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CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS DETERMINATION OF SIMVASTATIN AND SITAGLIPTIN IN COMBINED DOSAGE FORM

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ABSTRACT: To develop and validate simple and precise methods RP – HPLC (Method A) and Dual wavelength method (Method B) for simultaneous estimation of Simvastatin (SIM) and Sitagliptin (SITA) in combined dosage form. Methods used are Method A, the chromatographic separation was achieved by using mobile phase acetonitrile: methanol: water (50:30:20), a C_{18} column. The mobile phase was pumped at a flow rate 1 ml /min and the eluents were monitored at 250 nm. Method B, two wavelengths were selected for each drug in such a way that the difference in absorbance is zero for the second drug. At wavelengths 225 and 248 nm SITA has equal absorbance values, therefore, these two wavelengths have been used to determine SIM, on similar basis 254 and 274 nm were selected to determine SITA in the combined formulation.

INTRODUCTION: 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) -5, 6- dihydro [1,2,4]triazolo [4,3-a]pyrizine – 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 harmones (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 estimation [2], UPLC [3], Spectrophotometry [4, 5], bio-analytical [6], mass spectrometry [7], spectrofluorimetry [8].

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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-{2-[(2r, 4r)-4-hydroxy-6-oxotetrahydro-2H-pyran-2yl] ethyl}-3, 7-dimethyl-1, 2, 3, 7, 8.8a-hexahydronaphthalen-1-yl 2, 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 path way, the metabolic path way 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-HPLC [9-12], derivative Spectrophotometry[13], spectrophotometry [14-15], second order derivative[16], LC-MS/MS [17].

SIM and SITA can be estimated simultaneously by RP-HPLC [18-20] but this method was fond to be more precise than previous methods.

MATERIALS AND METHODS:

SIM and SITA standard drug was obtained as a gift sample. Acetonitrile, methanol, water (HPLC grade), methanol (AR grade) were purchased from Merck, India. Double distilled water was used for all purpose.

Instrumentation:

HPLC system (AGILENT HPLC Model-1220 Infinity-LC with **Ezchromelite** Software) with gradient elution containing C_{18} column with UV-detector. 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.

Method A: Estimation by RP-HPLC:

Chromatographic conditions:

The mobile phase was a mixture of Acetonitrile, Methanol and Water (50:30:20), the flow rate was fixed at 1 mL/min and the detection wavelength was monitored at 250 nm.

Standard solutions and calibration graphs for chromatographic measurement:

Weigh accurately 10 mg of SIM and SITA were dissolved in 10 ml of HPLC grade methanol to obtain the concentration of 1000 µg/ml. The solutions were further diluted with mobile phase to obtain the desired concentrations. The linearity was determined for SIM and SITA at 2-10 and 20-60 µg/mL respectively. Samples in triplicates were made for each concentration and peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Sample preparation:

Twenty tablets were taken and weighed and weight equivalent to 10 mg of SIM was taken and dissolved in 10 ml of HPLC grade methanol. The solution was further diluted with mobile phase to obtain the desired concentration.

Method B: Dual wavelength method:

The principle for dual wavelength method is that the absorbance difference at two points on the spectra is directly proportional to the component of interest, independent of the interfering component. It can be utilized to a great extent without much complication to calculate the unknown concentration of the component of interest in a mixture. The pre-requisite for dual wavelength method is the selection of two such wavelengths where the interfering component shows the same absorbance while the component of interest shows significant difference in absorbance with concentration.

Standard solutions:

Weigh accurately 10 mg of SIM and SITA were dissolved in 10 ml of methanol to obtain the concentration of 1000 µg/ml. The solutions were further diluted with water to obtain the desired concentrations. The linearity was determined for SIM and SITA at 3-15 and 50-150 µg/mL respectively. Samples in triplicates were made for each concentration and absorbances were plotted against the corresponding concentrations to obtain the calibration graphs.

Sample preparation:

Twenty tablets were taken and weighed and weight equivalent to 10 mg of SIM was taken and dissolved in 10 ml of methanol. The solution was further diluted with water to obtain the desired concentration.

Spectral characteristics and wavelength selection:

The absorption spectra of 15 µg/mL of SIM and 150 µg/Ml of SITA in water were recorded over the range 200–400 nm using water as blank. The overlain spectra were observed for selection of the suitable wavelengths of the developed method.

RESULTS:

Method development and optimization:

Method A, to develop a suitable method for the estimation of SIM and SITA, different mobile phases were employed to achieve the best separation and resolution. The method development was initiated with using a mobile phase Phosphate buffer pH 3.6: Methanol (50:50) and then changed to Phosphate buffer pH 3.6: Methanol: Acetonitrile (40:40:20) and finally the mobile phase consisting of acetonitrile: methanol: water (50:30:20) mixture was found to be appropriate allowing good separation of compound at a flow rate of 1mL/min using C_{18} column at a detection wavelength of 250

nm. Retention times were 3.39 min and 4.3 min for SIM and SITA respectively **(Figure1, 2 and 3)**.

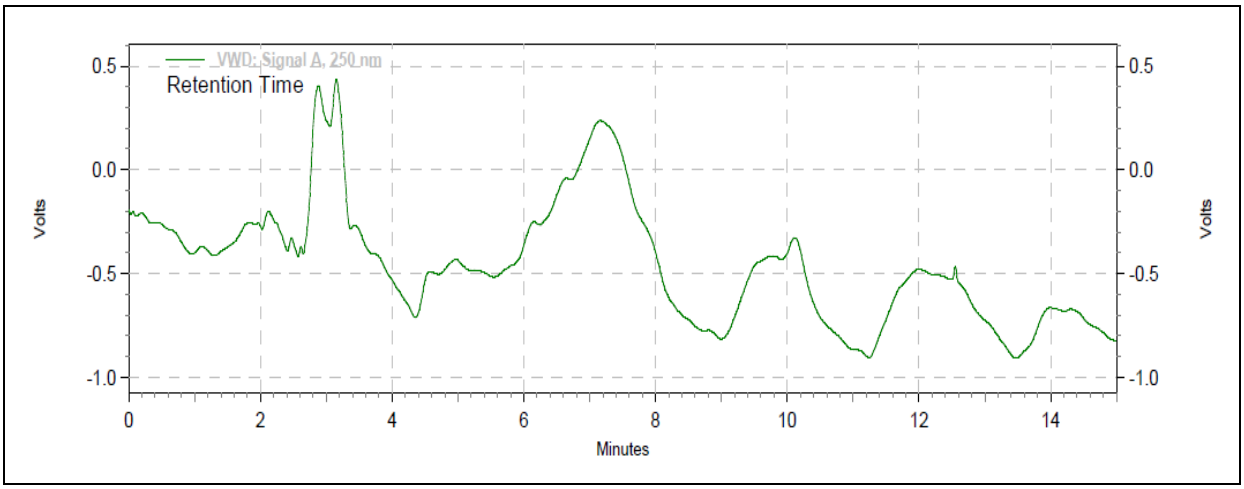

FIGURE: 1 CHROMATOGRAM OF BLANK

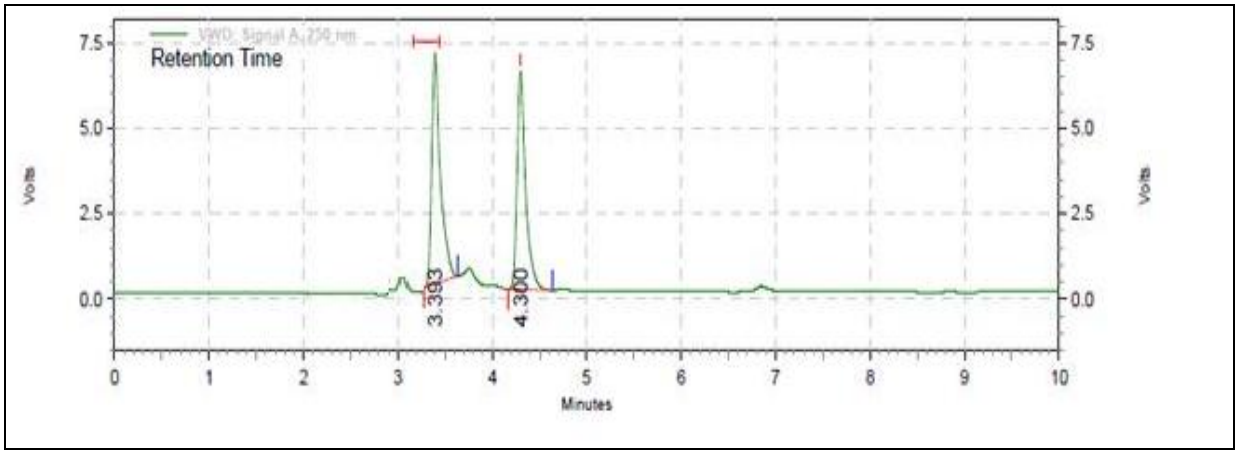

FIGURE: 2 CHROMATOGRAM OF STANDARD MIXTURE CONTAINING SIM (RT: 4.30) AND SITA (RT: 3.39)

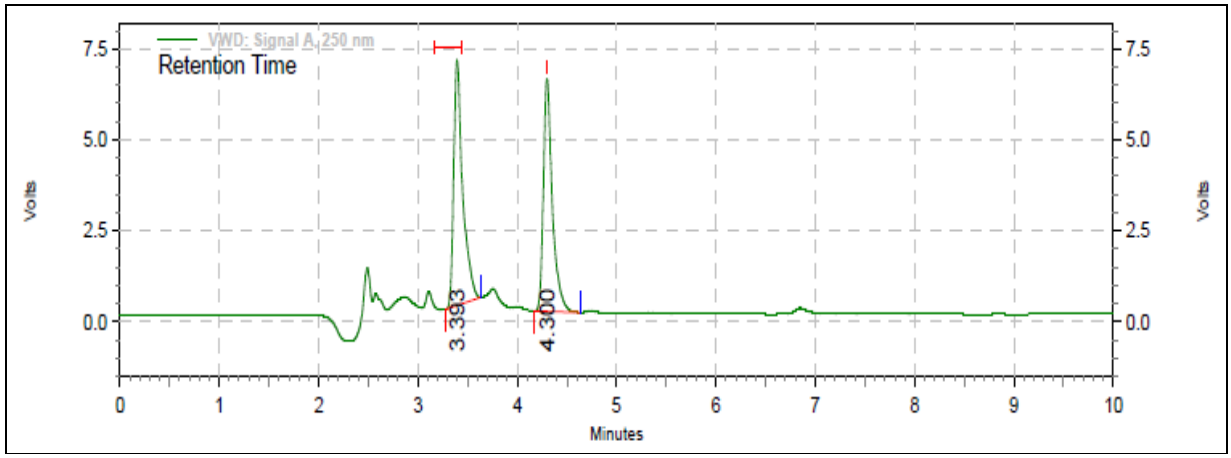

FIGURE: 3. CHROMATOGRAM OF FORMULATION MIXTURE CONTAINING SIM (RT: 4.30) AND SITA (RT: 3.39)

Method B, Standard solutions of both SIM and SITA in the range of 3-15 µg/mL and 50-150 µg/mL were separately prepared by appropriate dilutions of their respective working standard solutions in water and then were scanned in the range of 200–400 nm. Absorbance values at both 254 and 274 nm (for SIM) and at both 225 and 248 nm (for SITA) were measured **(Figure 4)**. SIM was determined by plotting the difference in absorbance at 254 and 274 nm (difference is zero for SITA) against its corresponding concentration. Similarly for determination of SITA, the difference in absorbance at 225 and 248 nm (difference is zero for SIM) was plotted against the corresponding

concentration. The concentrations of the two drugs were calculated each from the corresponding calibration curve equation.

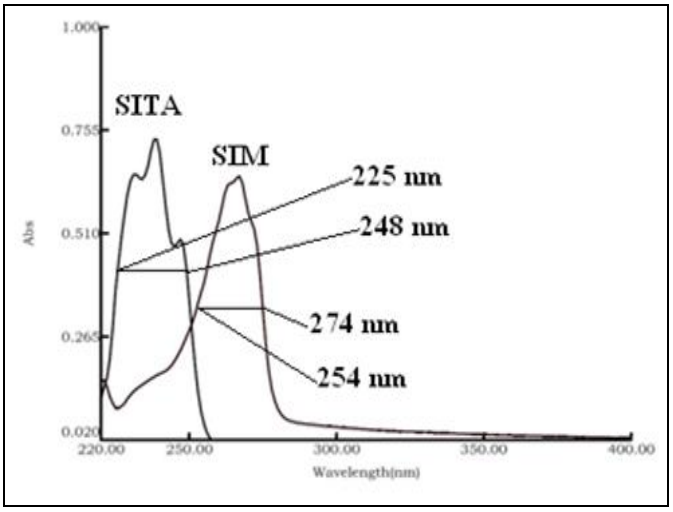

FIGURE 4: ABSORPTION SPECTRA FOR DUAL-WAVELENGTH.

Method validation:

Linearity and range:

For the construction of calibration curves, for method A five calibration standard solutions were prepared over the concentration range of 2-10 µg/mL for SIM and 20-60 µg/mL for SITA. The results, summarized in **Table1**. For method B five calibration standard solutions were prepared over the concentration range of 3-15 µg/mL for SIM and 50-150 µg/mL for SITA. The results, summarized in **Table 2**.

TABLE 1: LINEARITY PARAMETERS AND METHOD VALIDATION RESULTS OF METHOD A FOR STUDIED COMPOUNDS.

Parameter	SIM	SITA
Linearity range (µg/mL)	2-10 µg/mL	20-60 µg/mL
Equation Y=mX	Y=49189X	Y=20358X
Slope	49189	20358
Correlation coefficient (r)	0.997	0.996
Retention time	4.30 min	3.39 min
Theoretical plates	12168	7536
Asymmetry	1.64	1.73
Intra-day precision	1.171	0.427
Inter-day precision	1.12	0.393
Accuracy (%R.S.D.)	0.827	0.63
LOD (µg/mL)	2.306	0.543
LOQ (µg/mL)	6.990	1.646

TABLE 2: LINEARITY PARAMETERS AND METHOD VALIDATION RESULTS OF METHOD B FOR STUDIED COMPOUNDS

Parameter	SIM	SITA
Linearity range (µg/mL)	3-15 µg/mL	50-150 µg/mL
Equation Y=mX	Y=0.0045X	Y=0.003X
Slope	0.0045	0.003
Correlation coefficient (r)	0.99	0.995
Intra-day precision	1.106	1.042
Inter-day precision	1.94	1.08
Accuracy (%R.S.D.)	0.83	1.66
LOD (µg/mL)	0.3102	0.333
LOQ (µg/mL)	0.94	1.01

Accuracy and precision:

The accuracy was evaluated by the recovery of SIM and SITA. The summary of the results and average mean of recovery data for each level of both active pharmaceutical ingredients (API) was within acceptance range. The average results of repeatability and inter-day precision was within the limit and % R.S.D was less than 2.

Sensitivity:

The limit of detection and limit of quantitation decide about the sensitivity of the method. Tests for the procedure were performed on samples containing very low concentrations of analytes based on the visual evaluation method. In this method, LOD is determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected. Accordingly, the LOQ is determined by the analysis of samples with known concentration of analytes and by establishing the minimum level at which the analyte can be quantified. The LOD and LOQ values were found to be, for Method A 2.306µg/mL and 6.99 µg/mL for SIM, 0.543 µg/mL and 1.346 µg/mL for SITA and for Method B, 0.31 µg/mL and 0.94 µg/mL for SIM, 0.33 µg/mL and 1.01 µg/mL for SITA respectively.

Label claim recoveries from Juvisync tablets:

The proposed methods were evaluated in the assay of commercially available tablets containing 10 mg of SIM and 100 mg of SITA. The label claim found was to be 99.4, 100.21 for Method A and 100.01, 99.56 for Method B, for SIM and SITA respectively. (**Table 3**)

TABLE 3: ESTIMATION OF SIM AND SITA IN JUVISYNC TABLETS.

S.No.	Parameter	Method A		Method B	
		SIM	SITA	SIM	SITA
1.	% found	99.4	100.21	100.01	99.56
2.	SD	± 0.253	± 0.178	± 0.198	± 0.329
3.	% RSD	0.621	0.812	0.418	0.917

Retention times were 3.39 min and 4.3 min for SIM and SITA respectively. Linearity of SIM and SITA was established in the range of 2 - 10 and 20 – 60 µg/ml for Method A. For Method B the drugs obey beers lamberts law in the concentration range of 3 - 15 and 50 -150 µg/ml SIM and SITA respectively. The methods were validated in terms of accuracy, precision, and linearity, limit of detection and limit of quantification.

DISCUSSION: Validation of analytical method is the process by which it is established by laboratory studies, and the performance characteristics of the methods meet the requirements for the intended analytical application. Validation is required for any new or amended method to ensure that it is capable of giving reproducible and reliable results. Precision is a measure of the reproducibility of the whole analytical method (including sampling, sample preparation and analysis) under normal operating circumstances. Accuracy indicates the deviation between the mean value found and the true value. It is determined by applying the method to samples to which known amounts of analyte have been added these should be analyzed against standard and blank solutions to ensure that no interference exists.

Linearity is the ability of the method to obtain results which or either directly, or after mathematical transformation proportional to the concentration of the analyte within the given range. This is determined by calculating the regression value using a mathematical treatment of the results. Limit of detection is important for impurity test and the assays of dosages containing low drug levels and placebos. Limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy.

The proposed methods have been evaluated over the accuracy, precision and linearity and proved to be more convenient and effective for the quality control and identity of SIM and SITA in pharmaceutical dosage forms.

CONCLUSION: The proposed two methods were found to be efficient, accurate, precise and economic and are suitable for routine quality control analysis.

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