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[Research article]

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Sitagliptin and Simvastatin in Bulk and Tablet Dosage Form

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ABSTRACT

A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of Sitagliptin and Simvastatin in bulk and tablet dosage form. Chromatographic analysis was performed on a Nucleosil C_{18} (150X4.6 mm, 5µm) column ambient temperature with a mixture of phosphate buffer and Acetonitrile in the ratio 30:70 (phosphate buffer preparation; 0.01 N Potassium dihydrogen phosphate, pH 3.5 adjust with triethylamine) as mobile phase, at a flow rate of 1 mL min^{-1}. UV detection was performed at 254 nm. The method was validated for accuracy, precision, specificity, linearity and sensitivity. The retention times of Sitagliptin and Simvastatin were 3.242 min and 6.492 min, respectively. Calibration plots were linear over the concentration ranges 25-150 µg mL^{-1} and 5-30 µg mL^{-1} for Sitagliptin and Simvastatin respectively. The Limit of detection was 1.305 µg mL^{-1} and 0.257 µg mL^{-1} and the quantification limit was 3.941µg mL^{-1} and 0.77µg mL^{-1}Sitagliptin and Simvastatin for respectively. The accuracy of the proposed method was determined by recovery studies and found to be 99.20% to 100.94%.

Keywords: Sitagliptin, Simvastatin, RP-HPLC, Validation.

INTRODUCTION

Sitagliptin is chemically (*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4] triazolo [4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl) butan -2-amine (Figure:1). It is a Dipeptidyl peptidase - 4 (DPP-4) inhibitor. This enzyme breaks down the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal. Simvastatin is a hypolipidemic drug used to control elevated cholesterol, or hypercholesterolemia. (Figure 2),It is chemically(1*S*,3*R*,7*S*,8*S*,8a*R*)-8-{2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate .Very few reports are there on simultaneous estimation of Sitagliptin and Simvastatin. In tablets they were estimated using spectrophotometry, HPTLC, and HPLC methods. Till date, to the best of our knowledge, two method has been reported in the literature. This manuscript describes the development and validation, in accordance with ICH guidelines, of rapid, economical, precise and accurate isocratic reversed-phase HPLC method for analysis of Sitagliptin and Simvastatin in bulk and table dosage form.

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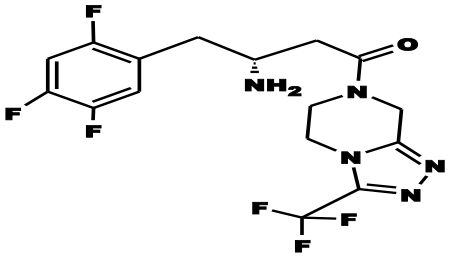

Figure-1 Sitagliptin

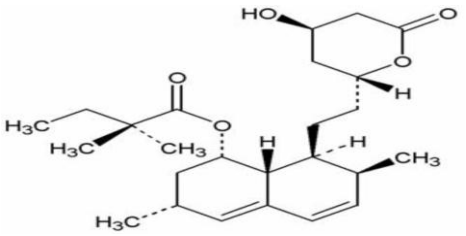

Figure-2 Simvastatin

MATERIALS AND METHODS

Chemicals

Sitagliptin and Simvastatin obtained from Bio Leo. lab.Pvt.Ltd, Hyderabad, as a gift samples. Potassium dihydrogen phosphate & Disodium hydrogen phosphate (AR Grade), Ortho-phosphoric acid (AR Grade), Acetonitrile (HPLC Grade), were purchased from Merck (India) Ltd., Worli, Mumbai, India. Tablet formulation (Juvisync) was purchased from local market, containing Sitagliptin (50 mg), Simvastatin (10 mg). Double distilled water was used throughout the experiment.

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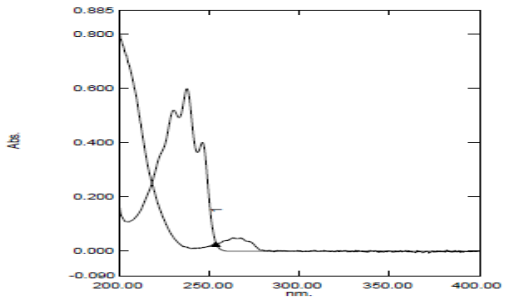
Instruments

Waters HPLC e 2695 series consisting 4 pumps. Auto sampler with 5 racks, each rack has 24 vials holding capacity with temperature control. Auto injector has capacity to inject 5µL to 500µL. UV-Vis Detector with PDA. Thermostat column compartment connected it has a capacity to maintain 5°C to 60°C column temperature.

Waters (alliance) HPLC System is equipped with Empower-2 software

ANALYTICAL METHOD DEVELOPMENT

Optimization of UV conditions

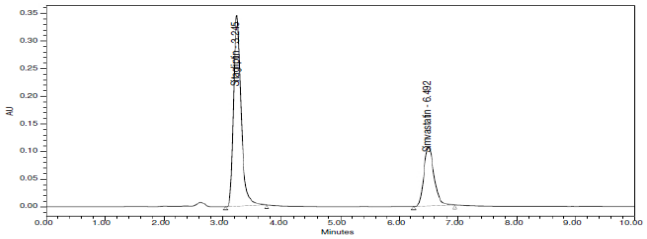


No.	Wavelength	Absorbance	Description
1	254.00	0.024	Sita&Simvast

Figure-3 Isobestic point of Sitagliptin and Simvastatin

Chromatographic Conditions

A waters nucleosil C-18 column (150 mm x 4.6 mm i.d.,5-µm) was used for chromatographic separation. The mobile phase composed of Acetonitrile and phosphate buffer (70:30 *v/v*); pH adjusted to 3.5 with triethylamine at a flow rate of 1 mL min-1 with run time of 20min. Mobile phase and sample solutions were filtered through a 0.45 µm membrane filter and degassed. The detection of both drugs was carried out at 254 nm.



	Peak Name	RT	Area	Height	% Area	USP Plate Count	USP Resolution	USP Tailing
1	Sitagliptin	3.245	3095888	346636	70.805	3384.22		1.14
2	Simvastatin	6.492	1264555	105542	29.195	6836.45	11.88	1.10

Figure-4 Optimized Chromatogram

METHODOLOGY

Mobile phase preparation

Buffer preparation

0.01 N Potassium dihydrogen phosphate adjust pH to 3.5 with triethylamine. Mix buffer and Acetonitrile at 30:70 ratio sonicate the resulting solution and degauss it using vacuum filtration through 0.45 micron membrane filter.

Standard stock solution preparation

Weigh and transfer50 mg of Sitagliptin working standard and 10 mg of Simvastatin working standard into 50 mL volumetric flask, add 50 mL of diluent and sonicate to dissolve and dilute to volume with diluent.

Standard preparation

Transfer10 mL of standard stock solution into 100 mL volumetric flask and dilute to volume with diluent.

Sample Preparation

Finely grind pre weighed 20 tablets. Transfer grinded sample quantitatively equivalent to 50 mg of Sitagliptin and 10 mg Simvastatin of in to 100 mL volumetric flask add 50 mL of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through filter paper. Dilute 10 ml of filtrate to 100 ml with mobile phase.

Procedure

Inject 20 µL of blank solution, placebo solution, Standard solution, Disregard peaks due to blank and placebo if any.

VALIDATION OF METHOD:

The HPLC method was validated in accordance with ICH guidelines.

Precision

The system precision of the method was verified by six replicate injections of standard solution containing Sitagliptin and Simvastatin. The method precision was carried out the analyte six times using the proposed method. Repeatability was measured by multiple injections of a homogenous sample of Sitagliptin and Simvastatin

Accuracy

Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analyzed sample solution of Sitagliptin and Simvastatin; a known amount of standard drug powder of Sitagliptin and Simvastatin were added at 80, 100 and 120 % level.

Specificity and Selectivity

Specificity of the method was determined through study of resolution factor of drug peak from the nearest resolving peak. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.

Limit of detection and Limit of quantitation

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 x ASD/S and LOQ = 10 x ASD/S, Where, 'ASD' is the average standard deviation and 'S' is the slope of the line.

Robustness

Robustness was evaluated by making deliberate variations in few method parameters such as variation of wave length; flow rate and variations in temperature. The robustness of the method was studied for Sitagliptin and Simvastatin

RESULTS AND DISCUSSION

Selection of Chromatographic Conditions and Optimization of Mobile Phase:

Mobile phase was optimized to separate Sitagliptin and Simvastatin using nucleosil C-18 column (150 mm x 4.6 mm i.d., 5µm). Initially, ACN and phosphate buffer in the ratio of (70:30) were tried as mobile phase but the splitting of the peaks for both these drugs was observed. Therefore, after adjustment of pH of mixed phosphate buffer to 3.5 with Triethyle amine, and mobile phase composition (ACN and phosphate buffer in 70:30 % v/v*)* was tried for resolution of both drugs. Good resolution and symmetric peaks were obtained. The flow rate of the mobile phase was 1 mL min-1. Under optimum chromatographic conditions, the retention time for Sitagliptin and Simvastatin were found to be 3.242 and 6.497 min, respectively when the detection was carried out at 254 nm. A typical chromatogram of two drugs is shown in (**Figure 3**).

LINEARITY DATA

The Linear detector response for Sitagliptin and Simvastatin is demonstrated by concentration versus Area. Over the range of 25 to 150% with respect to the target concentration (Dosage).

Table-1 For Peak Area of Sitagliptin

% Linearity	Conc(mcg)	Area
25	25	766916
50	50	1531453
75	75	2287590
100	100	3048305
125	125	3786765
150	150	4579050

Figure- 5 Calibration curve for sitagliptin

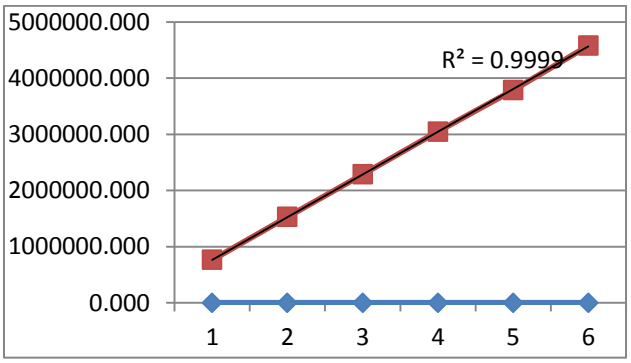


Table-2 For Peak Area of Simvastatin

% Linearity	Conc(mcg)	Area
25	5	310546
50	10	624181
75	15	939359
100	20	1248779
125	25	1563474
150	30	1890091

Figure-6 Calibration curve for Simvastatin

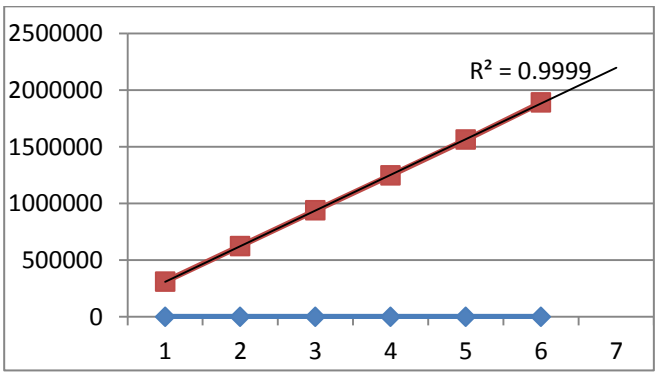


Table-3 PRECISION

S No	Name	Sita		Simva	
		RT	Area	RT	Area
1	S-Precision-1	3.245	3088245	6.504	1270021
2	S-Precision-2	3.243	3091365	6.501	1267588
3	S-Precision-3	3.244	3100702	6.497	1272347
4	S-Precision-4	3.242	3082899	6.492	1258526
5	S-Precision-5	3.242	3096365	6.488	1275423
6	S-Precision-6	3.242	3100568	6.488	1273320
Average		3.243	3093357	6.495	1269538
Standard Deviation		0.0013	7133.9	0.007	6035.35
RSD		0.0390	0.231	0.10	0.48

Table-4 Method Precision

S No	Name	Sita		Simva	
		RT	Area	RT	Area
1	M-Precision-1	3.242	3092232	6.500	1264535
2	M-Precision-2	3.246	3091365	6.502	1265545
3	M-Precision-3	3.241	3092623	6.502	1271365
4	M-Precision-4	3.245	3095865	6.492	1264531
5	M-Precision-5	3.244	3096445	6.502	1268435
6	M-Precision-6	3.241	3091545	6.495	1263452
Average		3.243	3093346	6.499	1266311
Standard Deviation		0.0021	2230.7	0.004	3004.58
RSD		0.0659	0.072	0.066	0.237

Acceptance criteria

The % of RSD for Area and RT from Repeated injections should not be more than 2.0**%.**

ACCURACY

Accuracy data.

The accuracy of the test method is demonstrated by % of recovery. The sample preparations are spiked with known amount of standard at three concentration levels and injected three times (Like 80% 100% and 120%).

Table-4 Recovery studies of Sitagliptin by RP-HPLC method

S.No	Spike level	Peak area	Amount Added (µg/ml)	Amount Recovered (µg/ml)	%Recovery	Avg	% RSD
		2484463	80	79.47	99.34	99.24	0.76
1	80%	2457436	80	78.64	98.30		
		2497082	80	80.12	100.16		
		3127747	100	100.01	100.01	99.48	0.38
2s	100%	3093509	100	99.1	99.1		
		3106925	100	99.3	99.35		
		3786765	120	121.38	101.15	100.9	0.17
3	120%	3774773	120	120.86	100.72		
		3784305	120	121.08	100.86		

Table-5 Recovery studies of Simvastatin by RP-HPLC method

S.No	Spike level	Peak area	Amount Added (µg/ml)	Amount Recovered (µg/ml)	%Recovery	Avg	% RSD
		1056502	16	16.05	100.35	99.4	0.71
1	80%	1037119	16	15.78	98.65		
		1047401	16	15.87	99.20		
		1282182	20	19.83	99.15	99.2	0.69
2	100%	1282777	20	19.68	98.42		
		1305643	20	20.02	100.1		
		1563474	24	23.78	99.10	99.2	0.16
3	120%	1570685	24	23.86	99.45		
		1570685	24	23.86	99.45		

Table-5 Results of global % recovery studies

Different level in %	Sitagliptin	Simvastatin
80	99.24	99.43
100	99.48	99.20
120	100.9	99.29
Average	99.87	99.30
SD	0.732	0.094
%RSD	0.74	0.095

Acceptance criteria

The % of recovery should be between 98 to 102%.

LIMIT OF DETECTION (LOD)

Table-6 Limit Of detection results.

S.NO	Name	LOD Value (µg/ml)
1.	Sitagliptin	1.305
2.	Simvastatin	0.257

Table-7 Limit of Quantitation (LOQ) results.

S.NO	Name	LOQ Value(µg/ml)
1.	Sitagliptin	3.941
2.	Simvastatin	0.77

ROBUSTNESS

Robustness for Sitagliptin and Simvastatin

The robustness of test method is demonstrated by carrying out intentional method variations like mobile phase flow changes, mobile phase compositions and column oven temperature variations etc...

Table-8 Robustness for Sitagliptin and Simvastatin

S No		Sitagliptin		Simvastatin	
		RT	Area	RT	Area
1	Standard	3.245	3095888	6.492	1264555
2	Robustness-Flow Change-1	2.89	2863684	5.777	1197338
3	Robustness-Flow Change-2	3.7	3667137	7.4	1530950
4	Robustness-Column Oven Temperature-1	3.23	3208756	6.084	1353173
5	Robustness-Column Oven Temperature-2	3.253	6.908	3204189	1329066

ASSAY

Assay for Sitagliptin and Simvastatin

Standard preparation

Transfer 10 ml of standard stock solution in to 100 mL volumetric flask and make up to volume with diluent.

Sample Preparation

Transfer sample quantitatively equivalent to 50 mg of Sitagliptin and 10 mg of Simvastatin in to 50 mL volumetric flask add 50 mL of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through filter paper. Dilute 10 ml of filtrate to 100 ml with mobile phase.

Procedure

Inject 20 µL of blank solution, standard solution, and sample solution record the chromatogram. And calculate percentage of assay.

Table-9 Assay

		Sitagliptin		Simvastatin	
S No	Name	RT	Area	RT	Area
1	Standard-1	3.256	3133162	6.491	1296594
2	Standard-2	3.250	3127115	6.489	1285416
Avg		3.253	3130139	6.490	1291005
3	Sample-1	3.254	3099385	6.498	1278425
4	Sample-2	3.253	3128408	6.492	1287541
Avg		3.254	3113897	6.495	1282983

Table-10Results for Sitagliptin

3113897	50	10	50	100	99.82	360	**mg/Tab**	**%Assay**
3130139	50	100	360	10	100		**49.74**	**99.48**

Table- 11Results forSimvastatin

1282983	10	10	50	100	99.75	360	**mg/tab**	**%Assay**
1291005	50	100	360	10	100		**9.94**	**99.38**

SYSTEM SUITABILITY PARAMETERS

Table-12 System suitability parameters results for Sitagliptin and Simvastatin

Parameters	Results	
	Sitagliptin	Simvastatin
Tailing factor	0.61	1.45
Theoretical plates per column	0.79	0.4835

CONCLUSION

The developed RP-HPLC method is simple, precise, accurate, selective and reproducible. The method has been found to be adequately robust and can be used for simultaneous determination of Sitagliptin and Simvastatin in bulk and tablet formulation. The method was validated as per ICH guidelines.

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