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ORIGINAL RESEARCH ARTICLE

Development of UV-SPectrophotometry and RP-HPLC Method and Its Validation for Simultaneous Estimation of Sitagliptin Phosphate and Simvastatin in Marketed Formulation

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ABSTRACT

Two methods are described for the determination of Sitagliptin Phosphate and Simvastatin in binary mixture. The first method was based on spectrophotometric determination of two, using simultaneous equation method. It involves absorbance measurement at 267.0 nm (λ_{max} Sitagliptin Phosphate) and 238.0 nm (λ_{max} Simvastatin) in methanol: water in a ratio of 90:10(v/v); linearity was obtained in the range 10-50 µg/ml and 5 –25µg/ml for both the drugs respectively. The second method was based on separation of the two in reverse phase mode using Cosmosil C_{18} column. Linearity was obtained in the concentration range 50-250µg/ml for Sitagliptin and 10-50 µg/ml for Simvastatin. Both these methods were validated according to ICH guidelines and can be successively applied to pharmaceutical formulation.

Key words: Sitagliptin, Simvastatin, UV-Spectrophotometry, Reverse Phase HPLC.

INTRODUCTION

Sitagliptin (STG), [(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] (**Fig 1**) is a well known hypoglycemic drug. STG is a novel oral hypoglycemic drug of the dipeptidyl peptidase 4 inhibitor class[1]. Sitagliptin increased incretin levels (GLP-1 and GIP) which inhibit glucagon release, in turn decreases blood glucose, but more significantly increases insulin secretion[2].The determination of STG has been carried out in tablet by RP-HPLC by UV Spectrophotomerty[3], RP-HPLC [4], UPLC [5], Laser diode thermal desorption tendem mass spectrometry [6], capillary electrophoresis [7].

Simvastatin (SMV), 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(**Fig 2**). It acts by inhibiting HMG CoA reductase and is used for the treatment of hypercholesterolemia. After oral administration, this prodrug 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 [2].

The determination of Simvastatin has been carried out in tablets by UV-Spectrophotometry [8-14], RP-HPLC [15,16], HPLC[17], HPTLC [17].

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. 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.

Fig 1: Structure of Sitagliptin

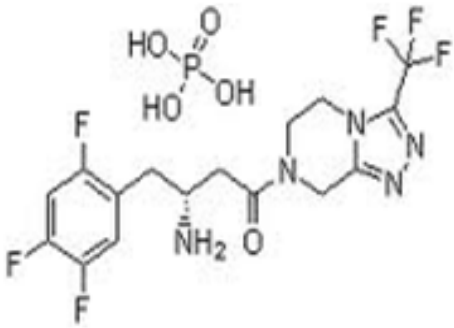

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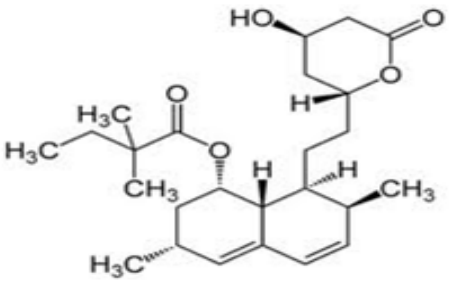
Fig 2: Structure of Simvastatin



MATERIALS AND METHODS

Reagents

Sitagliptin phosphate, Simvastatin, pharmaceutical preparation of combination of Sitagliptin phosphate and Simvastatin is Juvisync tablet, Methanol of analytical grade, distilled water Ammonium di hydrogen orthophosphate, Acetonitrile and water (HPLC grade) and ortho phosphoric acid (GR grade). Commercial samples of tablets containing the drugs were purchased from the local pharmacy.

Equipments and apparatus

Spectral runs were made on Systronics (Double beam) UV-Visible spectrophotometer, model- 108 of 1 cm matched quartz cell having band width of 0.1 nm to develop analytical method over the range of 200-400 nm.

Different kinds of equipments LIKE Analytical weighing balance, HPLC system (YL9100 with YL Clarity Software), Injector (Rheodyne,20µl), Sonicator, pH meter, Vacuum filter pump, Millipore filtration kit, mobile phase reservoir, Water bath, Sample filtration assembly and glassware's were used throughout the experiment.

UV- Spectrophotometry

Selection of common solvent

Methanol of analytical reagent grade was selected as common solvent for developing spectral characteristics of drug. The selection was made after assessing the solubility of both the drugs in different solvents.

Preparation of Standard Stock Solution

Standard stock solutions containing Sitagliptin phosphate (STG) and Simvastatin (SMV) were prepared individually by dissolving 10 mg of STG and 10 mg of SMV separately in 3 ml of methanol. Then sonicated for 15 minutes and final volume of both the solutions were made up to 10 ml with methanol to get stock solutions containing 1000 µg/ ml each of STG and SMV in two different 10 ml volumetric flasks.

Procedure

Determination of Absorption Maxima

By appropriate dilutions of two standard drug solutions with methanol, solutions containing 10 µg/ ml of STG and 10 µg/ ml of SMV were scanned separately in the range of 200- 400 nm to determine the wavelength of maximum absorption for both the drugs. STG and SMV showed absorbance maxima at 267 nm (λ_1) and 238 nm (λ_2) respectively. The overlain spectra showed λ_{max} of both drugs and also isoabsorptive points at 252 nm (**Fig 3**).

Simultaneous equation method

Two wavelengths selected for the method are 267 nm and 238 nm that are absorption maximas of STG and SMV respectively in methanol. The stock solutions of both the drugs were further diluted separately with methanol to get a series of standard solutions of 10-50 µg/ml and 5-25 µg/ml concentrations of STG and SMV respectively. The absorbances were measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs at both wavelengths were determined as mean of three independent determinations. Concentrations in the sample were obtained by using following equations-

$$C_x = \frac{A_1 ay_2 - A_2 ay_1}{ax_1 ay_2 - ax_2 ay_1} \quad \text{.....Eq. (i)}$$

$$C_y = \frac{A_1 ax_2 - A_2 ax_1}{ay_1 ax_2 - ay_2 ax_1} \quad \text{.....Eq. (ii)}$$

Where, A_1 and A_2 are absorbances of mixture at 208 nm and 237.5 nm respectively, ax1 and ax2 are absorptivities of STG at λ_1 and λ_2 respectively and ay_1 and ay_2 are absorptivities of SMV at λ_1 and λ_2 respectively. Cx and Cy are concentrations of STG and SMV respectively.

Application of the proposed method for the determination of STG and SMV in tablets:

Twenty tablets of marketed formulation Juvisync tablet (Merck & Co., India) containing STG 100 mg and SMV 20 mg were weighted, and finely powdered. Tablet powder equivalent to 100 mg sitagliptin (which will contain SMV equivalent to 20 mg) was weighed and transferred to a 100 ml volumetric flask and volume was made up to 100 ml with diluent (Methanol : water 90:10 v/v) to obtain concentration of 1000µg/ml. Resultant solution was filtered through Whatmann filter paper. 1 ml of filtrate was taken in 10 ml volumetric flask and volume was made up to 10 ml with diluent to obtain concentration of 100µg/ml. appropriate aliquots of STG and SMV within the Beer's law limit was taken. Absorbance of the sample solutions at 267.0 nm and 238.0 nm was measured and from the absorbance values, the concentration of drugs in the sample solution was determined by using Vierodt's formula.

Table 1: linear regression analysis of calibration curves with their respective absorptivity values.

Parameters	STG	SMV
Beer's law limit (µg/ ml)	10-50 (µg/ ml)	5-25 (µg/ ml)
Correlation coefficient (r)	0.986	0.998
Molar absorptivity (lit/mole/cm)	1.857×10^4	2.038×10^4
Sandell's sensitivity (mcg/ Sq.cm/0.001)	3.5×10^{-6}	4.97×10^{-5}
Slope	0.0412	0.049
Intercept	-0.0317	-0.001

Table 2: Results of analysis of tablet samples.

Brand name	Sitagliptin		Simvastatin	
	Label Claim (mg)	% Purity*	Label Claim (mg)	% Purity*
Juvisync	100	99.5	20	99.8

HPLC METHOD

Chromatographic conditions

Analysis was carried at 235nm using a ODS Cosmosil C_{18} reverse phase column of 250x 4.60mm i.d., 5µm dimensions at ambient temperature. The mobile phase consisted Ammonium dihydrogen orthophosphate: ACN (Ph3 with OPA) in the ratio 50:50v/v that was set at a flow rate of1.0ml/min.

Preparation of Mobile phase

Mobile phase is prepared by mixing 500ml of Dihydrogen orthophosphate and 500ml of Acetonitrile. (50:50). The mobile phase is then sonicated using Ultra-Sonicator to remove the impurities and dissolved gases, as they may lead to unwanted peaks in the chromatogram.

Diluent Preparation

Use the mobile phase as diluent.

Preparation of standard solution

10mg of Sitagliptin and 10mg of Simvastatin was weighed accurately and transferred to separate 10ml volumetric flask, and the volume was adjusted to the mark with the mobile phase (Ammonium dihydrogen orthophosphate : ACN (Ph3 with OPA)in the ratio of 50:50) to give a stock solution of 1000ppm.

Preparation of working standard solution

From stock solutions of Sitagliptin 1 ml was taken and diluted up to 10 ml. from this solution 5.0, 10.0, 15.0, 20.0, 25.0 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 100 ml with mobile phase, gives standard drug solution of 50, 100, 150, 200, 250 µg/ ml concentration. And from stock solutions of Simvastatin 1.0, 2.0, 3.0, 4.0, 5.0 ml solutions were transferred to 100 ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 10, 20, 30, 40, 50 µg/ ml concentration

Preparation of sample solution

For analysis of the Tablet Formulation, equivalent to weight 100 mg of Sitagliptin was transferred to 100 ml volumetric flask and dissolved in HPLC grade methanol. The solution was shaking vigorously for 10 mins and filtered through Whatmann filter paper no.41, then volume was made up to mark with methanol. From the above solution 0.5 ml of solution was taken and diluted to 100 ml with mobile phase (Ammonium Dihydrogen orthophosphate : ACN (Ph3 with OPA)in the ratio of 50**:**50) to get a solution containing 50 µg/ml of Sitagliptin and corresponding concentration of Simvastatin 10 µg/ml. The solution contains Sitagliptin and Simvastatin in the proportions of 50:10. The amounts of Sitagliptin and Simvastatin were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with Tablet formulation

METHOD VALIDATION

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 50 to 250 µg/ml and 10 to 50 µg/ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve) and the standard calibration curve of the drugs are shown From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration.

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

Repeatability

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis.Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out.

Robustness

As per ICH norms, small, but deliberate variations, by altering the pH and / or concentration of the mobile phase were made to check the method capacity to remain unaffected. The effect of change in pH of mobile phase, flow

rate, mobile phase ratio on the retention time, theoretical plates, area under curve and percentage content of Sitagliptin and Simvastatin were studied.

Table 3: Results of Analysis of Formulation

Parameters	STG	SMV
Label Claim	100	20
Drug content[a]	100.05	100.48
± S.D[b]	0.1069	0.0.561
% R.S.D[c]	0.1068	0.5592

**Each reading is mean reading of three batch of formulation*

Table 4: Recovery Studies

Drug	% of raw material added	Recovery[a]	% R.S.D
STG	80	99.96	0.451
	100	100.3	0.622
	120	99.88	0.196
SMV	80	99.37	0.628
	100	100	1.000
	120	99.97	0.455

Table 5: System Suitability Parameters

Parameters	Sitagliptin phosphate	Simvastatin
Tailing factor	1.173	1.20
Theoretical Plates	2768	2574
AUC	20985.11	1425.255
Retention Time	8.1033	5.471

Table 6: Summary of % R.S.D Values of Repeatability, Precision and Ruggedness

Parameter	STG	SMV
Repeatability[a]	0.001	0.194
Precision		
Intra day[b]	0.189	0.8107
Inter day[b]	1.155	1.662
Ruggedness		
Analyst 1[c]	0.028	0.017
Analyst 2[c]	0.014	0.026

Table 7: Result of Robustness of Formulation

Compound	% RSD in Normal	Changed Condition n= 6	
Temperature		- 5 °C	+ 5 °C
SITA		0.089	0.097
SIM	0.045	0.087	0.095
Flow rate		(-10%)	(+10%)
SITA	0.067	0.123	0.114
SIM	0.078	0.141	0.145
Mobile phase ratio		- 2 %	+ 2 %
SITA	0.34	0.88	0.25
SIM	0.49	0.43	0.22

Fig 3: Overlay Spectra of Simvastatin and Sitagliptin

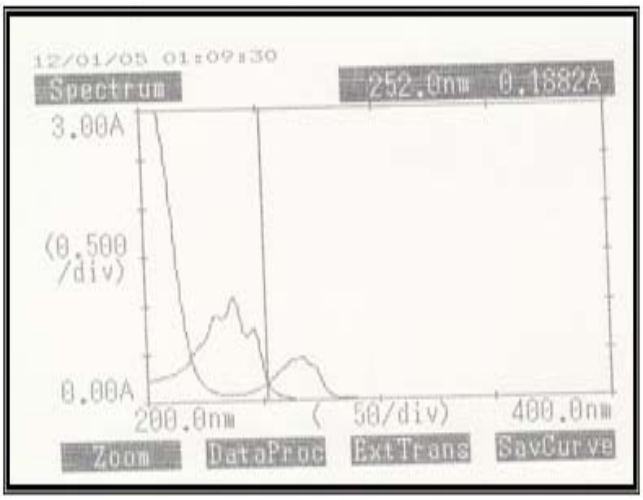


Fig 4: Calibration Graph of Sitagliptin

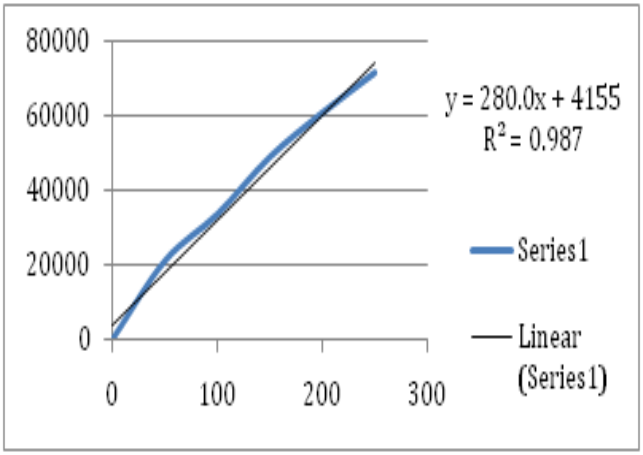


Fig 5: Calibration Graph of Simvastatin

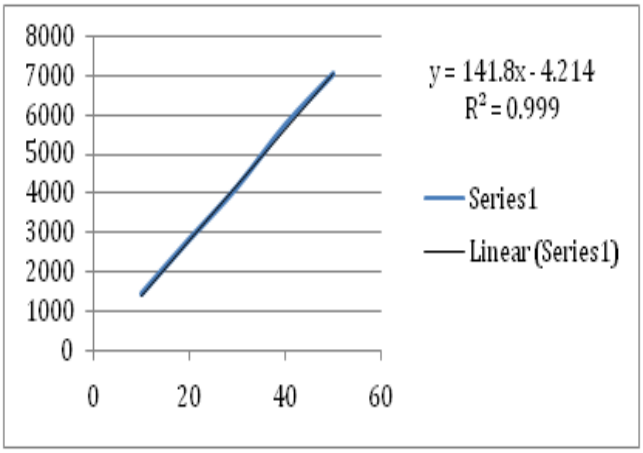


Fig 6: Chromatogram of Sitagliptin

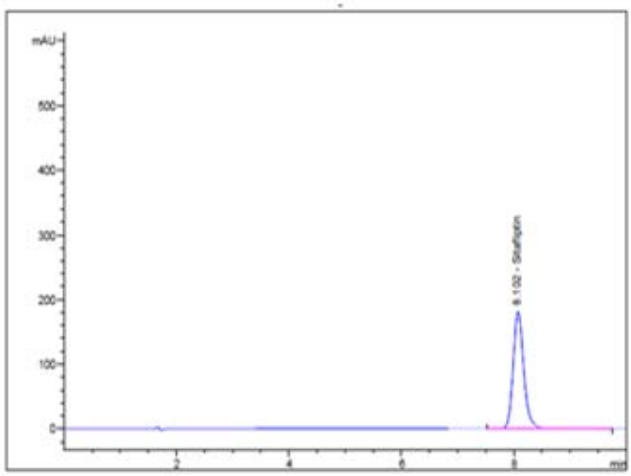


Fig 7: Chromatogram of Simvastatin

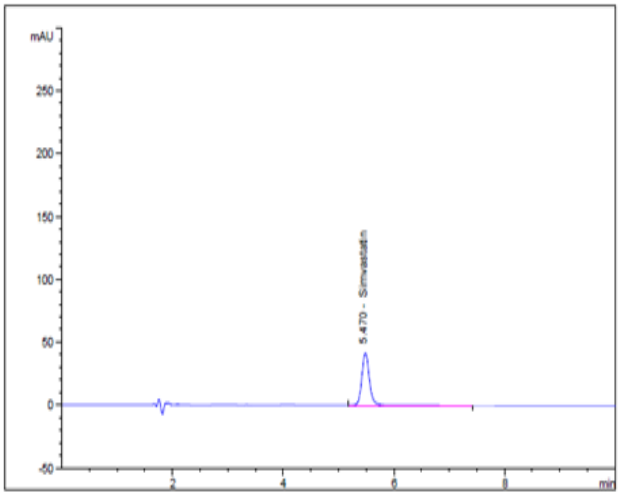
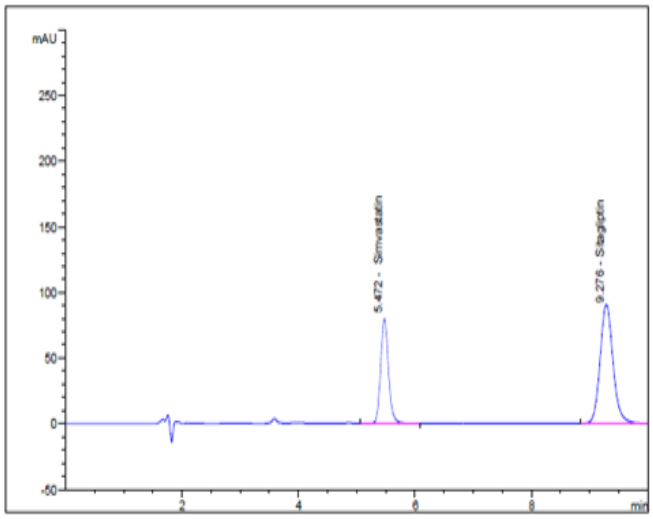


Fig 8: Chromatogram of Sitagliptin and Simvastatin



CONCLUSION

As the drug combination is available in market, hence, the research article is towards the method development for analysis. The Spectrophotometry provides versatile techniques for analyse drug in multi component pharmaceutical formulation in presence of various interferences as simple, specific, linear, precise and accurate while RPHPLC method has been developed and validated for quantitative determination of Phosphate and Simvastatin in new tablet formulation. The proposed methods are accurate, simple, rapid and selective for the simultaneous estimation of Phosphate and Simvastatin in bulk and in tablet dosage form by external standard calibration method.

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