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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF SITAGLIPTIN PHOSPHATE AND SIMVASTATIN IN TABLET DOSAGE FORM

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ABSTRACT

A simple and accurate method of analysis to determine Sitagliptin Phosphate (STG) and Simvastatin(SMV) in combined dosage forms was developed using simultaneous equation method at 267.0nm for Simvastatin and 238.0 nm for Simvastatin in spectra of their solutions in methanol: water(90:10). The calibration curves were linear [correlation coefficient (r) = 0.986 for STG and 0.998 for SMV] in concentration range of 10-50 µg/ml for STG and 5-25 µg/ml for SMV. The method was validated and found to be accurate, precise, and specific. The method was successfully applied to the estimation of STG and SMV in combined dosage tablet formulations.

Keywords: Simvastatin, Sitagliptin Phosphate, λmax, Simultaneous equation method.

INTRODUCTION

Sitagliptin Phosphate (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] 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. It acts by inhibiting HMG CoA reductase and is used for the treatment of hypercholesterolemia. After oral administration, this prodrug is converted into ß 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.

MATERIALS AND METHODS

Apparatus: Spectral runs were made on Shimadzu (Double beam) UV-Visible spectrophotometer, model- 1700 of 1 cm matched quartz cell having band width of 0.1 nm to develop analytical method over the range of 200-400 nm.

Reagents and Materials: Analytically pure Sitagliptin phosphate was procured from Bioplus life science, Bangalore while Simvastatin was provided by Ranbaxy, Dewas.

The pharmaceutical preparation of combination of Sitagliptin phosphate and Simvastatin is Juvisync tablet (Merck & Co., India). Methanol of analytical grade was purchased from Merck & Co. All the solutions were protected from light and were analyzed on the day of preparations. Glasswares used in each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven.

***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) and were prepared individually by dissolving 10 mg of STG and 10 mg of SMV separately in 3ml of methanol. It was then sonicated for 15 minutes and the 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.

Determination of Absorption Maxima: By appropriate dilution of two standard drug solution 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 lmax of both drugs and also isoabsorptive points at 252 nm (Figure 1).

***Simultaneous equation method*:** Two wavelengths selected for the method are 267 nm and 238 nm that are absorption maxima 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-

$$Cx = A_1\, ay_2 - A_2\, ay_1 \quad \text{.....Eq. (i)}$$
$$Ax_1\, ay_2 - ax_2\, ay_1$$
$$Cy = A_1\, ax_2 - A_2\, ax_1 \quad \text{.....Eq. (ii)}$$
$$Ay_1\, ax_2 - ay_2\, ax_1$$

Where, A_1 and A_2 are absorbances of mixture at 208 nm and 237.5 nm respectively, ax_1 and ax_2 are absorptivities of STG at λ_1 and λ_2 respectively and ay_1 and ay_2 are absorptivities of SMV at $\lambda_1$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.

Method Validation: The method was validated for accuracy, precision, specificity, and robustness by the following procedures.

Accuracy: The accuracy of the method was determined by calculating recoveries of STG & SMV by the methods of standard additions.

This study was performed by addition of known amounts of Sitagliptin and Simvastatin to a known concentration of the commercial tablets. The amount of standard recovered was calculated in the terms of mean recovery with the upper and lower limits of percent relative standard deviation.

Precision: The experiments were repeated for three times a day for intraday precision and on three different days for inter day precision. The developed

method was found to be precise for intraday and inter day precision on the basis of % RSD values for Sitagliptin and Simvastatin.

RESULTS AND DISCUSSION

The overlain spectra of STG and SMV exhibit lmax of 267 nm and 238 nm for STG and SMV respectively which are quite separated from each other. Additionally one isoabsorptive point was observed at 252 nm.

This wavelength was selected for simultaneous estimation of STG and SMV and it is assume to be sensitive wavelength. Standard calibration curves for STG and SMV were linear with correlation coefficients (r) values in the range of 0.986- 0.998 at all the selected wavelengths and the values were average of three readings with standard deviation in the range of 0.002 – 0.003. The method was repeated for three different days and average % RSD was found to be 0.13 for STG and 0.39 for SMV.

CONCLUSIONS

The developed spectrophotometric method is simple, accurate, precise and reliable for the simultaneous estimation of Sitagliptin Phosphate and Simvastatin in combined dosage form. The relative std. deviation (RSD) for all parameters was found to be less than one, which indicates the validity of method and assay results are also within the limit so the proposed method can be used for routine quantitative simultaneous estimation of both the drugs in multi-component pharmaceutical preparation.

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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/mol/cm)	1.857×10^4	2.038×10^4
Sandell's sensitivity(mcg/Sq.cm/0.001)	3.5×10^{-6}	4097×10^{-5}
Slope	0.0412	0.049
Intercept	-0.0317	-0.001

Table 2: Results of analysis of tablet samples

Brand name	Sitagliptin Label Claim (mg)	% Purity*	Simvastatin Label Claim (mg)	% Purity*
Juvisync	100	99.8	20	99.1

Table 3: Statistical Validation of Recovery Studies

Level of Recovery (%)	Drug	% Recovery	Standard Deviation*	% RSD
80	**STG**	101.17	2.013	1.989
	SMV	95.96	4.080	4.251
100	**STG**	100.13	0.498	0.497
	SMV	100.0	1.632	1.632
120	**STG**	99.88	0.160	0.160
	SMV	97.22	3.357	3.452

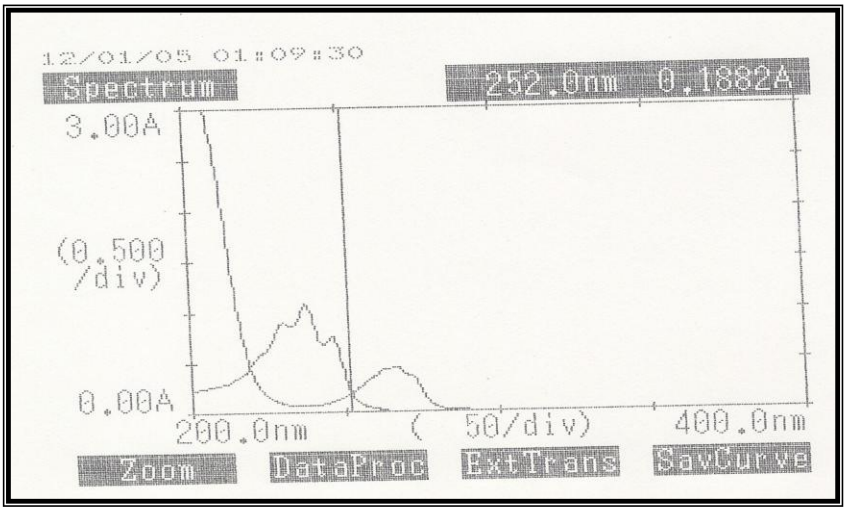

Figure 1: Determination of Absorption Maxima

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