there is only few HPLC methods are reported for the simultaneous estimation of these drugs in combined dosage forms. I got better results than already published one. The aim of the present study was A New Rp-Hplc Method for Simultaneous Estimation of Sitagliptin and Simvastatin in Its Bulk and Tablet Dosage Form and Its Force Degradation Studies as Per Ich.

MATERIALS AND METHODS:

Chemicals and Reagents: Sitagliptin and Simvastatin were Purchased from Hetero drugs. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 241 nm with column Inertsil ODS 3V column,C18(150x4.6 ID) 5µm, dimensions at 25^{0}C temperature. The optimized mobile phase consists of Mixed buffer: MeOH :ACN (30:50:20). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Preparation of standard stock solution of Sitagliptin:

10 mg of Sitagliptin was weighed and transferred in to 10ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of Simvastatin:

10 mg of Simvastatin was weighed in to 10ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of mixed standard solution

weigh accurately 98 mg of Sitagliptin and 102 mg of Simvastatin in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 98 µg/ml of Sitagliptin and 102 µg/ml of Simvastatin is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample: **5tablets (each tablet contains Simvastatin 102mg**

Sitagliptin -98 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Simvastatin and Sitagliptin (µg/ml) were prepared by dissolving weight equivalent to 102 mg of Simvastatin and 98 mg of Sitagliptin and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and sonicated for 5 min and dilute to 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 102 µg/ml of Simvastatin and 98µg/ml of Sitagliptin was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Sitagliptin and Simvastatin peaks and calculate the %Assay by using the formulae**.**

RESULTS AND DISCUSSION:

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Inertsil ODS 3V column, C18(150x4.6 ID) 5µm, the mobile phase of composition Mixed buffer: MeOH :ACN (30:50:20) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1,2.

Table 1: System suitability parameters of Sitagliptin

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor	Resolution
1	3.773	295.884	4667	1.111	3.247
2	3.733	290.743	4813	1.143	3.306
3	3.733	292.910	4813	1.176	3.540
4	3.770	293.024	4908	1.206	3.531
5	3.733	290.900	4813	1.176	3.247

6	3.748	292.692	4667	1.111	3.306
Mean	0.0049	55.704	-	-	-
SD	**0.14**	**0.64**	-	-	-
%RSD	3.773	295.884	-	-	-

Table 2: Results for system suitability of Simvastatin

Injection	**Retention time (min)**	**Peak area**	**Theoretical plates**	**Tailing factor**	**Resolution**
1	4.680	8815.579	2994	1.596	3.247
2	4.837	8708.391	2058	1.627	3.306
3	4.683	8510.447	2436	1.907	3.540
4	4.670	8553.080	2422	1.952	3.531
5	4.680	8815.579	2994	1.596	3.247
6	4.690	8708.391	2058	1.627	3.306
Mean	4.707	8685.245	-	-	-
SD	0.064	128.893	-	-	-
%RSD	**1.36**	**1.48**	-	-	-

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Sitagliptin and Simvastatin in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-3.

Table 3: Assay results for Sitagliptin and Simvastatin

	SITAGLIPTIN		**SIMVASTATIN**	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	753.879	766.992	1981.613	2032.155
Injection-2	764.681	772.321	2015.949	2014.800
Injection-3	768.971	762.114	2020.613	2011.565
Injection-4	767.503	763.828	2024.578	2035.008
Injection-5	759.196	755.593	2014.021	2018.668
Average Area	762.846	764.1696	2011.355	2022.439
Standard deviation	6.170728		10.52615	
%RSD	0.008075		0.005205	
Assay(%purity)	**100.17**		**100.55**	

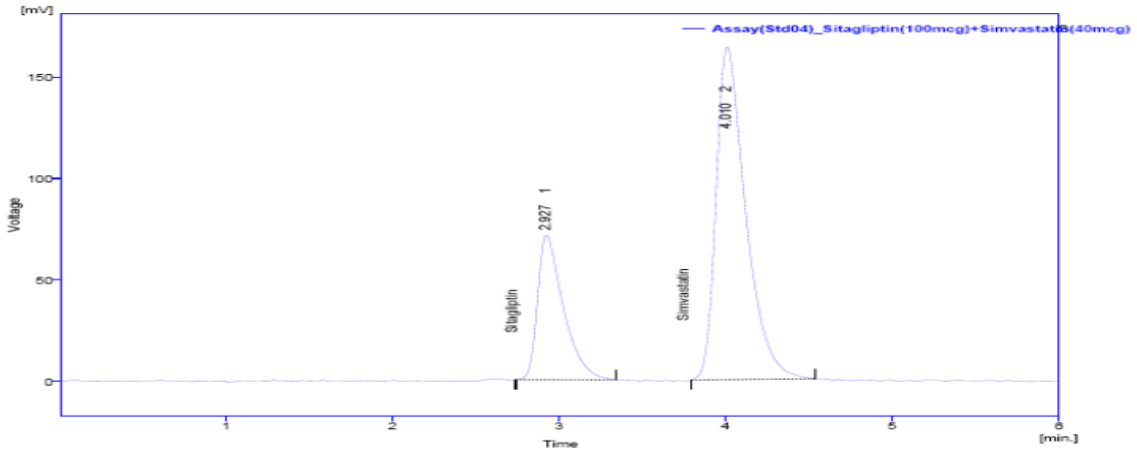

Figure 3: Standard chromatogram

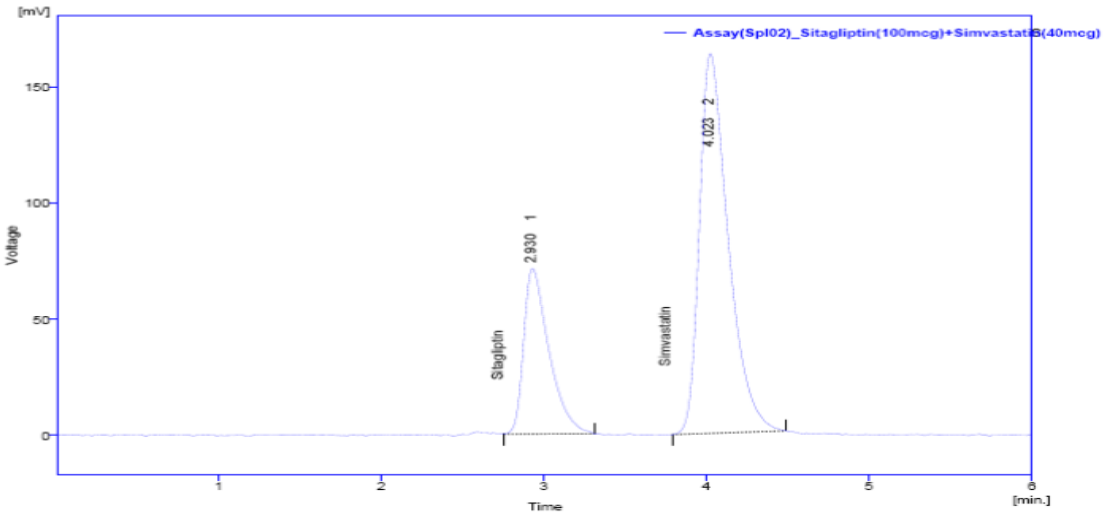

Figure 4: Sample chromatogram

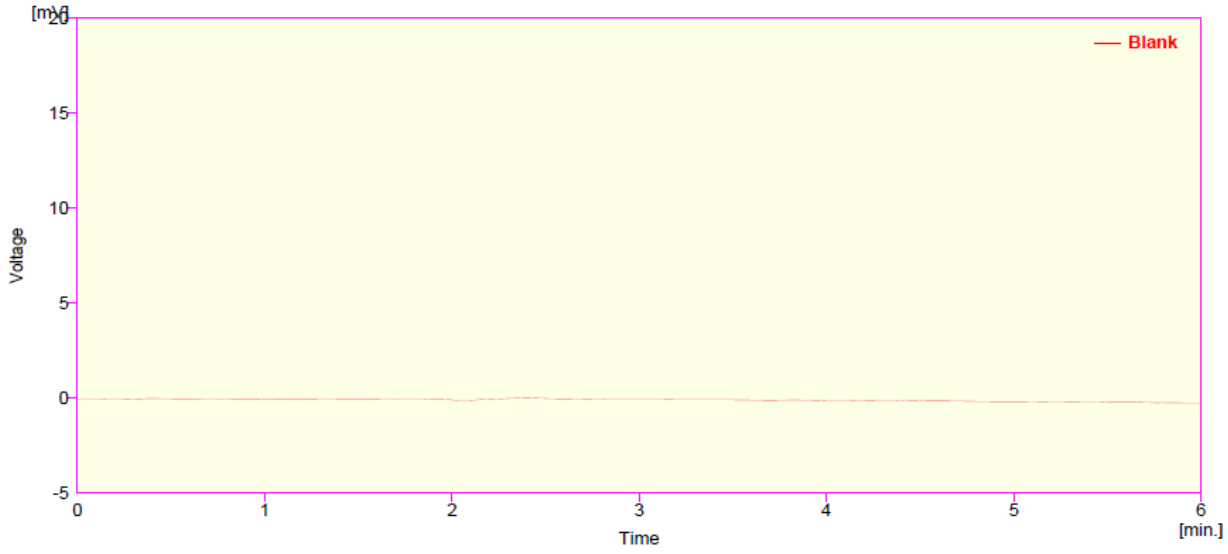

Figure 5: Blank chromatogram

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 58.8 µg/ml to 137.2 µg/ml and 61 µg/ml to 142 µg/ml level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The resulte are shown in table 4,5.

Table 4: Linearity results of Sitagliptin

S.No.	Conc.(µg/ml)	Area
1	58.8	485.279
2	78.4	697.105
3	98	768.173
4	117.6	1062.233
5	137.2	1245.814

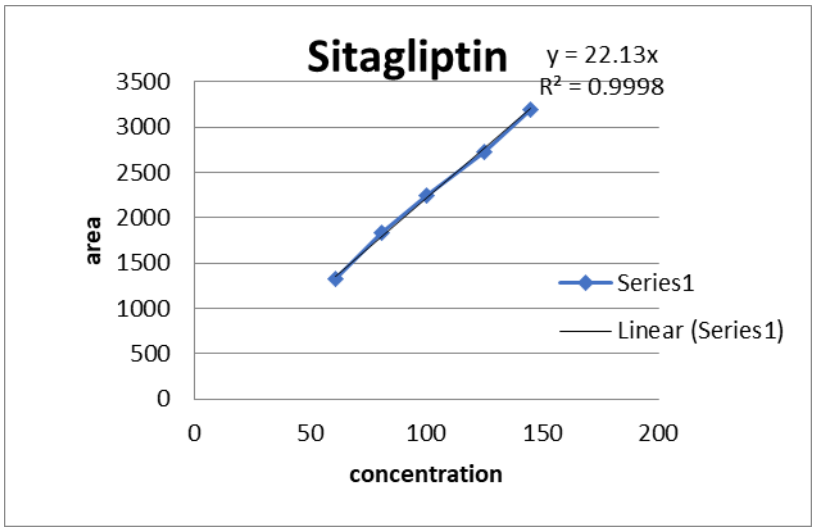

Figure 6: Linearity graph for Sitagliptin

Table 5: Linearity results of Simvastatin

S.No.	Conc.(µg/ml)	Area
1	61	1331.154
2	81	1859.595
3	102	2010.885
4	122	2728.440
5	142	3196.923

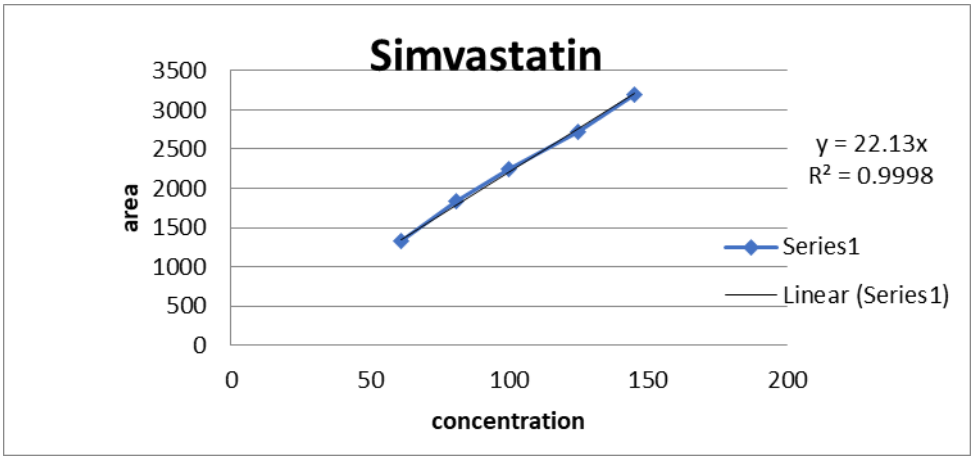

Figure 7: Linearity graph for Simvastatin

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 100%, 120%, 140% and 100%, 120%, 140% Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Sitagliptin and Simvastatin and calculate the individual recovery and mean recovery values. The results are shown in table 6,7.

Table 6: Showing accuracy results for Sitagliptin

Recovery level	**Accuracy Sitagliptin**					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
100%	98	747.051	758.681	31.78	99.45	99.60
	98	760.464				
	98	768.530				
120%	117.6	1062.233	1057.388	45.95	91.48	
	117.6	1050.067				
	117.6	1059.864				
140%	137.2	1245.814	1247.078	152.29	107.89	
	137.2	1242.233				
	137.2	1247.189				

Table 7: Showing accuracy results for Simvastatin

Recovery level	**Accuracy SIMVASTATIN**					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
100%	102	2012.830	2015.409	31.78	100.201	98.08
	102	2009.765				
	102	2023.634				
120%	122.4	2728.440	2738.442	44.76	89.85	
	122.4	2737.172				
	122.4	2749.714				
140%	142.8	3196.923	3175.268	148.78	104.19	
	142.8	3166.488				
	142.8	3162.393				

Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 8.

Table 8: Precision results for Sitagliptin and Simvastatin

Injection	Area for Sitagliptin	Area for Simvastatin
Injection-1	765.269	2012.58
Injection-2	760.464	2009.765
Injection-3	768.53	2023.634
Injection-4	763.825	2028.946
Injection-5	762.172	2031.51
Injection-6	775.496	2035.765
Average	765.9593	2023.7
Standard Deviation	5.42459	10.50642
%RSD	0.007082	0.005192

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on Analyst. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 9.

Table 9: Ruggedness results of Sitagliptin and Simvastatin

SITAGLIPTIN	%Assay	SIMVASTATIN	%Assay
Analyst 01	99.33	**Analyst 01**	99.69
Analyst 02	99.33	**Analyst 02**	99.69

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.8 ml/min to 1.2 ml/min. The results are shown in table 10.

Table 10: Flow variation results for Sitagliptin

Parameter	SITAGLIPTIN		SIMVASTATIN	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow Rate **0.8 ml/min** **1.2 ml/min**	 3.480 2.523	 2.343 2.107	 4.767 3.460	 2.093 1.882
Wavelength **239nm** **243nm**	 2.940 2.940	 2.333 2.300	 4.050 4.050	 2.000 2.000

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 11.

LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

Table 11: LOD, LOQ of Sitagliptin and Simvastatin

Drug	LOD	LOQ
Sitagliptin	0.72	2.20
Simvastatin	1.56	4.74

CONCLUSION:

A simple and selective LC method is described for the determination of Sitagliptin and Simvastatin in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 80 volumes of methanol and 20 volumes of water with detection of 241 nm. Linearity was observed in the range 60-140µg /ml for Sitagliptin (r^2 =0.997) and 61-155µg /ml for Simvastatin (r^2 =0.997) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

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