SIMULTANEOUS ESTIMATION OF SIMVASTATIN AND SITAGLIPTIN BY USING DIFFERENT ANALYTICAL METHODS

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Abstract
Simple accurate and precise Spectrophotometric methods have been developed for simultaneous estimation of Simvastatin (SIM) and Sitagliptin (SITA) by employing three methods. Method A was simultaneous equation method; the method involves formation and solving the simultaneous equation using 238 nm and 267 nm as two wavelengths for SIM and SITA respectively. Method B was first order derivative spectrophotometry. The first order derivative absorption at 230 nm (zero crossing point of SITA) was used for SIM and 275nm (zero crossing point of SIM) was used for SITA. Method C involved Q-absorption analysis based on the measurement of absorbance at two wave lengths that is the \( \lambda_{max} \) of SITA 267 nm and isoabsorptive point of both drugs at 250 nm. The three methods obeyed the Beer’s law in the concentration range of 3-15 µg/ml for SIM and 50-150 µg/ml for SITA. The methods were validated statistically and recovery studies were carried out to confirm the accuracy of the method.

Keywords: Simvastatin, Sitagliptin, Simultaneous Equation Method, First Derivative Spectrophotometry, Q-Absorption Analysis

1. Introduction
1.1. Sitagliptin: SITA is the first of a new class of drugs for the treatment of type two diabetes, chemically it is known as (R)-4-oxo – 4- [3-(triflouromethyl) -dihydro [1,2,4]triazolo [4,3-a]pyrazine -7(8H)-yl ]-1-(2,4,5-trifloro phenyl )butan-2-amine. It reduces blood glucose concentrations by enhancing the effect of incretins. Incretins are hormones (chemicals) which are produced by the (bowel) in response to food. These drugs are therefore also known as incretin enhancers. SITA can be estimated by different analytical and bio-analytical techniques, they are first order derivative ¹, simultaneous estimation², UPLC³, Spectrophotometry⁴, bio-analytical⁶, mass spectrometry⁷, spectrofluorimetry⁸.

1.2. Simvastatin: SIM belongs to a class of drugs called HMG-coA reductase inhibitors commonly called statins. It is chemically known as (1S, 3R, 7S, 8S, 8aR)-8-[(2r, 4r)-4-hydroxy-6-oxotetrahydro-2H-pyran-2yl] ethyl]-3, 7-dimethyl-1, 2, 3, 7, 8,8a-hexahydropyridazin-1-yl2, 2-dimethyl butanoate. All statins act by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A HMG-CoA reductase, the rate limiting enzyme of the HMG-CoA reductase pathway, the metabolic pathway responsible for the endogenous production of cholesterol mainly used for the treatment of dislipidimia and the prevention of cardiovascular diseases. SIM can be estimated by different analytical techniques, they are RP-HPLC⁹,¹⁰,¹¹,¹², derivative Spectrophotometry¹³, spectrophotometry¹⁴,¹⁵, second order derivative¹⁶, LC-MS/MS¹⁷.

2. Experimental
2.1. Materials (Instrumentation): LABINDIA UV 3092 UV-Visible double beam spectrophotometer with a fixed slit width 1 nm and 1 cm matched quartz cells was used for all the spectral measurements.

2.2. Reagents: Analytical grade Alcohol, distilled water.

2.3. Preparation of standard stock solutions:
A standard SIM stock solution (100µg/ml): Standard SIM (10 mg) was dissolved in 10 ml of alcohol. The solution was further diluted with water to obtain 100 µg/ml.

A standard SITA solution (100µg/ml): Standard SITA 10 mg was sonicated in 10 ml of alcohol. The solution was diluted with water to obtain 100 µg/ml.

Standard stock solution SITA solution (100µg/ml): Standard SITA 10 mg was sonicated in 10 ml of alcohol. The solution was diluted with water to obtain 100µg/ml.

2.4. Sample preparation: 10 tablets were taken and accurately weighed and weight equalent to 10 mg of SIM was taken and dissolved in 10 ml of alcohol.

2.5. Method A
Simultaneous Equation Method: For selection of analytical wavelength for Simultaneous Equation Method; standard solutions of SIM (100 µg/ml) and SITA (100 µg/ml) were scanned in the range of 220 to 400 nm. Fig C
represents the overlain spectrum of both drugs. Wavelengths 238 and 267 nm i.e. the $\lambda_{max}$ of SIM and SITA respectively for the simultaneous equation (fig A & B). All the solutions were measured at both the wavelengths 238 and 267 nm respectively. Absorptivites at each wavelength for SIM and SITA were determined and used to form the equation, the absorbance and the absorptivites values at particular wavelengths were submitted in the following equation to obtain the concentration.

$$
C_x = \frac{(A_2y_1 - A_1y_2)}{(ax_2 - ay_1)}
$$

$$
C_y = \frac{(A_1ax_2 - A_2ax_1)}{(ax_2ay_1 - ax_1ay_2)}
$$

$C_x$ = concentration of SIM

$C_y$ = concentration of SITA

$A_1$ = absorbance of samples at 238 nm.

$A_2$ = absorbance of samples at 267 nm.

$ax_1$ is the absorbivitiy of SIM at 238 nm.

$ax_2$ is the absorbivitiy of SIM at 267 nm.

$ay_1$ is the absorbivitiy of SITA at 238 nm.

$ay_2$ is the absorbivitiy of SITA at 267 nm.

2.6. Method B: Standard stock solutions (100 $\mu$g/ml) of SIM and SITA were prepared separately with water to obtain concentration range of 3-15 $\mu$g/ml for SIM and 50-150 $\mu$g/ml for SITA. For all solutions the derivative spectra were obtained over 220-400 nm range. From the overlain spectrum of both the drugs (Fig.1) wavelengths were selected. At 230 nm there were well developed first order derivative absorption spectra for varying concentrations of SIM for its determinations (fig.2) and no interference was observed by SITA as $D_1=0$ (fig.2a) so any change in SITA concentration has no effect on quantitative determination of SIM.

To determine sita the first order derivative spectra were used by making measurement at 275 nm (fig3) at which $D_1=0$ for SIM. NO SIM interference was found even at different concentrations (fig3a) for quantitative determination of SITA. The calibration curves were constructed by plotting drug concentration versus absorbance values of first derivative spectrum ($D_1$) 230 nm for SIM and 275 nm for SITA. Statistical data for calibration curve is depicted in (Table 1). The concentration of individual drugs present in the mixture (Fig 4) was determined from the calibration curve in quantization method.

2.7. Method C: Absorbance Ratio method: The working standard stock solutions of SIM and SITA were scanned in the range of 220-400 nm against water as blank. Isoabsorptivity point was found at 250 nm (Fig C) and another wavelength used was 267 nm which is $\lambda_{max}$ of SITA calibration curve was plotted over a concentration range of 3-15 $\mu$g/ml for SIM and 50-150 $\mu$g/ml for SITA. The absorbance of each solution was measured at both the wavelengths 250nm and 267 nm. Concentrations of SIM and SITA were determined using the following simultaneous equation.

$$
C_x = \frac{(QM-QY)*A_1}{(QX-QY)*ax_1}
$$

$$
C_y = \frac{A_1}{ax_1} - C_x
$$

Where $A_1$ and $A_2$ are absorbance’s of the mixture at 250 nm and 267 nm respectively; $ax_1$ and $ay_1$ are absorptivites of SIM and SITA respectively at 250 nm and $ax_2$ and $ay_2$ are absorptivites of SIM and SITA respectively at 267 nm ;

$$
QM = \frac{A_2}{A_1}, \quad QX=\frac{AX_2}{AX_1} \quad AND \quad QY=\frac{AY_2}{AY_1}.
$$

3. Results and Discussion

3.1. Validation:

3.1. A. Linearity range: Calibration curves constructed were linear over the selected range of 3-15 $\mu$g/ml and 50-150 $\mu$g/ml for SIM and SITA respectively. Each concentration was repeated three times. The assays were performed according to experimental conditions and the linearity of the calibration graphs were validated by the high value of the correlation coefficient and the intercept value.

3.1. B. Accuracy: Accuracy of the methods was assured by standard addition technique involving analysis of samples to which certain amounts of authentic drugs were added. The resulting mixtures were assayed and compared with those expected. Recovery studies were given in (Table 2).

3.1. C. Precision: For evaluations of precision repeatability of the results were evaluated by 6 replicate determinations. The coefficient of variations (%CV) values at these concentration levels were calculated.

3.1. D. Limit of Detection & Limit of Quantification: Limit of detection for SIM and SITA were found to be 0.121$\mu$g/ml, 0.213$\mu$g/ml and 0.314$\mu$g/ml. Limit of quantification for SIM and SITA were found to be 0.265$\mu$g/ml, 0.456$\mu$g/ml, 0.654$\mu$g/ml.

3.1. E. Application to the pharmaceutical dosage form: The proposed methods were validated successfully to determine SIM and SITA in tablets. No interference of excipients were made, therefore the proposed methods were employed for simultaneous estimation of SIM and SITA.
Conclusion
The proposed methods are simple accurate and precise to determine the quantitative estimation of SIM and SITA.

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Fig A. spectrum of SIM at 238 nm.

Fig B. spectrum of SITA at 267 nm

Fig C: overlaid spectra of SIM and SITA showing iso-absorptive point at 275 nm

Fig 1: First order derivative overlain spectra of SIM (SIM, 6 µg/ml) and SITA (SITA 75 µg/ml)

Fig 2: First order derivative spectra for SIM (3, 6, 9, 12 and 15 µg/ml) at 275 nm

Fig 2a: First order derivative spectra for SITA (75 µg/ml) and SIM (3, 6, 9, 12 and 15 µg/ml)

Fig 3: First order derivative spectra for SITA (50, 75, 100, 125 and 150 µg/ml) at 230 nm
Fig 3b: First order derivative spectra for SIM (6 µg/ml) and SITA (50, 75, 100, 125 and 150µg/ml)

Fig 4: First order derivative spectra of (a) 50, (b) 75, (c) 100, (d) 125 and (e) 150 µg/ml solution of SITA and (v) 3, (w) 6, (x)9, (y) 12, and (z)15 µg/ml solution of SIM

Table 1: optical and regression characteristics and validation parameters of SIM and SITA

| Parameters                  | SIM | SITA |
|-----------------------------|-----|------|
| Method A 238 | Method B 267 | Method C 238 | Method A 267 | Method B 238 | Method C 267 |
| Beers law limit (µg/ml)     | 3-15 | 3-15 | 3-15 | 3-15 | 3-15 | 3-15 |
| Regression equation (y=mx)  | y=0.048x | y=0.001x | y=0.002x | y=0.004x | y=0.001x | y=0.002x |
| Slope (m)                   | 0.048 | 0.001 | 0.002 | 0.023 | 0.004 | 0.001 |
| Correlation coefficient(R²) | 0.997 | 0.996 | 0.994 | 0.998 | 0.996 | 0.996 |
| Relative standard deviation(%RSD) | 0.412 | 0.514 | 0.513 | 0.613 | 0.313 | 0.368 |
| Precision                   |      |      |      |      |      |      |
| Intraday(n=5) (%CV)         | 0.121 | 0.132 | 0.714 | 1.120 | 0.214 | 0.141 |
| Interday(n=5) (%CV)         | 0.241 | 0.262 | 0.825 | 0.320 | 0.514 | 0.331 |

Table 2. Results of recovery studies

| Recovery level (%) | Amount spiked (µg/ml) | Amount recovered (µg/ml) | % Mean |
|--------------------|-----------------------|--------------------------|-------|
|                    | SIM | SITA | SIM | SITA | SIM | SITA |
| Method A           |     |      |     |      |     |      |
| 80                 | 7.2 | 80   | 7.19 | 80.10 | 99.86 | 100.125 |
| 100                | 9   | 100  | 9.09 | 100.05 | 101 | 100.05 |
| 120                | 10.8 | 120 | 10.807 | 119.90 | 100.60 | 99.91 |
| Method B           |     |      |     |      |     |      |
| 80                 | 7.2 | 80   | 7.205 | 79.59 | 100.06 | 99.48 |
| 100                | 9   | 100  | 8.98 | 100.50 | 99.77 | 100.50 |
| 120                | 10.8 | 120 | 10.79 | 120.05 | 99.90 | 100.04 |
| Method C           |     |      |     |      |     |      |
| 80                 | 7.2 | 80   | 7.18 | 79.89 | 99.72 | 99.86 |
| 100                | 9   | 100  | 8.96 | 99.89 | 99.55 | 99.89 |
| 120                | 10.8 | 120 | 10.76 | 120.20 | 99.62 | 100.16 |