International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 04-02-2021; Accepted: 12-03-2021: Published: 20-03-2021
www.botanyjournals.com
Volume 6; Issue 2; 2021; Page No. 255-260

A new derivative and non-derivative UV-spectroscopic approach for quantification of simvastatin and sitagliptin in bulk and pharmaceutical formulation

Rama Rao Nadendla[1*], Abhinandana Patchala[2]
[1] Professor and Principal, Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur, Andhra Pradesh, India
[2] Assistant Professor, Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur, Andhra Pradesh, India

Abstract
Simple accurate and precise spectrophotometric methods have been developed for the simultaneous estimation of simvastatin (SIMV) and sitagliptin (SITA) by employing four different analytical UV-Spectroscopic methods. From them method A was simultaneous equation method involves formation and solving the simultaneous equation using 238 nm and 267 nm as two wavelengths for simvastatin and sitagliptin respectively. Method B related to first order derivative spectrophotometry. The first order derivative absorption at 230 nm (zero crossing point of SITA) was used for SIMV and 275nm (zero crossing point of SIMV) was used for SITA. Method C is simultaneous estimation of simvastatin and sitagliptin by using dual-wavelength method. Method D involved in Q-absorption analysis based on the measurement of absorbance at two wavelengths that is the λ_{max} of SITA 267 nm and iso -absorptive point of both drugs at 250 nm. Two wavelengths were selected for each drug in such a way that the difference in absorbance was zero for the second drug. At wavelengths 225 and 248 nm SITA had equal absorbance values; therefore, these two wavelengths have been used to determine SIMV; on a similar basis 254 and 274 nm were selected to determine SITA in their binary mixtures. The four methods were obeyed the Beer's law in the concentration range of 3-15 µg/ml for SIMV and 50-150 µg/ml for SITA.

Keywords: sitagliptin (SITA), simvastatin (SIMV), dual-wavelength, q-absorption analysis, first order derivative, spectrophotometry

Introduction

Sitagliptin [(S)-5-methoxy-2-[(4-methoxy-3, 5 dimethylpyridin-2-yl) Methyl sulfinyl]-3H-benzoimidazole] is Soluble in water (42.2mg/ml) and slightly soluble in methanol. It works competitively to inhibit the enzyme dipeptidyl peptidase 4 (DPP-4). This enzyme breaks down the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal.

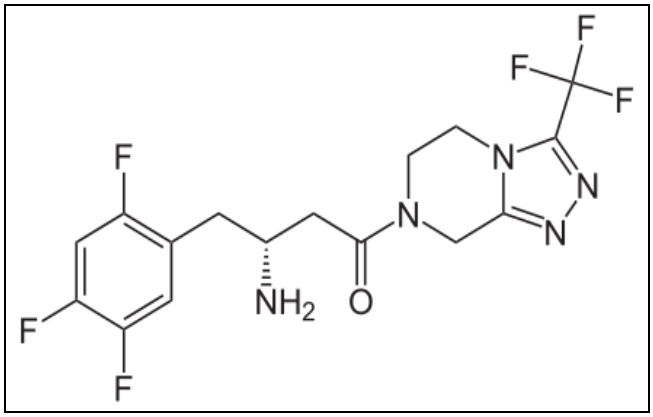

Fig 1: Stucture of Sitagliptin

Simvastatin [(1*S*,3*R*,7*S*,8*S*,8a*R*)-8-{2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate] is practically insoluble in water and freely soluble in chloroform, methanol and ethanol.

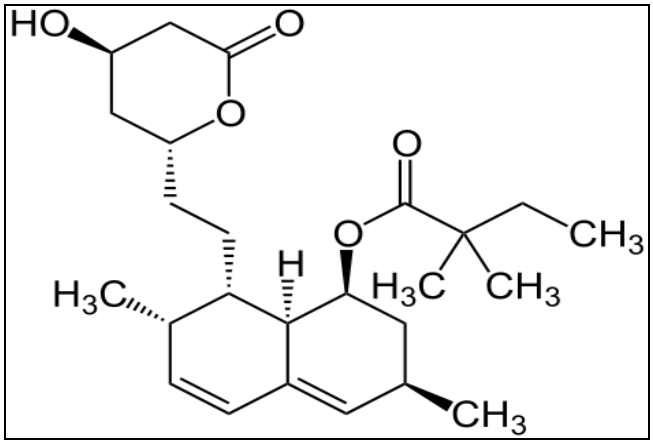

Fig 2: Stucture of simvastatin

The 6-membered lactone ring of simvastatin is hydrolyzed *in vivo* to generate the beta, delta-di hydroxy acid, an active metabolite structurally similar to HMG-CoA (hydroxy methylglutaryl CoA).

Instruments and Chemicals

Different instruments and chemicals were utilized in this study are given below.

Instruments

LABINDIA-UV 3092 UV/VIS spectrophotometer
Oscar ultrasonic's – ultra probe sonicator.
Vacuum filtration unit
Potentiometer-Titrasys 352.

Chemicals
Methanol, Acetonitrile
HPLC grade Methanol
Ferric chloride, Distilled water

Method A
Simultaneous Estimation Done By Using Simultaneous Equation Method
By appropriate dilution of two standard solutions with water, solutions containing 100 µg/mL of SIMV and 100 µg/mL of SITA were scanned separately in the range of 200-400 nm to determine the wavelength of maximum absorption for both the drugs. They were scanned in the wavelength range of 400−200 nm and the overlain spectrum was obtained. Two wavelengths 238 nm (λ max of Simvastatin) and 267 nm (λ_{max} of Sitagliptin) were selected for the formation of Simultaneous equation. The calibration curves were found to be linear in the concentration range of 3−15 µg/mL, for SIMV and 50-150 µg/mL for SITA. The absorptivity coefficients of each drug at both wavelengths were determined. The concentrations of two drugs in the mixture were calculated using equations.
C_X = (A2ay1-A1ay2)/ (ax2ay1-ax1ay2) CX = concentration of SIMV
C_Y = (A1ax2-A2ax1)/ (ax2ay1-ax1ay2) CY = concentration of SITA
A_1=absorbance of samples at 238 nm.
A_2= absorbance of samples at 267 nm.
ax_1 is the absorbivity of SIMV at 238nm.
ax_2 is the absorbivity of SIMV at 267 nm.
ay_1 is the absorbivity of SITA at 238 nm.
ay_2 is the absorbivity of SITA at 267 nm.

Preparation of standard solution
Preparation of standard solution of simvastatin
Standard solution of SIMV (10 mg) was dissolved in 10 ml of methanol to obtain the concentration of 1000 µg/ml. The solution was further diluted with water to obtain the desired concentrations 3-15 µg/ml.

Preparation of standard solution of Sitagliptin
Standard solution of SITA (10mg) was dissolved in 10 ml of methanol to obtain the concentration of 1000 µg/ml. The solution was further dilution with water to obtain the desired concentrations 50-150 µg/ml.

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

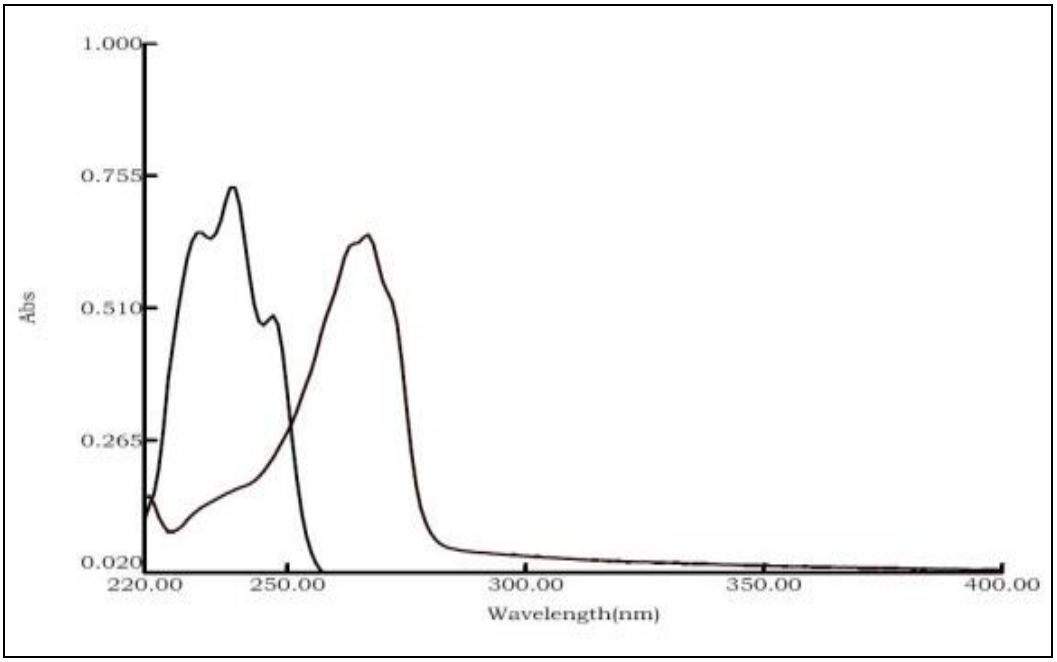

Fig 3: Overlain spectrums of SIMVV and SITA

Results and discussion
Linearity: The proposed simultaneous equation method shows good linearity. The linearity of calibration curves in pure solution was checked over the concentration ranges of about 3-15 µg/ml for SIMV and 50-150 µg/ml for SITA. The calibration graphs of SIMV and SITA at 238 nm and 267 nm are given below.
Precision: Method reproducibility was demonstrated by repeatability and intermediate precision measurements. The experiments were repeated for six 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 SIMV and SITA. The results obtained for repeatability studies (intraday precision) were calculated and showed in and for intermediate precision are presented in The observed results comply with the acceptance criteria.
Accuracy: The accuracy of the method was determined by calculating recoveries of SIMV and SITA by the methods of standard additions. This study was performed by addition of known amounts of SIMV and SITA 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. Table 1 shows that the overall percent recoveries of SIMV and SITA at 80, 100 and 120% of the

test concentration. The method shows excellent mean recoveries, standard deviation and good consistent recoveries for SIMV and SITA.

LOD and LOQ

Limit of detection (LOD) and Limit of quantitation (LOQ) were calculated according to the 3s/m and 10s/m criterions, respectively, where s is the standard deviation of the absorbance of the sample and m is the slope of the corresponding calibration curve. The values of LOD and LOQ are given in (Table 1).

Results of analysis of commercial formulations

Applicability of the method was tested by analyzing the commercial available formulation. Tablets containing 10 mg SIMV and 100 mg of SITA were used.

The values of % recovery from formulations (Table 1) are found to be very close to each other as well as to the label value of commercial formulation.

This shows that the method is applicable for SIMV simultaneous determination of SIMV and SITA from their binary mixture formulation.

Table 1: Results for Simultaneous Estimation Done by Using Simultaneous Equation method

Results for Simultaneous Estimation Done By Using Simultaneous Equation Method					
Parameters		**SIMV**		**SITA**	
		238 nm	267 nm	238 nm	267 nm
Linearity		3-15 µg/mL	3-15 µg/mL	50-150 µg/mL	50-150 µg/mL
Correlation coefficient		0.997	0.996	0.999	0.996
Intra- day precision (%RSD, n=3)		0.5542	1.63	1.838	0.634
Inter-day precision (%RSD, n=3)	Day-1	0.4179	1.9425	1.287	0.2657
	Day-2	0.3212	1.7218	1.554	0.3236
	Day-3	1.294	1.9598	1.6255	1.3087
Accuracy	80 %	100.273		100.75	
	100%	100.586		99.4	
	120 %	100.33		100.54	
LOD &		0.162	0.323	1.380	9.487
LOQ		0.492	0.98	4.182	28.75
Analysis of commercial formulation		99.3		99.76	

Method B: Simultaneous estimation of Simvvastatin and Sitagliptin by first order derivative spectroscopy using zero crossing point

Method

From standard stock solutions (100 µg/ml) of SIMV and SITA aliquots solutions were prepared separately with water to obtain concentration range of 3-15 µg/ml for SIMV and 50-150 µg/ml for SITA. Working standard stock solutions were scanned in the range of 220-400 nm. The absorption spectrum thus obtained was derivatized to first order. For all solutions the derivative spectra were obtained over 220-400 nm range.

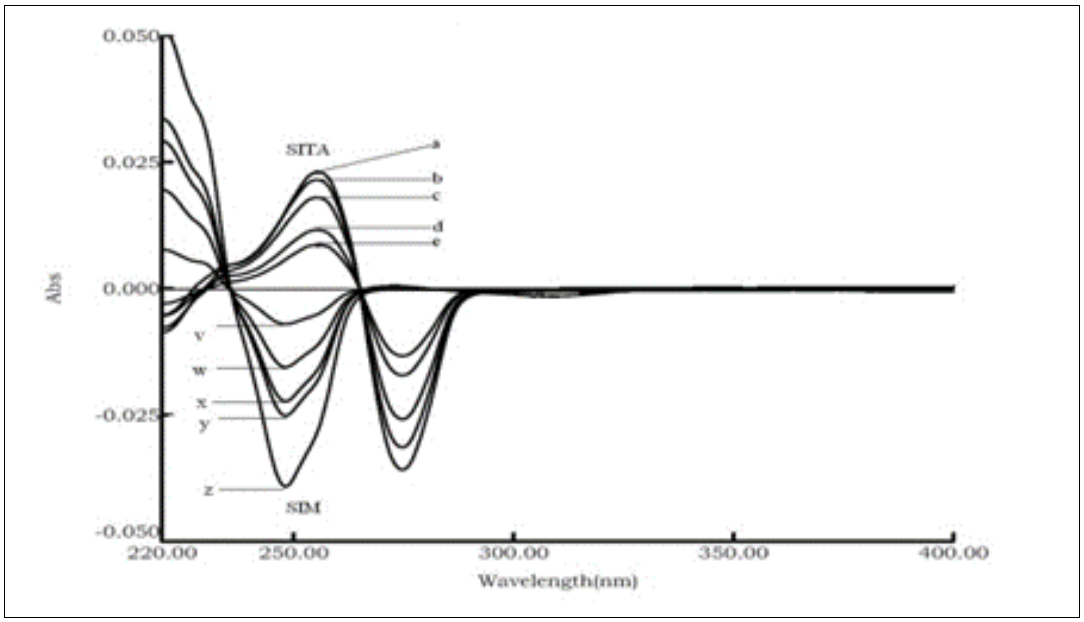

Fig 4: First order derivative spectra of (a) 50, (b) 75, (c) 100, (d) 125 and (e) 150 µg/ml solution of SITA and (v) 3, (w) 6, (x) 9, (y) 12, and (z) 15 µg/ml solution of SIMV

Table 2: Simultaneous estimation of simvvastatin and sitagliptin by first order derivative spectroscopy using zero crossing point.

Method B: Simultaneous estimation of simvvastatin and sitagliptin by first order derivative spectroscopy using zero crossing point		
Parameters	**SIMV**	**SITA**
	230 nm	275 nm
Linearity	3-15 µg/mL	50-150 µg/mL

Correlation coefficient		0.994	0.996
Intra- day precision (%RSD, n=3)		1.0972	0.5656
Inter-day precision (%RSD, n=3)	Day-1	1.251	1.2592
	Day-2	0.8402	1.5552
	Day-3	0.6151	1.5552
Accuracy	80 %	100.89	100.12
	100%	99.44	98.67
	120 %	100.32	100.69
LOD &		0.308	0.935
LOQ		0.465	1.41
Analysis of commercial formulation		100.5	99.37

Method C

Simultaneous estimation of simvastatin and Sitagliptin by using dual-wavelength method

Method

Standard solutions of both SIMV 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 SIMV) and at both 225 and 248 nm (for SITA) were measured. SIMV 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 SIMV) was plotted against the corresponding concentration. The concentrations of the two drugs were calculated each from the corresponding calibration curve equation.

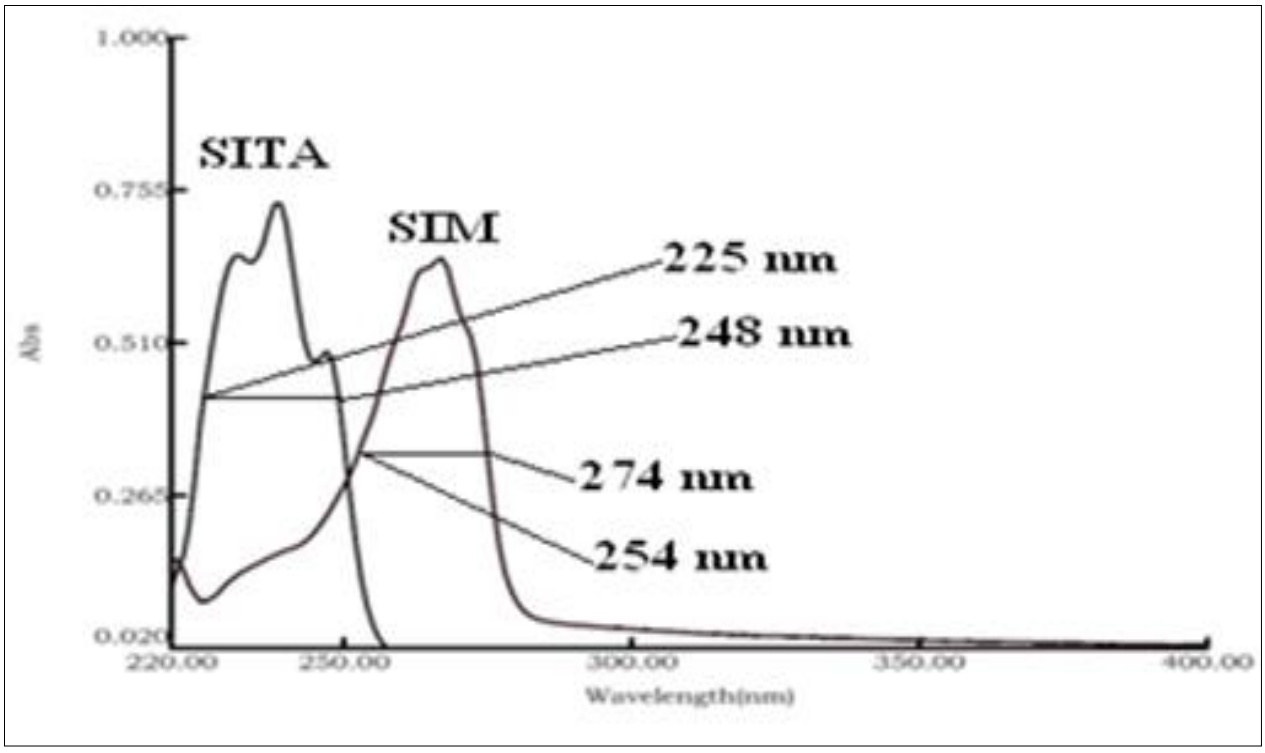

Fig 5: Spectrum of dual wavelength method

Results and discussion

Linearity: SIMV and SITA and Summarized in table (3)

Precision: Precision was assessed as % RSD at different levels and given in table 3.

Accuracy: Accuracy was calculated as the percentage recoveries of blind samples of pure SIMV and SITA and it indicated the agreement between obtained results and those accepted as true, detailed results are presented in (Table 3). Percentage recoveries for SIMV and SITA were found to be acceptable**.**

LOD and LOQ: They were calculated from the standard deviation (d) of the response and the slope of the calibration curve (S) in accordance to the following equations: LOD = 3.3 (d/S) and LOQ =10 (d/S). Results presented in Table 3, indicated that the method is sensitive for determination of the studied drugs.

Results of analysis of commercial formulations

Applicability of the method was tested by analyzing the commercial available formulation.

Tablets containing 10 mg SIMV and 100 mg of SITA were used for the study. The values of % recovery from formulations (Table 3) are found to be very close to each other as well as to the label value of commercial formulation. This shows that the method is applicable for Simultaneous determination of SIMV and SITA from their binary mixture formulation.

Table 3: Simultaneous estimation of simvastatin and Sitagliptin by using dual-wavelength method

Method C: Simultaneous estimation of simvastatin and sitagliptin by using dual-wavelength method			
Parameters		**SIMV**	**SITA**
		225 nm	248 nm
Linearity		3-15 µg/mL	50-150 µg/mL
Correlation coefficient		0.99	0.995
Intra- day precision (%RSD, n=3)		1.1061	1.0423
Inter-day precision (%RSD, n=3)	Day-1	1.8204	0.87409
	Day-2	1.944	1.0847
	Day-3	0.4160	0.9523
Accuracy	80 %	99.58	100.38
	100%	100.33	100.33
	120 %	100.467	98.94
LOD &		0.3102	0.333
LOQ		0.94	1.01
Analysis of commercial formulation		101.2	101.3

Method D

Simultaneous estimation of Simvvastatin and Sitagliptin by q-absorbance ratio method.

Method

The working standard stock solutions of SIMV and SITA were scanned in the range of 220-400 nm against water as blank. Iso-absorptive point was found at 250 nm and another wavelength used was 267 nm which is λmax of SITA calibration curve was plotted over a concentration range of 3-15 µg/ml for SIMV and 50-150 µg/ml for SITA. The absorbance of each solution was measured at both the wavelengths 250 nm and 267 nm. Concentrations of SIMV and SITA were determined using the following Simultaneous equation.

$C_X = (Q_M-Q_Y)*A_1/ (Q_X-Q_Y)*aX_1$

$C_Y=A_1/ax_1-C_X$

Where A1 and A2 are absorbances of the mixture at 250 nm and 267 nm respectively; ax_1 and ay_1 are absorptivites of SIMV and SITA respectively at 250 nm and ax_2 and ay_2 are absorptivites of SIMV and SITA respectively at 267 nm.

$Q_M = A_2/A_1$, $Q_X=AX_2/AX_1$ AND $Q_Y=AY_2/AY_1$

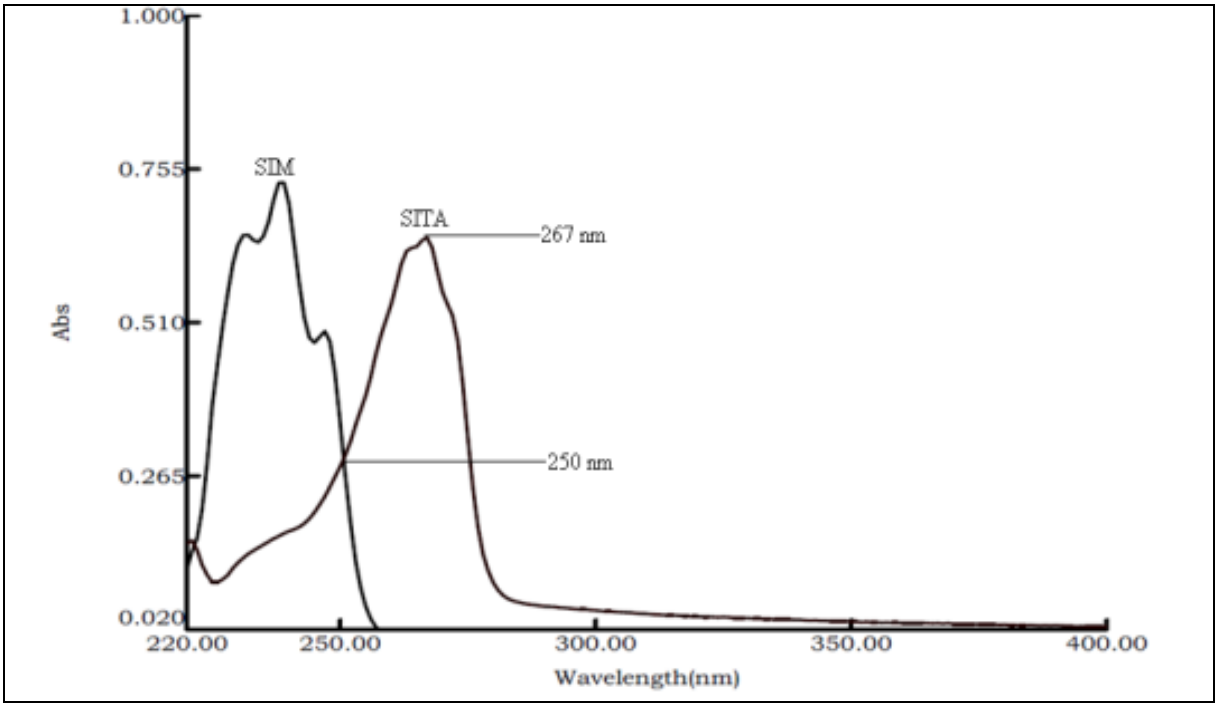

Fig 6: Overlain spectrums of SIMV and SITA which shows Iso-Absorptive point at 250 nm

Results and discussion

Linearity: The linearity of the developed methods was evaluated by analyzing different concentrations of standard solutions of SIMV and SITA and given tables and figures below.

Precision: Intra-day precision and inter-day was Preformed and tabulated the %RSD.

Accuracy: For studying the accuracy of the proposed methods, and for checking the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. The excellent mean recoveries and standard deviation can be find in Table4.

LOD and LOQ: LOD and LOQ were calculated and presented in Table 4.

Results of analysis of commercial formulations

The values of % recovery from formulations (Table 4) are found to be very close to each other as well as to the label value of commercial formulation. This shows that the method is applicable for Simultaneous determination of SIMV and SITA from their binary mixture formulation.

Table 4: Simultaneous estimation of Simvvastatin and sitagliptin by q-absorbance ratio method

Method D: Simultaneous estimation of simvvastatin and sitagliptin by q-absorbance ratio method			
Parameters		**SIMV**	**SITA**
		250nm (Iso Absorptive point	250nm (Iso Absorptive point
Linearity		3-15 µg/mL	50-150 µg/mL
Correlation coefficient		0.998	0.996
Intra- day precision (%RSD, n=3)		1.896	1.79
Inter-day precision (%RSD, n=3)	Day-1	1.2135	1.154
	Day-2	1.844	1.446
	Day-3	1.0348	1.7
Accuracy	80 %	99.92	100.4
	100%	100.55	100.68
	120 %	100.13	99.53
LOD &		0.556	10.56
LOQ		1.686	32
Analysis of commercial formulation		99.4	100.08

Acknowledgement

The authors would like to thank to Chalapathi drug testing laboratory, Chalapathi institute of pharmaceutical sciences, Guntur for providing all the facilities to carry out the research work.

Ethical approval

This is a research work, without clinical research involved.

Funding

The authors declare that there were no sources of funding.

Conflict of interest

Authors declared no conflict of interest.

References

1. Sharma BK. Instrumental methods of chemical analysis. 19th ed. Meerut: Goel Publishing House, 2000, P1- 4.
2. Sethi PD. Quantitative Analysis of Drugs in Pharmaceutical Formulations. 3rd ed. Delhi: CBS Publisher s and Distributor s, 2005, P7-27.
3. Michael E, Schartz IS, Krull. Analytical method development and validation. New Delhi: CBS Publishers and Distributors, 2004, P25-46.
4. Willard HH, Merritt LL, dean JA, Frank AS. Instrumental methods of Analysis. 7th ed. New Delhi: CBS Publishers and Distributer s, 1986, P1-19.
5. ICH, Q2A, Text on Validation of Analytical Procedures, International7. Conference on Harmonization, Geneva; October, 1994, 1-5.
6. ICH, Q2B, Validation of Analytical Procedures: Methodology, International. Conference on Harmonization, Geneva; November, 1996, 1-8.
7. http://en.wikipedia.org/wiki/Sitagliptin
8. http://www.mims.com/India/drug/info/sitagliptin/
9. Indian pharmacopoeia New Delhi: The Indian pharmacopoeia commission Indian pharmacopoeia, 2010; 3:2103-2104.
10. The United States Pharmacopoeia**,** the Official Compendia of Standards, 29th ed., Rockville, MD, USP convention Inc, 2006, 1683-1684.
11. Sushil P Narkhede, Gali Vidyasagar, Anil G Jadhav, Sachin B Narkhede, Atul R Bendale. Development and validation of reverse phase HPLC method for determination of Simvastatin and Ezetimibe in tablet dosage form. *Pelagia* Research Library Der Pharmacia Sinica. 2011; 2(1):p49-56.
12. Naga Raju K, Sudheer Babu I, Jyothi R, Prathyusha S. Spectrophotometric methods for estimation of Simvastatin in bulk drug and its dosage form. *IJPCBS.* 2012; 2(1):p124-129.
13. Vinod kumar K, Sudhakar M, Dr Reddy YP, AVSS Kumar. Development and validation of UV spectrophotometric method for simultaneous estimation of Simvastatin and Ezetimibe in combined dosage form using simultaneous equation method. Journal of Pharmacy Research. 2011; 4(12):p4672-4674.
14. Stephen B, Rathinaraj Rajamanickam V, Joseph Mazarin Fiedlis P, Ashik Elahi AL, Rajveer Ch *et al*. Development and validation of analytical methods for simultaneous estimation of Simvastatin and Ezetimibe in combined dosage form. Journal of Advanced Pharmaceutical Research. 2010; 1(2):133-139.
15. Jat RK, Sharma S, Chipa RC, Singh Rambir, Alam Imran. Development and validation of Reversed Phase HPLC method for Estimation of Simvastatin in Pharmaceutical Dosage Form. Journal of Drug Delivery & Therapeutics. 2012; 2(3):121-124.
16. Arayne MS, Sultana N, Hussain F, Ali SA. Validated Spectrophotometric Method for Quantitative Determination of Simvastatin in Pharmaceutical Formulations and Human Serum. Journal of Analytical Chemistry. 2007; 62(6):536-541.
17. Stephen Rathinaraj B, Rajamanickam V, Rajveer Ch, Kumaraswamy1 D, Ganesh shehrao banglae3, Gajanan V Shinde3. Development and Validation of HPTLC Method for the Estimation of Simvastatin and Extimibe. Journal of Pharmaceutical Science and Technology. 2010; 2(8):p288-292.
18. oshi HV, Patel JK, Lata Kothapalli. Simultaneous derivative and multicomponent spectrophotometric determination of Simvastatin and Ezetimibe in tablets. Der Pharma Chemica. 2010; 2(2):152-156.

19. Madhukar AV, Swapna K, Nagasree S, Spoorthi B, Uma Maheshwari. Sensitive Analytical Method Development and Validation of Simvastatin Bulk Drug by RP-HPLC. Journal of Pharmacy Research. 2012; 5(2):p906-907.
20. Balaji S, Sunitha A. Development and Validation of Spectrophotometeric method for Simultaneous determination of Simvastatin and Ezetimibe in tablet formulations. Pak. J. Pharm. Sci. 2010; 23(4):p375-378.
21. Bhupendra Shrestha, Stephenrathinaraj B, Sita Sharan Patel, Verma NK, Mazumder R. Simultaneous HPTLC Estimation of Simvastatin and Ezetimibe in Tablet Dosage Form. E-Journal of Chemistry. 2010; 7(4):p1206-1211.
22. Jayapal Reddy Samaa C, Rama Rao Kalakuntlab, Surya Narayanac Rao V, Reddanna P. Simultaneous estimation of Simvastatin and Ezetimibe in pharmaceutical formulations by RP-HPLC method. J. Pharm. Sci. & Res. 2010; 2(2):p82-89.
23. Selvadurai Muralidharan1, Janaki Sankarachari Krishnan Nagarajan1, Sachin Singh, and Anil Dubala. Bioequivalence Study of Simvastatin. *J Bioanal Biomed Volume.* 2009; 1(1):028-032.
24. Anantha Kuma D, Sujan DP, Vijayasree V, Seshagiri Rao JVLN. Simultaneous Determination of Simvastatin and Ezetimibe in Tablets by HPLC. E-Journal of Chemistry. 2009; 6(2):541-544.
25. Nilesh Jain, Ruchi Jain, Hemant Swami, Deepak Kumar Jain. RP-HPLC Method for Simultaneous Estimation of Simvastatin and Ezetimibe in Bulk Drug and its Combined Dosage Form. Asian journal of research in chemistry. 2008; 1(1):p29-31.
26. Sreelakshmy N, Jayalakshmi B, Ramesh J, Vijay Amirtharaj R. A simultaneous method development and validation of Ezetimibe and Simvastatin in combined dosage form by RP-HPLC method. Journal of Pharmacy Research. 2011; 4(4):p1127-1128.