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Development and validation of first order and second order derivative UV spectrophotometric method for determination of sitagliptin phosphate and simvastatin in bulk and in formulation

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Abstract
Sitagliptin phosphate and simvastatin are used in combination for treatment of antidiabetic. The present work deals with simple spectrophotometric method development for simultaneous estimation of sitagliptin phosphate (SITA) and simvastatin (SIMVA) in two component tablet formulation. The method employed first order derivative spectroscopy and second order derivative spectroscopy. For determination of SITA and SIMVA were scanned in 400-200 nm range and simvastatin shows zero crossing point at 252.5nm and sitagliptin shows zero crossing point at 273nm in first order derivative spectroscopy. The quantitative determination of the drug was carried out using second order derivative values measured at 282nm for SITA and 257nm for SIMVA. This method obeyed Beer's law in the concentration range of 10-60 µg /ml for sitagliptin and 2-12 µg /ml for simvastatin. The recovery studies confirmed accuracy of proposed method and low values of standard deviation confirmed precision of method. The method is validated as per ICH guidelines.

Keywords: simvastatin (SIMVA), sitagliptin phosphate (SITA), first and second derivative spectroscopy

Introduction

Sitagliptin chemically is (3R) -3-amino-1-[3- (trifluoromethyl)-6,8-dihydro-5h- [1, 2, 4] triazolo [3,4-c] pyrazin-7-yl]-4-(2,4,5-trifluorophenyl) butan- 1-one, Fig. 1 shows the structure of Sitagliptin Phosphate, an oral Anti-diabetic agent that blocks Dipeptidyl peptidase-4 (DPP-4) activity. Sitagliptin increased incretin levels (GLP-1 and GIP) which inhibit glucagon release, in turn decreases blood glucose, but more significantly increases insulin secretion [1-3].

Simvastatin (SIMVA) is chemically is 2,2-Dimethyl butanoic acid (1S,3R,7S,8S,8aR)-1,2,3,7,8,8ahexahydro-3,7-dimethyl-8-[2-[(2R,4R)- tetrahydro-4- hydroxy-6 oxo2H pyran-2yl]ethyl]1-napthalenyl ester ;Fig. no: 2 shows the structure of Simvastatin. Simvastatin used as a HMG-CoA reductase inhibitors Simvastatin is used in the treatment of primary hypercholesterolemia and is effective in reducing total and LDL-Cholesterol as well as plasma triglycerides and apolipoprotein B [4-6].

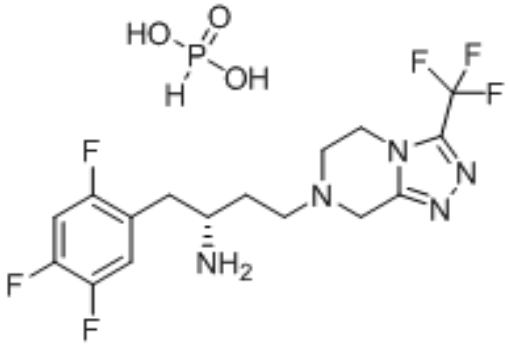

Fig 1: Structure of sitagliptin phosphate

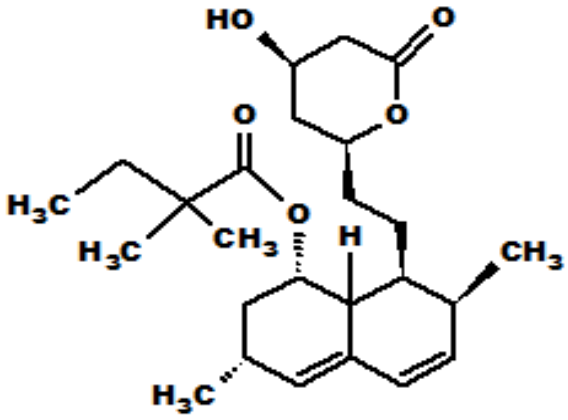

Fig 2: Structure of simvastatin

The literature reveals that there are some of the methods have been reported for Sitagliptin UV, HPLC, and Spectrophotometry [7-9]. For Simvastatin also have been reported UV, HPLC, and Spectrophotometry [10-12]. As on Sitagliptin and Simvastatin one method was reported on spectrophotometry [13].

Experimental Materials and Methods

A Jasco UV/Visible double beam spectrophotometer (UV model– 1700) and 1cm UV matched quartz cells were used. Gift samples of SITA and SIMVA were obtained from Merck Pharmaceutical, Sinner, Nasik, and Maharashtra, India. All chemicals and reagents used were of analytical grade and purchased from Fine Chemicals, Mumbai, India. Marketed formulation Juvisync tablet containing Sitagliptin phosphate 100mg and Simvastatin 40 mg was used as sample; purchased

from local pharmacy Pune. Calibration glassware's were used throughout the work.

Preparation of standard stock solution

Accurately weighed 10 mg of SITA and SIMVA was transferred to 100 ml volumetric flask separately, dissolved in 40 ml Methanol and 60ml distilled water by sonicater, sonicate up to 10 minute. The volume was adjusted with the same up to the mark to give final strength i.e. 100 µg/ml.

Selection of wavelength for analysis [14-15]

Method A

By appropriate dilutions with distilled water were prepared for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm and their spectra were overlaid. For First order derivative spectra at n=1, selected wavelength were 239nm and 267nm, which were selected for quantitation of SIMVA and SITA respectively. SIMVA shows zero crossing point at 252.5nm and SITA shows zero crossing point at 273nm.

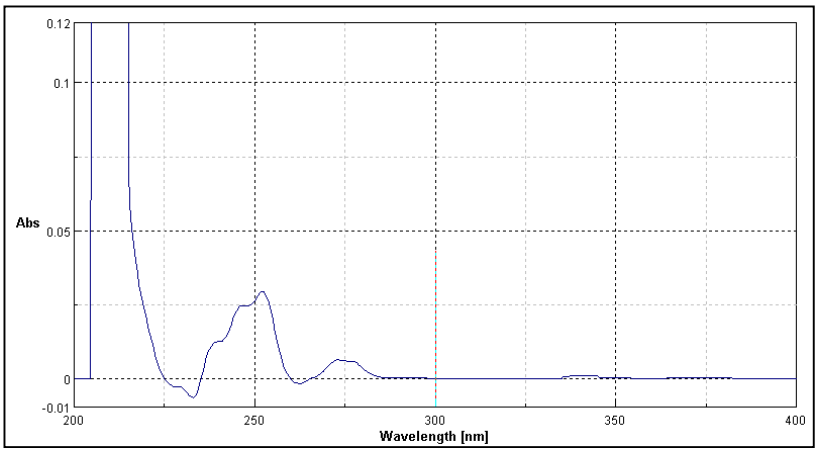

Fig 3: first order derivative spectra of mixture

Method B

By appropriate dilutions with distilled water were prepared for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm and their spectra were overlaid. For Second order derivative spectra at n=1 SIMVA shows zero crossing point at 257.5 nm and SITA shows zero crossing point at 282nm.

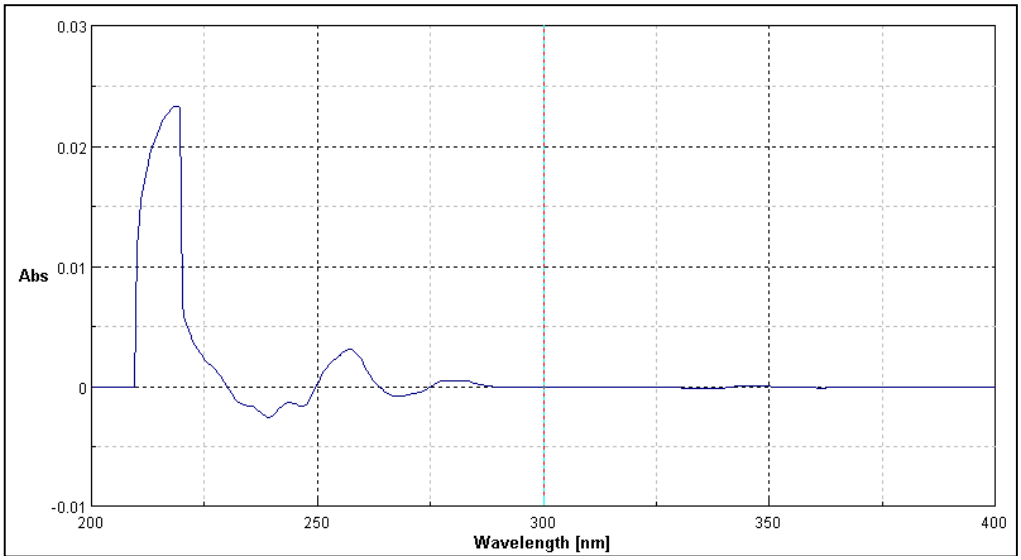

Fig 4: second order derivative spectra of mixture

Linearity

For each drug, appropriate aliquots of standard stock solutions were transferred to a series of 10 ml volumetric flasks. The volume was made up to the mark with distilled water to obtain working standard solutions for each drug of concentrations of 1-100 µg / ml for both SITA and SIMVA. Six sets of each concentration of the drugs were prepared separately. The absorbances of the working standard solutions of each concentration were measured at the selected analytical wavelengths. The standard calibration curves of Absorbance Vs Concentration were plotted using the mean of these six independent observations. The concentration range over which the drugs obeyed Beer- Lambert's law was found to be between 10 to 60 µg/ml for SITA and 2 to 12 µg/ml for SIMVA Fig. 5 and 6.

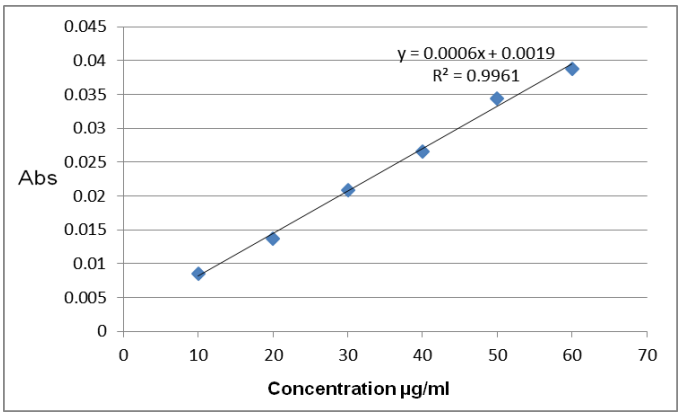

Fig 5: standard calibration curve of SITA

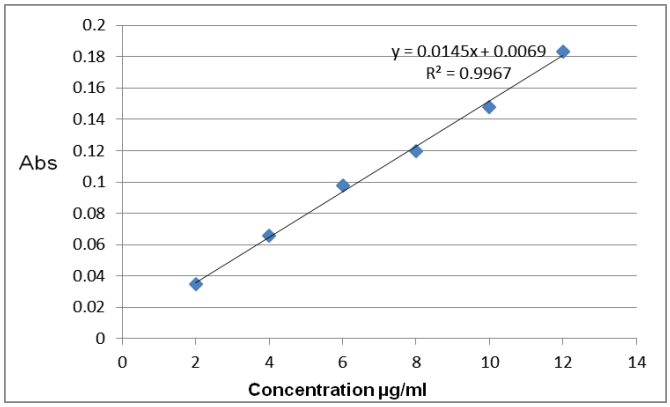

Fig 6: standard calibration curve of SIMVA

Validation of the method

Marketed tablets containing 40 mg of SIMVA and 100 mg of SITA were used. Twenty tablets were weighed and average weight was calculated. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 100 mg of SITA and 40 mg of SIMVA were transferred to 100 ml volumetric flask and dissolved in 40 ml of Methanol solution by sonicating for 10 mins and volume was then adjusted up to 60 ml with distilled water. The solution was filtered through Whatmann filter paper No. 41.

Sensitivity

The sensitivity of measurements of SITA and SIMVA by the use of the proposed method was estimated in terms of the Limit of Quanfication (LOQ) and Limit of Detection (LOD). The LOQ and LOD were calculated using equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where, 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

Repeatability

Repeatability was determined by analyzing 20 µg/ml and 8 µg/ml concentration of SITA and SIMVA solution for six times.

Accuracy

To the preanalysed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80%, 100% and 120%. The solutions were reanalyzed by proposed method.

Precision

Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing the 20 µg/ml of SITA and 8 µg/ml of SIMVA solutions for three times in the same day. Inter-day precision was determined by analyzing the 20 µg/ml of SITA and 8 µg/ml of SIMVA solutions daily for three days over the period of week.

Result and discussion

Method Validation

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

Linearity studies

The linear regression data for the calibration curves showed good linear relationship over the concentration range 10-60 µg/ml for SITA and 2-12 µg/ml for SIMVA. Linear regression equation was found to be y = 0.000x + 0.001 (r2 = 0.996) of SITA and for SIMVA y = 0.014x + 0.006 (r2 = 0.996) The result is expressed in table no 1.

Table 1: Optical Characteristics of SITA and SIMVA:

Parameters	SITA (267 nm)	SIMVA (239 nm)
Slope*	0.000	0.04
Intercept*	0.001	0.006
Correlation coefficient*	0.996	0.996
Linearity range µg/ml)	10-60	2-12

*Average of six determinations

Sensitivity The LOD and LOQ for SITA and SIMVA for method A and B; shown in table no.2

Repeatability

Repeatability was determined by analyzing 20 µg/ml concentration of sitagliptin solution and 8 µg/ml concentration of simvastatin for six times and the % amount with % R.S.D. less than 1.shown in table no.2.

Table 2: summary of validation parameter:

Sr.no.	Parameters	Method A *first order derivative method*		Method B *second order derivative method*	
		Sita Result	Simva Result	Sita Result	Simva Result
1	Accuracy (% recovery± *SD)	100.01 ± 0.056	99.9±0.007	100.03± 0.56	100.1 ± 0.65
2	Precision (% CV± *SD)	100.03 ± 0.007	100± 0.181	100.2±0.0014	100±0.007
3	Repeatability (% mean± *SD)	99.54±0.013	99.59±0.0178	99.68 ± 0.0089	99.76 ±0.0044
4	*LOD	0.00029	0.00541	0.0029	0.0241
5	*LOD	0.00087	0.0164	0.00097	0.00364

*Average of six determinations

Accuracy

The solutions were reanalyzed by proposed method; results of recovery studies are reported in table 3 which showed that the % amount found was 100.01% and 99.9 for SITA and SIMVA for Method A and Method B 100.03% and 100.1 with %R.S.D. >2 of SITA and SIMVA.

Table 3: Determination of Accuracy by percentage recovery method for SITA and SIMVA

Method	Ingredients	Tablet amount (µg/ml)	Amount added (µg/ml)	Level of addition	Amount recovered (µg/ml)	Percentage recovery %	Average % recovery *S.D.
Method A	SITA	20	16	80%	36	85.70	99.99±0.056
		20	20	100%	40	100.1	
		20	24	120%	44	111.24	
	SIMVA	8	6.4	80%	14.4	85.62	99.39±0.007
		8	8	100%	16	99.9	
		8	9.6	120%	17.6	112.67	
Method B	SITA	20	16	80%	36	81.21	99.81±0.16
		20	20	100%	40	100.03	
		20	24	120%	44	115.2	
	SIMVA	8	6.4	80%	14.4	83.07	99.32±0.65
		8	8	100%	16	100.1	
		8	9.6	120%	17.6	111.79	

*Average of six determinations

Precision

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These result shows reproducibility of the assay. The % R.S.D. values found to be less than 2, so that indicate this method precise for the determination of both the drugs in formulation shown in table no 2 and 4.

Table 4: Inter-Day and Intra-Day Precision

Method	Drugs	Inter-day			Intra-day		
		Mean	*S.D.	*%R.S.D.	Mean	*S.D.	*%R.S.D.
Method A	SITA	100.03	0.007	0.007	100.01	0.07	0.06
	SIMVA	100	0.181	0.181	99.82	0.007	0.006
Method B	SITA	100.2	0.0014	0.0014	100.01	0.029	0.029
	SIMVA	100	0.007	0.007	99.98	0.013	0.013

*Average of six determinations

Conclusion

The results of our study indicate that the proposed UV spectroscopic methods are simple, rapid, precise and accurate. The developed UV spectroscopic methods were found suitable for determination of SITA and SIMVA as bulk drug and in marketed solid dosage formulation without any interference from the excipients. Statistical analysis proves that, these methods are repeatable and selective for the analysis of SITA and SIMVA. It can therefore be concluded that use of these methods can save much time and money and it can be used in small laboratories with accuracy.

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