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DEVELOPMENT AND VALIDATION OF SITAGLIPTIN AND SIMVASTATIN TABLETS BY USING RP-HPLC METHOD

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Abstract
A simple, precise and accurate RP-HPLC method has been developed for simultaneous estimation of Sitagliptin phosphate and Simvastatin. In RP-HPLC method, mixture of pH 4.0 sodium phosphate buffer and acetonitrile in the ratio of 20:80 v/v was selected as a mobile phase and equal proportions of water and acetonitrile with one drop of phosphoric acid was selected as solvent which gives good resolution and good peak shapes for Stagliptin and Simvastatin. The flow rate was set at 1.0 ml/min, and the detection was carried out with UV detector at 250 nm. Sunfire C18 250 × 4.6 mm, 2.5 µm column was used for the separation. The total run time required was 20 mins. The linearity and range was established over the range of 25-150 µg/ml and 10-60 µg/ml concentration range Stagliptin and Simvastatin. The correlation coefficient of Stagliptin and Simvastatin was found to be 1. The method validation data showed excellent results for accuracy, precision, linearity, specificity, limit of detection, limit of quantification and robustness. The present method can be successfully used for routine quality control analysis.

Key words: Sitagliptin, Simvastatin, RP-HPLC, Validation.

INTRODUCTION

Sitagliptin (Fig. 1) 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.Sitagliptin is a Dipeptidyl peptidase-4 (DPP-4) inhibitor. This enzyme splits the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal[1].

Simvastatin (Fig. 2) is chemically (1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl 1,2,3,7,8,8a hexahydro naphthalen-1-yl 2,2-dimethylbutanoate. It is a hypolipidemic drug used to control elevated cholesterol, or hypercholesterolemia. It is a specific inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes the conversion of HMG-CoA to mevalonate [2].

A survey of literature reveals that very few simultaneous analytical methods were available for determination of Sitagliptin and Simvastatin by using RP-HPLC method[3,4,5,6,7]. This document describes the development and validation, in accordance with ICH guidelines, of simple, economical, precise, rapid, and accurate isocratic reversed-phase HPLC method for analysis of Sitagliptin and Simvastatin in table dosage form.

MATERIALS AND METHODS

Instrument:

Waters HPLC e-module 2695 series consisting 4 pumps and auto sampler with 5 racks, each rack has 24 vials holding capacity with temperature control. Auto injector has capacity to inject 5 µl to 500 µl. UV Visible 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.

Chemicals and reagents:

Sitagliptin and Simvastatin drug samples were obtained from Aurobindo Pharma 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., 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.

Analytical method development:

Chromatographic conditions:

A sunfire C18 column (250 mm x 4.6 mm ID, 2.5 µm) was used for chromatographic separation. The mobile phase composed of acetonitrile and sodium phosphate buffer (80:20 v/v); pH adjusted to 4 with triethylamine. Finally the detection is at a flow rate of 1 ml/min with run time of 25 minutes. 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 250 nm.

Mobile phase preparation:

Mix Sodium phosphate buffer and acetonitrile at 20:80 v/v; pH adjusted to 4 with triethylamine and sonicates the resulting solution and degausses it using vacuum filteration through 0.45 µm membrane filter.

Preparation of standard stock solution:

An accurately weighed quantity of 100 mg of Sitagliptin and 40 mg of Simvastatin were transferred into a 100 ml volumetric flask. Dissolved with 70 ml of diluent and sonicated for 15 minutes and made volume up to the mark with the same solvent.

Standard preparation:

Transfer 10 ml of standard stock solution into 100 ml volumetric flask and dilute to volume with diluent.

Preparation of sample stock solution:

Twenty tablets were weighed and ground to a fine powder. An amount of powder equivalent to 100 mg of Sitagliptin and 40 mg of Simvastatin was weighed accurately and transferred into a 100 ml volumetric flask containing 70 ml of diluent and sonicated for 30 min. and diluted to 100 ml with the same solvent.

Preparation of sample solution:

The sample stock solution was filtered through 0.45 µm membrane filter and 10 ml of the filtrate was taken into 100 ml volumetric flask and made up to the volume with diluent.

Procedure:

The column was maintained at a temperature of 27^0C. The run time was set at 6 minutes. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solutions. 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.

Linearity:

Several aliquots of standard solutions of Sitagliptin and Simvastatin were taken in six different 10 ml volumetric flasks and diluted up to the mark with diluents such that the final concentrations were in the range of 25-150 µg/ml for Stagliptin and 10-60 µg/ml for Simvastatin. The above solutions were injected into the HPLC system keeping the injection volume constant. The drugs were eluted with UV detector at 250 nm, peak areas was recorded for all the pecks. The linearity curves were constructed by plotting concentration of drugs against peak areas. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of drugs in tablet dosage forms.

Precision:

The standard solution of Sitagliptin and Simvastatin was injected for six times and the area for all six injections was measured in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:

To study the accuracy of the method, recovery studies were carried out, by adding a known quantity of the standard to the pre analyzed sample and recovery study were done. The recovery was carried out at 50%, 100%, 150% level and the contents were determined from respective chromatogram.

System suitability:

The system suitability parameters like retention time, theoretical plates and tailing factor were evaluated by six replicate analyses of Sitagliptin and Simvastatin and compared with standard values. The acceptance criteria are %RSD of peak areas not more than 2%, theoretical

plates numbers (N) at least 2000 per each peak and tailing factors not more than 2.0 for Sitagliptin and Simvastatin.

Specificity and Selectivity:

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. There should not be any peak in the chromatogram at the retention time of main analyte in the blank and placebo sample injection.

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:

For demonstrating the robustness of the developed method experimental conditions were purposely altered and evaluated. As part of the Robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

Assay:

Standard preparations are made from the bulk drug and sample preparations are made from commercial formulation. Both standard and sample solutions were injected in six homogeneous samples. 20 µl of sample solution was injected and from the peak areas of Sitagliptin and Simvastatin, amount of each drug in the sample were computed. The results were compared with the label claim of Sitagliptin and Simvastatin in tablet dosage forms. From the results the average %Assay was calculated.

RESULTS AND DISCUSSION

Mobile phase was optimized to separate Sitagliptin and Simvastatin using sunfire C18 column (250 mm x 4.6 mm ID, 2.5 µm). Initially, acetonitrile and sodium phosphate buffer in the ratio of (80:20 v/v) 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 4 with triethyle amine, mobile phase composition (acetonitrile and sodium phosphate buffer in 80:20 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. Under optimum chromatographic conditions, the retention time for Sitagliptin and Simvastatin were found to be 2.959 and 8.397 minutes, respectively when the detection was carried out at 250 nm. A typical chromatogram of two drugs is shown in (Figure 5).

The Linear detector response for Sitagliptin and Simvastatin is demonstrated by concentration versus area. Linearity range is obtained over the range of 25-150 µg/ml and 10-60 µg/ml concentration range for Stagliptin and Simvastatin respectively. The correlation coefficient (r^2) was found to be 1 for both Sitagliptin and Simvastatin respectively. The regression equation of the linearity plot of concentration of Sitagliptin over its peak area was found to be y = 7752.8091x + 1437.4667, where x is the concentration of Sitagliptin (µg/ml) and y is the corresponding peak area. The regression equation of the linearity plot of concentration of Simvastatin over its peak area was found to be y = 28380.736x + 4830.6, where x is the concentration of Simvastatin (µg/ml) and y is the corresponding peak area. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated. The linearity results were shown in Table 2 and the calibration curves were shown in Fig. 3 and Fig. 4.

The %RSD for 6 replicates for Sitagliptin and Simvastatin were found to be 0.095% and 0.082% respectively (limit %RSD<2.0%) and hence the method is precise. The precision data of Sitagliptin and Simvastatin were furnished in Table 3.

The %Recovery of the drugs Sitagliptin and Simvastatin were found to be 97.83 to 99.05% and 98.43 to 99.89% respectively and the high percentage of recovery of Sitagliptin and Simvastatin indicates that the proposed method is highly accurate. The results of accuracy studies of Sitagliptin and Simvastatin were shown in Table 4.

The retention times for the drugs Sitagliptin and Simvastatin was 2.959 minutes

and 8.397 minutes respectively. The number of theoretical plates calculated for Sitagliptin and Simvastatin was 3782 and 4311 respectively. The tailing factor for Sitagliptin and Simvastatin was 1.24 and 1.72 respectively, which indicates efficient performance of the column. The limit of detection (LOD) and limit of quantification (LOQ) for Sitagliptin were found to be 0.3132 µg/ml and 0.9776 µg/ml; 0.1096 µg/ml and 0.342 µg/ml for Simvastatin respectively, which indicate the sensitivity of the method. The summary of system suitability parameters and validation parameters were shown in Table 5.

The robustness studies indicated that no considerable effect on the determination of the drugs. Therefore the test method is robust for the quantification of the drugs. In all deliberately varied conditions, the % RSD for replicate injections of Sitagliptin and Simvastatin were found to be within the acceptable limits.

Validated method was applied for the simultaneous estimation of Sitagliptin and Simvastatin in commercial tablet dosage forms. The %Assay of Sitagliptin and Simvastatin were found to be 99.29% and 100.40% respectively. The results for the drugs assay showed good agreement with label claims. No interfering peaks were found in the chromatogram of the tablet formulation within the run time indicating that excipients used in tablet formulation did not interfere with the simultaneous estimation of the drugs Sitagliptin and Simvastatin by the proposed HPLC method. The assay results are shown in Table 6.

The chromatograms were checked for appearance of any extra peaks under optimized conditions, showing no interference from common tablet excipients and impurities. Also the peak areas were compared with standard and were found to be within limits. As shown in chromatogram, two analytes are eluted by forming symmetrical peaks. The typical chromatogram of Sitagliptin and Simvastatin standard were shown in Fig. 5.

CONCLUSION

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

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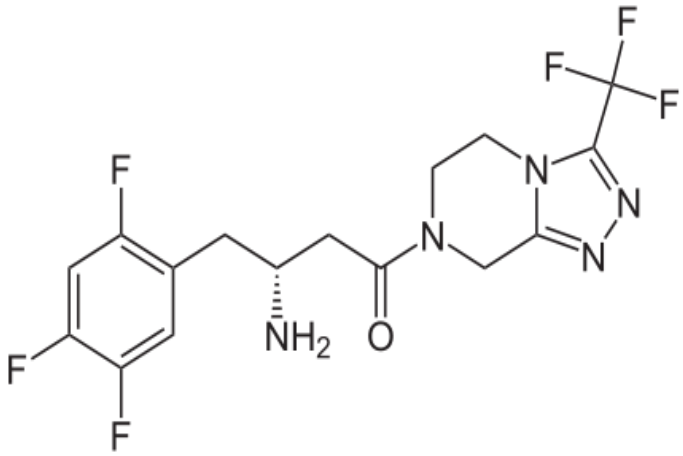

Fig. 1: Molecular structure of Sitagliptin

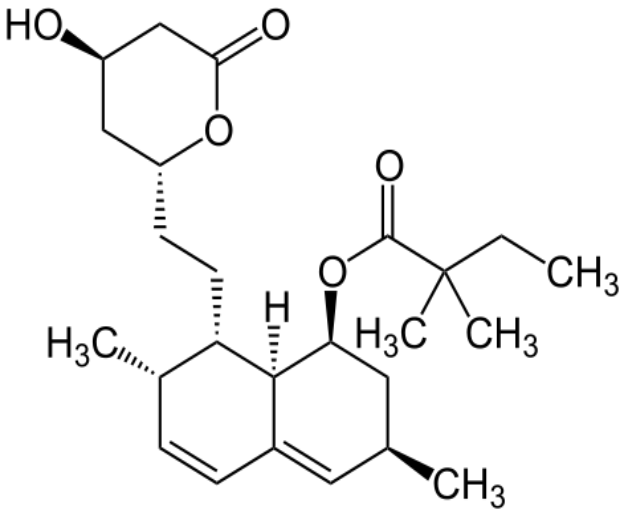

Fig. 2: Molecular structure of Simvastatin

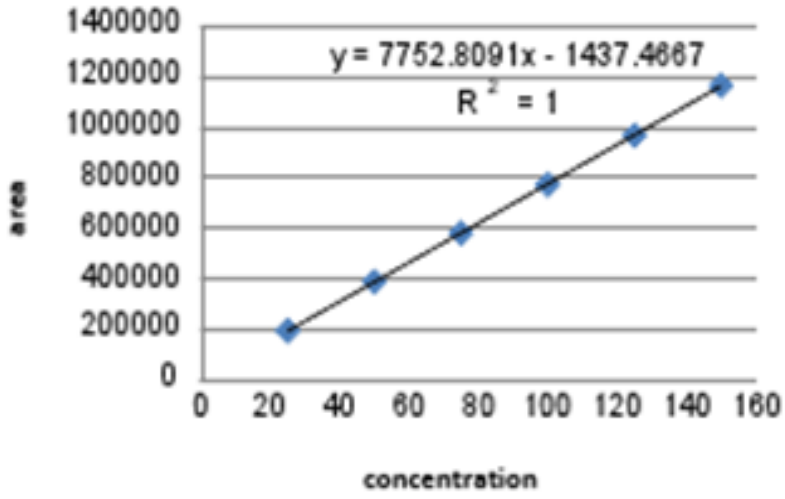

Fig. 3: Calibration curve of Sitagliptin

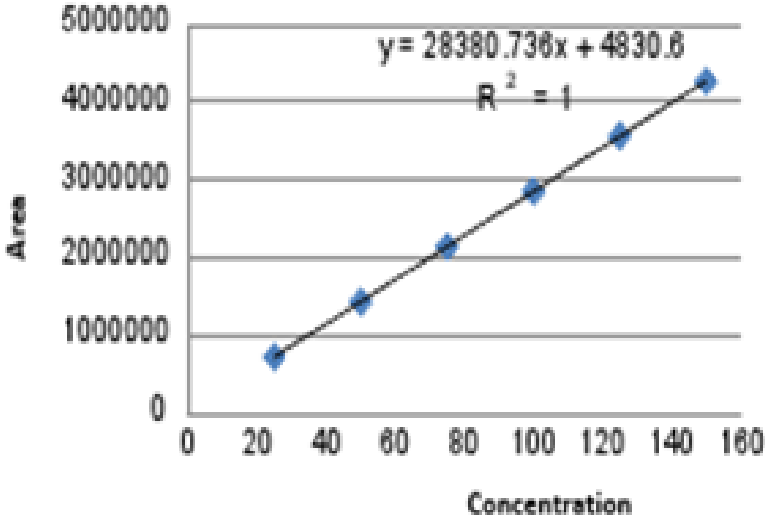

Fig. 4. Calibration curve of Simvastatin

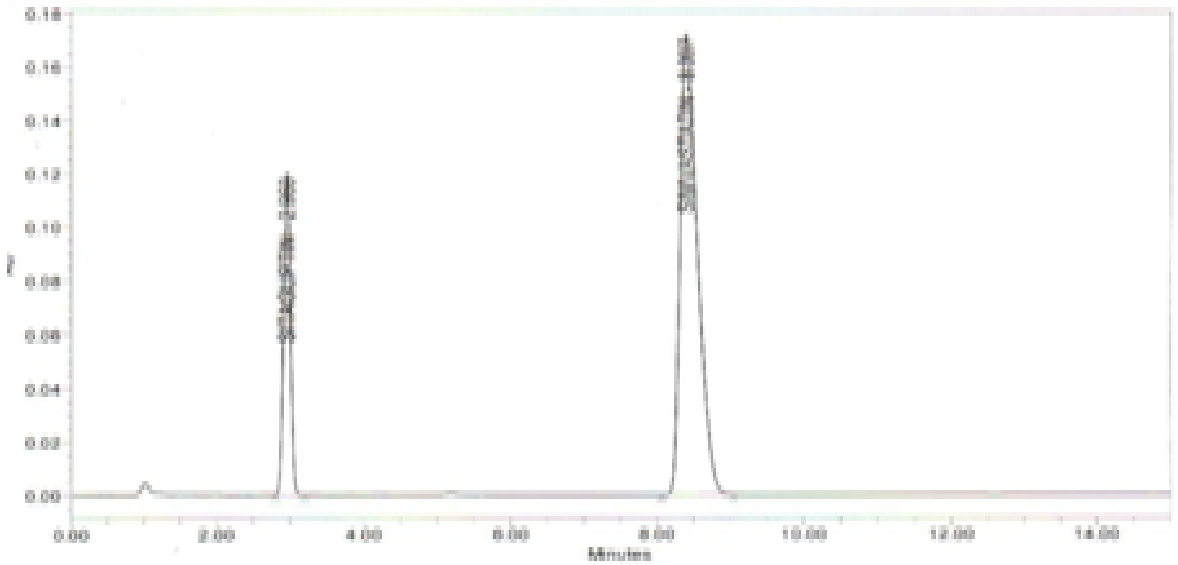

Fig. 5: Typical chromatogram of standard for Sitagliptin and Simvastatin

Table 1: Optimized chromatographic conditions

Mobile phase	Mixture of pH 4.0 sodium phosphate buffer and acetonitrile (20:80, v/v)
Column	sunfire C18 column (250 mm x 4.6 mm ID, 2.5 µm)
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	250 nm
Injection volume	20 µl
Run time	20 minutes
Retention time	2.959 min for Sitagliptin and 8.397 min for Simvastatin

Table 2: Linearity results of Sitagliptin and Simvastatin

S. No.	Sitagliptin		Simvastatin	
	Concentration (µg/ml)	Mean peak area	Concentration (µg/ml)	Mean peak area
1	25	193631	10	712736
2	50	386751	20	1423518
3	75	579136	30	2133594
4	100	771393	40	2844268
5	125	966793	50	3558533
6	150	1163896	60	4256221

Table 3: Precision data of Sitagliptin and Simvastatin

S. No.	Sitagliptin		Simvastatin	
	Rt (min.)	Area	Rt (min.)	Area
1.	2.948	772757	8.416	2844001
2.	2.951	770897	8.416	2846482
3.	2.950	771282	8.413	2843713
1.	2.951	771642	8.407	2846798
2.	2.954	772635	8.407	2848548
3.	2.954	771956	8.405	2849576

Avg.	2.95133	771861.5	8.410666	2846519.666
S.D	0.00234	738.0262190	0.0049261	2356.066948
%RSD	0.0792215	0.095616405	0.05856992	0.082770092

Table 4: Accuracy studies of Sitagliptin and Simvastatin

% Concentration level	Sitagliptin			Simvastatin		
	Conc. added (µg/ml)	Conc. found (µg/ml)	% Recovery	Conc. added (µg/ml)	Conc. found (µg/ml)	% Recovery
50%	50	49.45	98.90%	50	49.69	99.39%
100%	100	97.83	97.83%	100	98.43	98.43%
150%	150	148.57	99.05%	150	149.85	99.89%

Table 5: System suitability parameters of proposed method

S. No.	Parameters	Sitagliptin	Simvastatin
1	Linearity (µg/ml)	25-150	10-60
2	Correlation coefficient	1	1
3	Retention time (min.)	2.959	8.397
4	Tailing factor	1.24	1.72
5	Theoretical plates (N)	3782	4311
6	LOD (µg/ml)	0.3132	0.1096
7	LOQ (µg/ml)	0.9776	0.342

Table 6: Assay results of marketed formulations

	Sitagliptin		Simvastatin	
	Standard Area	Sample Area	Standard Area	Sample Area
Injection-1	758567	750426	2762521	2762082
Injection-2	758563	750424.5	2762518	2762080
Injection-3	758558	750423	2762511	2762072
Average Area	758562.66	750424.5	2762516.67	2762080
Tablet avg. weight	300		300	
Standard weight	100		40	
Sample weight	298		298	
Label amount (mg)	100		40	
Std. purity	99.7		99.75	
Amount found (mg)	99.29		40.16	
Assay (%purity)	99.29		100.40	