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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SIMVASTATIN AND SITAGLIPTIN

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ABSTRACT

A simple, specific, accurate, rapid, inexpensive isocratic Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the quantitative determination of Simvastatin and Sitagliptin pharmaceutical tablet dosage forms. RP-HPLC method was developed by using Inertsil ODS-3 C 18 (75 mm*4.6 mm) 5 microns Short column, Shimadzu LC-20AT Prominence Liquid Chromatograph. The mobile phase composed of 0.05 M Ammonium acetate: CAN 60:40. The flow rate was set to 1.0 mL.min^{-1} with the responses measured at 253 nm using Shimadzu SPD-20A Prominence UV-Visible detector. Retention time of simvastatin and sitagliptin were found to be 3.260 and 2.136 minutes. Linearity was established for simvastatin and sitangliptin in the range of 25 to 150 and 10 to 60 µg.mL^{-1} with correlation coefficient 1. The validation of the developed method was carried out for specificity, linearity, precision, accuracy, robustness, limit of detection, limit of quantitation. The developed method can be used for routine quality control analysis of in simvastatin and sitangliptin pharmaceutical tablet dosage form.

Keywords: simvastatin and sitangliptin , Isocratic RP-HPLC, UV-Vis detector, Method Validation.

INTRODUCTION

Simvastatin (SIM), a methylated analog of lovastatin, is -(+)-{1*S*,3*R*,7*S*,8*S*,8*aR*)-1,2,3,7,8,8*a*-hexahydro-3,7-dimethyl-8-[2-(2*R*,4*R*)-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl]-naphthyl-2,2-dimethyl butanoate (Figure 1)[1]. It acts by inhibiting HMG CoA reductase and is used for the treatment of hypercholesterolemia. After oral administration, this pro drug is converted into β hydroxy acid of simvastatin, which is a potent inhibitor of HMG CoA reductase, a key enzyme required for the synthesis of cholesterol in liver.

The determination of Simvastatin has been carried out in tablets by UV-Spectrophotometry[9,10], RP-HPLC[11-16], HPTLC. A literature review reveals that no analytical method (neither UV spectrophotometric nor any other method) is available for the simultaneous estimation of Sitagliptin and Simvastatin in tablet dosage form in pharmaceutical preparations, which prompted to pursue the present work. The objective of the present work is to develop and validate new analytical methods for simultaneous determination of Sitagliptin and Simvastatin in tablet dosage form[2-8]. This communication forms the first report of a simple, sensitive and reproducible method for the simultaneous estimation of Sitagliptin and Simvastatin from combined dosage form. Sitagliptin (SIT), [(2*R*)-1-(2,4,5-trifluorophenyl)-4- oxo-4-[3-(trifluoromethyl)-5,6 dihydro [1,2,4] triazolo [4,3-*a*]pyrazin-7(8*H*)-yl] butan-2-amine][1] is a well known hypoglycemic drug. STG is a novel oral hypoglycemic drug of the dipeptidyl peptidase 4 inhibitor class (Figure 2). Sitagliptin increased incretin levels (GLP-1 and GIP) which inhibit glucagon release, in turn decreases blood glucose, but more significantly increases insulin secretion. The determination of STG has been carried out in tablet by RP-HPLC by UV Spectrophotomerty[9,10].

MATERIALS AND METHODS

Instruments

Quantitative HPLC was performed on a isocratic high performance liquid chromatograph (Waters Alliance 2695) with a LC-20AT VP pump, manual injector with loop volume of 20 µL (Rheodyne), programmable variable wavelength Shimadzu SPD-20A Prominence UV-Vis detector and Inertsil ODS C_{18}(75 , 4.6 mm, 5 µ). The HPLC system was equipped with "Empower 2 software" software. In addition an electronic balance (sartorious bsa2245-cw), digital pH meter (micro processor model no- lp-1395), a sonicator (biotechnics India (model no- 9l250h mode), UV-Visible Spectrophotometer (Schimadzu) were used in this study.

Standards and Chemicals Used

Potassium dihydrogen orthophosphate (Analytical grade) and Ortho phosphoric acid (Analytical grade) from Merck Pharmaceuticals Private Ltd., Mumbai, India., while acetonitrile (HPLC grade) and triethylamine (HPLC grade) from Merck Pharmaceuticals Private Ltd., Mumbai, India. Sitagliptin and Simvastatin were procured from Merck Pharmaceuticals Private Ltd., Mumbai, India.

Preparation of Mobile Phase

3.85 g of ammonium acetate was dissolved in 1000 ml of milliQ water. The solution was adjusted to a pH of 4.0 with orthophosphoric acid. Then it was degassed in ultrasonicator and then filtered through 0.45 µ pore size membrane filter. Mix a mixture of above buffer 600 ml (60 %) and 400 ml of Acetonitrile HPLC (40 %) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Preparation of Calibration Standards

Preparation of standard stock solution for Simvastatin

In the case of Simvastatin, 40 mg of Simvastatin working standard was weighed accurately. They were transferred to 100 ml volumetric flask dissolved and made up to the volume with mobile phase to obtain 400 µg / ml solution.

Preparation of standard dilutions of Simvastatin

From the stock solution, 2.5 ml, 5 ml, 7.5 ml, 10 ml, 12.5 ml,15 ml were pipette out in to 100 ml volumetric flask and made up to the mark with mobile phase to obtain dilutions

with concentrations 10 µg / ml, 20 µg / ml, 30 µg / ml, 40 µg / ml, 50 µg / ml,60 µg / ml respectively.

Preparation of standard stock solution for Sitagliptin:
In the case of Sitagliptin, 100 mg of Simvastatin working standard was weighed accurately. They were transferred to 100 ml volumetric flask dissolved and made up to the volume with mobile phase to obtain 1000 µg / ml solution.

Preparation of standard dilutions of Sitagliptin
From the stock solution, 2.5 ml, 5 ml, 7.5 ml, 10 ml ,12.5 ml, 15 ml were pipette out in to 100 ml volumetric flask and made up to the mark with mobile phase to obtain dilutions with concentrations 25 µg / ml, 50 µg / ml, 75 µg / ml, 100 µg / ml, 125 µg / ml,150 µg / ml respectively.

Procedure
Inject each concentration in to the chromatographic system and measure the peak area. Plot the graph of peak area on y axis versus concentration on x axis. Calculate the correlation coefficient.

Validation Study of Sitagliptin and Simvastatin
An integral part of analytical method development is validation. Once the method has been developed, it is necessary to evaluate under the conditions expected for real samples before being used for a specific purpose. The method validation was performed as per ICH guidelines for the determination of Simvastatin and Sitagliptin in bulk and pharmaceutical dosage forms. The method was validated with respect to parameters including specificity, precision, accuracy, linearity, robustness, system suitability and limit of detection (LOD), limit of quantification (LOQ).

Specificity
The effect of wide range of excipients and other additives usually present in the formulations of Simvastatin and Sitagliptin in the determinations under optimum conditions is investigated. The specificity of the RP-HPLC method is established by injecting the mobile phase and placebo solution in triplicate and recording the chromatograms. The specificity results were presented in Table 4.

Precision
Precision of the method was performed as intraday and interday precision. To study the intraday precision, six replicate standard solutions of Simvastatin and Sitagliptin were injected. The percent relative standard deviation (% RSD) was calculated. The acceptable criteria are not more than 2.0.

Linearity
Linearity graphs for the proposed assay methods were obtained over the concentration range of 25-150 µg / ml and 10-60 µg / ml. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient values and results were presented in Table 2. The linearity graphs of Simvastatin and Sitagliptin were shown in Figure 5 and 6.

Accuracy (Recovery studies)
The accuracy of the method was determined by calculating recovery of by the method of addition at three different levels (80 %, 100 % and 120 %). Percent recovery for Simvastatin and Sitagliptin by all the methods was found in the range of Table 7. The mean percentage recovery of Simvastatin and Sitagliptin at each level was 99.5 %, 100.2 % which were in the acceptance limit of 98 to 103 % as shown in Table 6.

Robustness
Robustness of the proposed methods was evaluated by making small changes in flowrate (± 0.2 ml / min), wavelength (± 2 nm) of the solution. The results were presented in Table 7.

Ruggedness
Ruggedness of the method was evaluated by comparing the results of assay of Simvastatin and Sitagliptin obtained from two analysts, system and two columns. RSD was always found to be < 2 % which indicates the method was rugged.

Limit of Detection (LOD)
The limit of detection (LOD) is defined as the lowest concentration of the analyte that can be detected but not necessarily quantified. LOD is calculated using formula (3.3*S.D) / Slope.

Limit of Quantitation (LOQ)
The limit of Quantitation (LOQ) is defined as the lowest concentrations of the analytes that can be qualified with acceptable precision and accuracy. LOQ is calculated using formula (10*S.D) / Slope.

RESULTS AND DISCUSSIONS
An effort has been made to identify a Simple, Precise, Specific and Accurate method for estimation of Sitagliptin and Simvastatin in formulation by using RP-HPLC method. During the selection of mobile phase several solvents were tried at various levels and finally selected mobile phase system was Acetonitrile: 0.05 M Ammonium Acetate Buffer of pH 4.0 at ratio 40:60 at ambient temperature. The concentration of (10 µg / ml) of Sitagliptin and Simvastatin was prepared by using mobile phase. The above solution was scanned in the range of 200-400 nm by using UV-VIS spectrophotometer with mobile phase as reference. After considering all the system suitability parameters, Acetonitrile: 0.05 M Ammonium Acetate Buffer (40:60) of pH 4.0 was selected for analysis at optimized flow rate of 1.0 ml / min. The Retention time of SIT And SIM was found to be 2.1 minutes, 5 minutes respectively. The Linearity of SIT and SIM was carried out at different concentrations ranging from 25-150 µg / ml and 10-60 µg / ml and correlation coefficient was found to be 1 and 1 which indicates that the concentration had given good linearity. Accuracy was confirmed by Recovery Studies. The % recovery of Sitagliptin and Simvastatin was found to be 99.5 %, 100.2 % which were in the acceptance limit of 98 to 103 % as shown in Table 6. The Precision has done in two ways i.e., System Precision and Method Precision. The % RSD values of Sitagliptin and Simvastatin for System Precision and Method Precision was found to be 0.35 and 0.23 and 0.32 and 0.44 respectively as shown in the Table 5 Which were in the acceptance limit of less than 2 %. The Specificity for these drugs was determined by using 0.1 N Hcl, 0.1 N NaoH for 60 minutes. When drug was mixed with 0.1 N Hcl, 0.1 N NaOH it was found to be occurrence of irregular peak and peak elution was not good. Above results has shown good Specificity.

Table 1: Optimized Chromatographic Conditions for Simultaneous Estimation of Simvastatin and Sitagliptin by RP HPLC

Optimized Chromatographic Conditions	
Mode of separation	**Isocratic elution**
Mobile phase	0.05 M Ammonium Acetate: CAN (60: 40)
Column	Inertsil ODS C_{18} (75, 4.6 mm, 5 µ)
Flow rate	1 ml / min
Detector wavelength	253 nm
Injection volume	20 µl
Oven temperature	Ambient
Run time	12 minutes

Table 2: Linearity Results of SIM and SIT

Sitagliptin		Simvastatin	
Con. (µg / ml)	**Area**	**Con. (µg / ml)**	**Area**
25	440023	10	797145
50	886133	20	1595582
75	1312724	30	2400233
100	1752182	40	3192209
125	2188862	50	3978418
150	2632535	60	4753369
Correlation Coefficient	1	Correlation Coefficient	1

Table 3: Assay Results of SIM and SIT

Compound	Standard area	Sample area	Standard weight	Sample weight	Average weight	Label claim	Standard purity
Simvastatin	3148996	3154055	40 mg	301 mg	300 mg	40 mg	99.7 %
Sitagliptin	1732920	1742406	100 mg	301 mg	300 mg	100 mg	99.75 %

Table 4: Specificity Study

	Sample Weight (mg)	Simvastatin Area	Sitagliptin Area	% assay of Simvastatin	% assay of Sitagliptin
Acid degradation	301	2819999	1521520	88.8 %	87.3 %
Base degradation	301	2832183	1557970	89.18 %	89.40 %

Table 5: Results of Precision Study

Injection samples	System precision				Method precision			
	R_t	Area	S.D	% RSD	R_t	Area	S.D	% RSD
SIM	5.13	3206673	7326.146	0.23	5.081	3178938	14028.	0.44
	5.11	3213605			5.077	3177020	8	
	5.10	3202295			5.071	3163649		
	5.09	3194559			5.066	3146634		
	5.08	3200967			5.062	3179110		
	5.09	3194557			5.057	3184166		
SIT	2.139	1761847	6130.6	0.35	2.132	1747035	5645.9	0.32
	2.137	1762735			2.132	1741146		
	2.138	1768234			2.133	1757841		
	2.136	1753754			2.132	1747043		
	2.134	1753939			2.131	1744099		
	2.136	1753758			2.129	1747785		

Table 6: Recovery of SIM and SIT

Inj. Sample	Spike level	area	Amount present	Amount recovered	% recovered	Mean recovery
Simvasatatin	50 %	4733579.3	50 mcg	50.11 mcg	100.22 %	100.28 %
	100 %	6360163.3	100 mcg	101.51 mcg	101.51 %	
	150 %	7877120.6	150 mcg	148.70 mcg	99.13 %	
Sitagliptin	50 %	2605649.3	50 mcg	49.57 mcg	99.95 %	99.65 %
	100 %	3493723.6	100 mcg	100.68 mcg	99.41 %	
	150 %	4322112.6	150 mcg	148.19 mcg	99.59 %	

Table 7: Results of Robustness of SIM and SIT

Parameter Modifications		Plate count	Tailing	Rt	Plate count	Tailing	Rt
		SIMVASTATIN			SITAGLIPTIN		
Flow Rate (ml / min)	0.8	6647	1.05	5.286	3622	1.32	2.2
	1.2	6684	1.02	5.155	3465	1.45	2.0
Mobile phase Buffer: ACN	50:50	6616	1.04	5.1	3564	1.25	2.5
	70:30	6654	1.07	5.2	3479	1.47	1.986

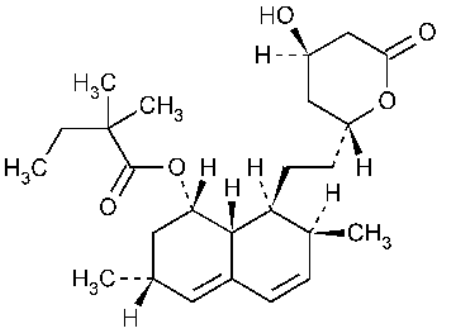

Figure 1: Structure of Simvastatin

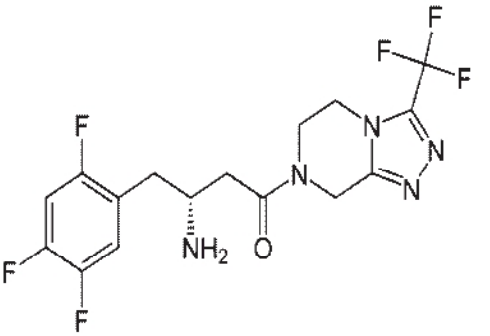

Figure 2: Structure of Sitagliptin

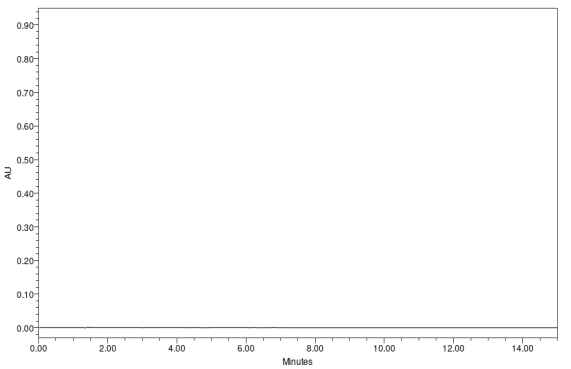

Figure 3: Chromatogram of Placebo

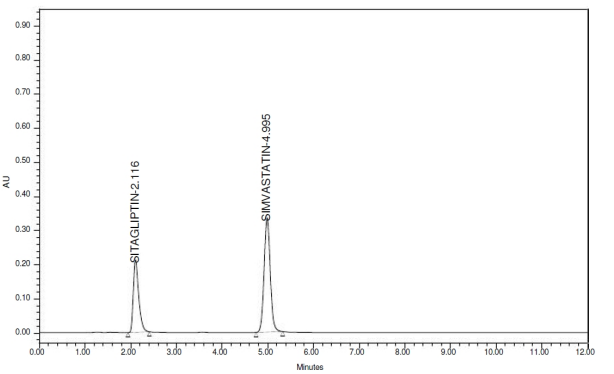

Figure 4: Chromatogram of SIT and SIM

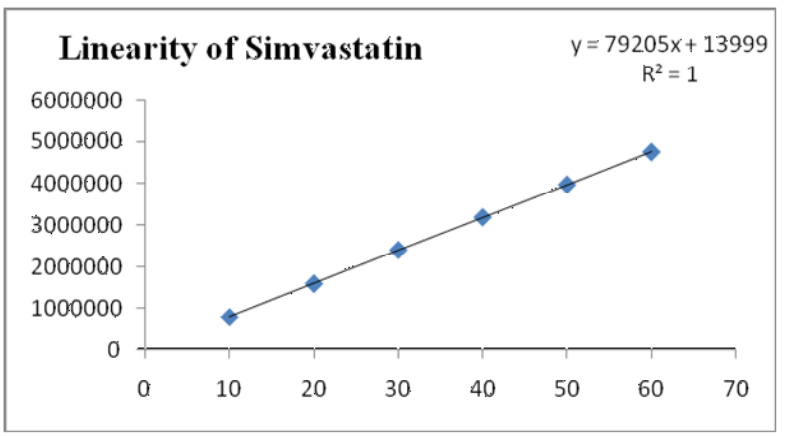

Figure 5: Linearity Curve of SIM

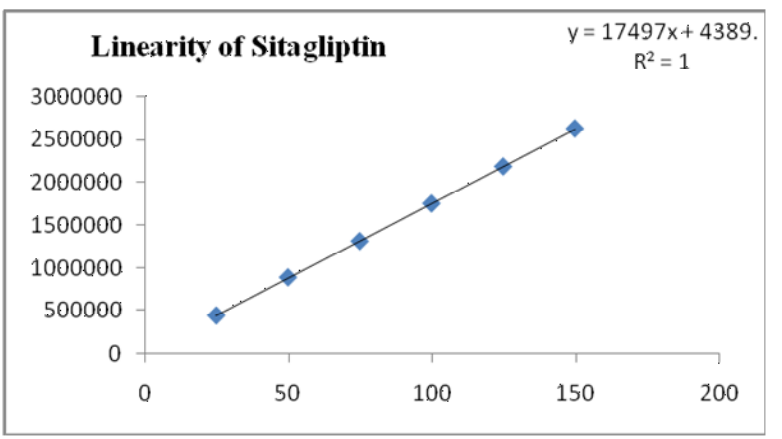

Figure 6: Linearity Curve of SIT

The Robustness of the method developed was validated by changing the flow Rate and mobile phase has shown in Table 7. The selected flow rate and mobile phase gives good separation of drugs. The proposed method was analyzed by two different analysts by conducting Ruggedness. Hence the proposed method has good repeatability. The tablet formulation was selected for analysis. The nominal concentration (100 %) considered and 20 µl of formulation was injected. The Assay percentage of SIT And SIM present in the sample was found to be 100.14 %, 99.5 % respectively. All the above parameters combined with the simplicity and ease of operation ensures that the RP-HPLC method can be applied for Simultaneous Estimation of Sitagliptin and Simvastatin in routine analysis of the two drugs in tablet dosage forms.

CONCLUSION

From the above experimental data and results, the developed HPLC method is having the following advantages:

- The standard and sample preparation requires less time.
- No tedious extraction procedure was involved in the analysis of formulation.
- Run time required for recording chromatograms were less than 10 minutes.
- Suitable for the analysis of raw materials, applicable to dissolution studies and can be used for the content uniformity studies.

Hence, the chromatographic method developed for the Simvastatin and Sitagliptin is said to be rapid, simple, specific, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories.

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