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Research

Method Development And Validation Of Sitagliptin And Simavastatin In Tablet Dosage Form By Rp-Hplc

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Abstract

A simple, sensitive, and precise RP-HPLC method for the simultaneous estimation of Sitagliptin (SIT) and Simvastatin (SIM) combined dosage form has been developed and validated. The components were well separated using Agilent C8 (4.6 x 150mm, 3.5µm) column using phosphate buffer (ph-6.26): Acetonitrile in the ratio 25:75 as mobile phase at a flow rate of 0.8 mL/min. The eluents were detected at 254 nm using UV detector. The retention times of SIT and SIM were found to be 2.475min and 6.528min for Simvastatin and Sitagliptin respectively. The linearity was observed in the range of 80-120 µg/mL for SIT and 16-24µg/mL for SIM. The marketed dosage form was analyzed by using the developed method and the percent content of SIT and SIM were found to be 99.67 – 100.27 % and 99.42 – 100.54%. The developed method was validated for parameters like system suitability, specificity, linearity, accuracy, precision, ruggedness and robustness as per ICH guidelines and the results were found to be within the limits. The validated method was used for the stability studies (short, long and auto sampler) and forced degradation studies (acidic, alkaline, oxidative and photolytic). Both SIT and SIM were found to be stable in all conditions. This validated method can be used for the routine quality control testing of SIT and SIM combined dosage form.

Keywords: Sitagliptin, Simvastatin, Simultaneous estimation.

INTRODUCTION

Chemically, an antidiabetic drug Sitagliptin (SIT) is (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[1] (Fig. 1). SIT competitively inhibit the dipeptidyl peptidase 4(DPP-4)[2]. Chemically, a HMG-COA inhibitor drug Simvastatin (SIM) is (1S,3R,7S,8S,8aR)-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-[(2R,4R)-tetrahydro-4-hydroxy-6 -oxo-2H-pyran-2-yl]-1-napthyl-2,2-dimethylbutanoate (Fig. 2). The 6-membered lactone ring of Simvastatin is hydrolyzed in vivo to generate the Dihydroxy acid, an active metabolite structurally similar to HMG-CoA (hydroxy CoA). Once hydrolyzed, Simvastatin competes with HMG-CoA for HMG-CoA1 microsomal enzyme. Interference with the activity of this enzyme reduces the mevalonic acid, a precursor of cholesterol. Sitagliptin is a Dipeptidyl peptidase-4(DPP-4) inhibitor, which is believed to exert its actions in patients with type2 diabetes by slowing

the inactivation of incretin hormones. Concentrations of the active intact hormones are increased by SITAGLIPTIN, thereby increasing and prolonging the action of these hormones It is frequently prescribed drug used to treat hypercholesterolemia with coronary heart disease. SIM is official in IP, BP and USP whereas SIT is not. Several methods have been reported for the assay of SIT, SIM in bulk, dosage forms with other drug combinations like HPLC, HPLC-MS, UPLC-MS and UV-Visible spectrophotometry. The literature review revealed that there is no chromatographic method available for the simultaneous estimation of this combination. Hence an attempt was made to develop a validated analytical method for the simultaneous estimation of SIT and SIM combined dosage form using RP-HPLC method.

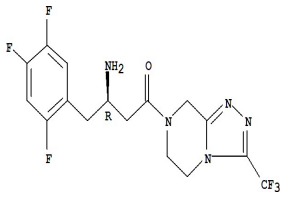

Fig 1: Structure of SIT

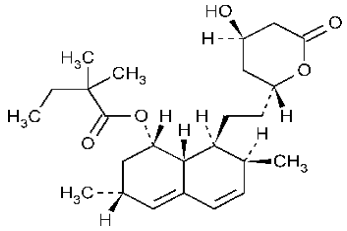

Fig 2: Structure of SIM

MATERIALS AND METHODS

Chemicals and Reagents

HPLC grade methanol, acetonitrile and all other analytical grade reagents were purchased from Merck, India. HPLC grade water was prepared using Milli-Q water purification system. Juvisync tablets were purchased from local markets. Class A glassware is used throughout the experiment. SIT and SIM gift samples were obtained from Dr. Reddy's Laboratories Ltd., Hyderabad and Aurobindo Pharma Ltd., Hyderabad, respectively.

Equipment & Chromatographic conditions

The chromatographic system consists of a Shimadzu class VP Binary pump LC-10ATvp Pump, SIL-10ADvp auto sampler, CTO-10Avp Column temperature oven, SPD- 10Avp UV-Visible detector. All the components of the system are controlled using SCL-10Avp system controller. Data acquisition was done using LC Solutions version 1.23 software. Chromatographic separations were carried out using Agilent C8 (4.6 x 150mm, 3.5µm) reverse phase column with a mobile phase consisting of phosphate buffer (pH-6.26): Acetonitrile in the ratio 25:75 as mobile phase at a flow rate of 0.8 mL/min. The eluents were detected at 254nm using UV detector.

Preparation of solutions Preparation of stock solution

Weighed 2.950 grams of potassium dihydrogen phosphate and 540mg of dipotassium hydrogen ortho phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and the pH was found to be 6.26.

Mobile Phase

Mixture of above buffer 250 ml (25%) and 750 ml of Acetonitrile HPLC (75%) are mixed and degassed in ultrasonic water bath for 5 minutes. Filtered through 0.45 µ filtered under vacuum filtration.

Preparation of Calibration standards and Quality controls

Preparation of stock solution

Accurately weighed and transferred 10 mg of Sitagliptin and Simvastatin working standard into a 10mL clean dry volumetric flask added about 7mL of Diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (80ppm of Sitagliptin & 16ppm of Simvastatin)

0.8ml of stock solution has taken in 10ml of volumetric flask diluted up to the mark wit diluent.

Preparation of Level – II (90ppm of Sitagliptin & 18ppm of Simvastatin)

0.9ml of stock solution has taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level – III (100ppm of Sitagliptin & 20ppm of Simvastatin)

1.0 ml of stock solution has taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level – IV (110ppm of Sitagliptin & 22ppm of Simvastatin)

1.1ml of stock solution has taken in 10ml of volumetric flask diluted up to the mark with diluent.

Preparation of Level – V (120ppm of Sitagliptin & 24ppm of Simvastatin)
1.2ml of stock solution has taken in 10ml of volumetric flask diluted up to the mark with diluent.

Procedure
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.
Acceptance criteria
The Linear Regression coefficient should not be less than 0.995.

Method Validation
The developed chromatographic method was validated for system suitability, linearity, accuracy and precision, specificity, ruggedness and robustness as per ICH guidelines19, 20.

RESULTS AND DISCUSSION

Method Development and Optimization
The standard solutions of SIT and SIM were scanned in UV-Vis Spectrophotometer and the λmax of SIT and SIM were found to be 268 nm and 238 nm respectively. The iso- absorptive point of both drugs at 254 nm was chosen for detection. Different permutations and combinations at different pH values ranging from 3 to 11 using various columns like Hypersil-BDS-C18, Symmetry C18, Ymc C18, Sperisorb C18, Phenomenox C18 and Ymc C4 have been tried with different buffers using ammonium acetate, ortho phosphoric acid, acetic acid, potassium dihydrogen phosphate along with acetonitrile and methanol. Good resolution, least tailing and high number of theoretical plates were obtained for the separation of the drugs using Agilent C8 (4.6 x 150mm, 3.5µm). The optimized mobile phase is phosphate buffer (ph-6.26): Acetonitrile in the ratio 25:75 v/v) at a flow rate of 0.8 mL/min. Under these conditions SIT and SIM were eluted at 2.475min and 6.528min respectively with run time of 9 min. A typical combined chromatogram of SIT and SIM is shown in Fig. 3.

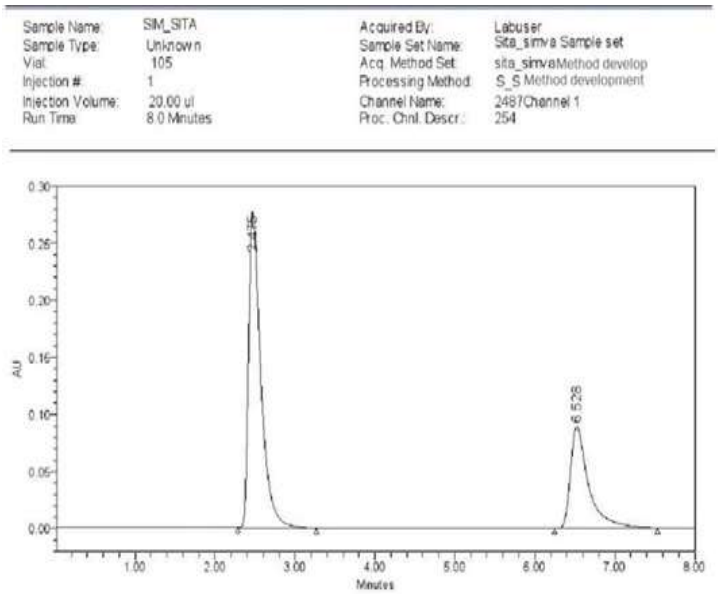

Fig 3: Chromatogram for system suitability

System Suitability
System suitability parameters are integral part of liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The key to achieving system suitability is an optimal instrumentation and an efficient analytical column. System suitability test is a Pharmacopoeial requirement. The % CV of peak areas and retention times (RT) for both the drugs are less than 2 % limits. Results were shown in Table 1.

Table 1: System suitability

S No	Parameter	Sitagliptin	Simvastatin	Acceptance criteria
1	Theoretical plates	2286.158	4608.944	Theoretical plate count should be more than 2000.
2	Retention time	2.232	5.6754	Retention time should be more than 2.
3	Resolution	11.27	11.27	Resolution betweentwo peaks must be more than 2
4	Tailing factor	1.264	1.316	Tailing factor must be less than 2.

Specificity

The specificity of the method was observed by injecting the blank, working standard solutions of SIT, SIM and the mixture. The specificity chromatogram was shown in Fig. 4. Where, the retention times of SIT and SIM were does not interfere with the other.

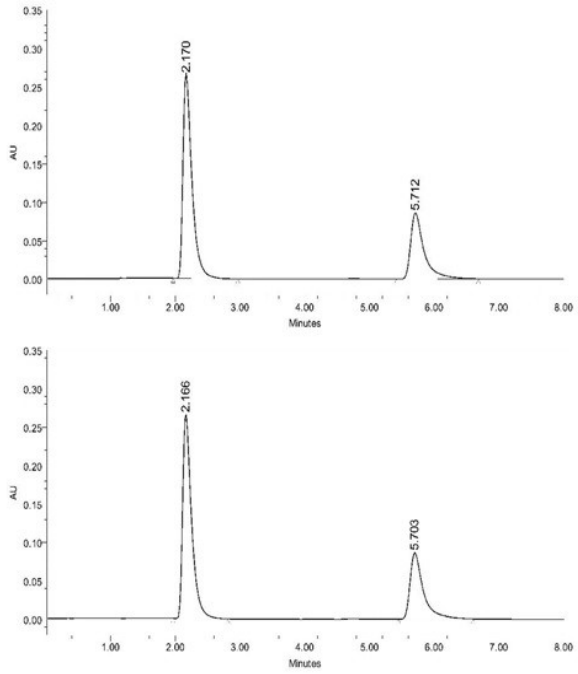

Fig 4: Chromatogram for specificity

Linearity

The calibration curves were constructed and evaluated by their correlation coefficients. The peak areas of SIT and SIM were found to be linear in the ranges of 50 - 500 µg/mL and 20 - 200 µg/mL. Calibration curves for SIT and SIM were shown in Fig. 5 & Fig. 6. The obtained results were shown in Table 2 & 3.

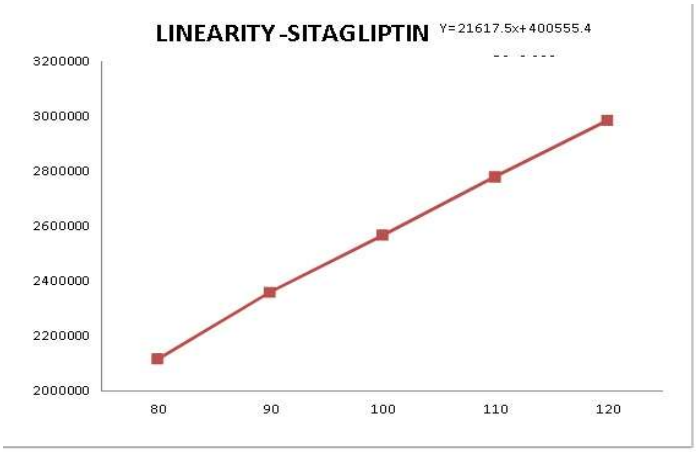

Fig 5: Linearity of SIT

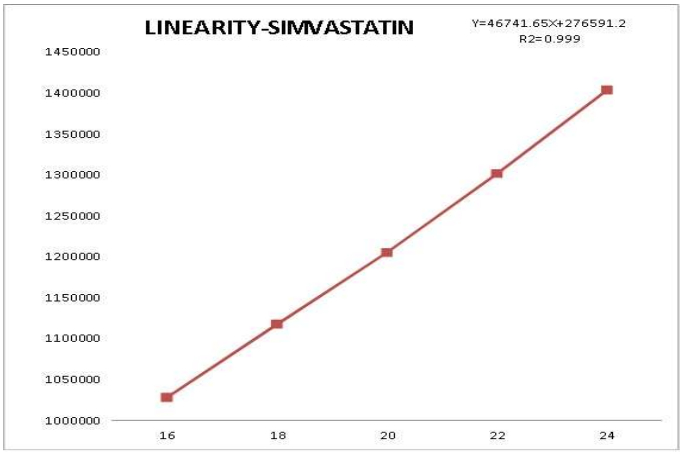

Fig 6: Linearity of SIM

Table 2: Results of regression analysis SIT

Sr.no	Concentration	Area	Average area
1	80ppm	2115841 2115843 2115844	2115842
2	90ppm	2360794 2360796 2360797	2360795
3	100ppm	2567566 2567568 2567569	2567567
4	110ppm	2780461 2780463 2780464	2780462
5	120ppm	2986885 2986887 2986888	2986886
Correlation coefficient			0.999

Table 3: Linearity Results: (for Simvastatin)

S.No	Concentration	Area	Average area
1	16ppm	1028432	1028433
		1028434	
		1028435	
2	18ppm	1117783	1117784
		1117785	
		1117786	
3	20ppm	1205381	1205382
		1205383	
		1205384	
4	22ppm	1301560	1301561
		1301562	
		1301563	
5	24ppm	1403960	1403961
		1403962	
		1403963	
Correlation coefficient		0.999	

Correlation coefficient should be not less than 0.999.

Accuracy and Precision

Accuracy and precision were calculated for the QC samples during the intra-day and inter-day run were given in Table 3 and Table 4. All the data were within the acceptance criteria of 5% (except 10% for LLOQ).

Limit of detection and quantification

LOD and LOQ were estimated from signal to noise ratio and were found to be 1 µg/mL and 3 µg/mL respectively for SIT and 0.5 µg/mL and 1.5 µg/mL respectively for SIM. The results were shown in Table 4,5.

Table 4: The accuracy results for Sitagliptin.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	2639988	10.0	10.21	102.14%	100.22%
100%	5160066	20.0	19.96	99.82%	
150%	7653938	30.0	29.61	98.71%	

The % Recovery for each level should be between 98.0 to 102.0%.

Table 5: The accuracy results for Simvastatin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1239691	10.4	10.47	100.69%	100.54%
100%	2359402	20.0	19.93	99.65%	
150%	3357441	28.0	28.36	101.29%	

The % Recovery for each level should be between 98.0 to 102.0%.

Calculation of S/N ratio for Sitagliptin
Average Baseline Noise obtained from Blank :95 µV Signal Obtained from LOD solution (0.13% of target assay concentration) : 285 µV S/N =285/95 = 3.00
S/N Ratio value shall be 3 for LOD solution.
Calculation of S/N ratio Simvastatin
Average Baseline Noise obtained from Blank : 95 µV Signal Obtained from LOD solution (0.35% of target assay concentration) : 298 µV S/N =298/95 = 3.14
S/N Ratio value shall be 3 for LOD solution.

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.

a. The flow rate was varied at 0.7 ml/min to 0.9ml/min.

Standard solution 100ppm of Sitagliptin & 20ppm of Simvastatin was prepared and analysed using the varied flow rates along with method flow rate. Results were shown in Table 6,7.

Table 6: Robustness results for Sitagliptin

S.No	Parameter variation		Area of Sitagliptin	% RSD
1.	Flow rate	0.7	2975037	0.1165
			2975108	
			2975087	
		0.9	232619	
			232605	
			232622	
2.	Organic phase	70	2563842	0.1185
			2563951	
			2563901	
		80	2644123	0.1321
			2645456	
			2643849	

Results for actual Mobile phase composition (75:25 Acetonitrile: Buffer) have been considered from Assay standard.

Table 7: Robustness results for Simvastatin

Serial number	Parameter variation		Area of Sitagliptin	% RSD
1.	Flow rate	0.7	1417227	0.124
			1417442	
			1417589	
		0.6	1104266	0.269
			1104236	
		0.9	1104671	
2.	Organic phase	70	1210154	0.198
			1211123	
			1242810	
		80	1229838	0.312
			1230152	
			1231268	

Results for actual Mobile phase composition (75:25 Acetonitrile: Buffer) have been considered from Assay standard.

CONCLUSION

The new RP-HPLC method was developed and validated for the simultaneous estimation of SIT and SIM in combined pharmaceutical dosage form and was found to be accurate, precise, simple, economic, rapid and having good specificity, selectivity and stability. The developed method can be used for the routine analysis of the combined formulation.

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