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Original Article

A Statistical Based Estimation of Candesartan and Simvastatin Using Simultaneous Estimation, First Order Derivative and Q Value Method by UV Spectrophotometry

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ABSTRACT

Aim/Background: When combined with simvastatin, candesartan is a more potent antihypertensive medication than statins by itself. Therefore, to estimate Candesartan and Simvastatin concurrently in the mixture, a quick, economical and straightforward analytical method is needed. In order to evaluate candesartan and simvastatin using UV spectrophotometry, the current study set out to create and statistically validate an estimating approach utilizing simultaneous estimation, the first order derivative method and the Q ratio method. **Materials and Methods:** All the methods were validated by following the ICH Q2 (R1) guidelines. Phosphate buffer of pH 7 was selected as a solvent because Simvastatin and Candesartan were soluble in it and provided stable absorbance with it. For simvastatin and candesartan, the wavelengths chosen were 239 nm and 250 nm, respectively. The accuracy, precision, robustness, linearity, limit of detection and limit of quantification of the suggested approach were all validated. ONE way ANOVA was applied **Results:** Simvastatin and candesartan concentrations varied from 2-10 µg/mL and 1-5 µg/mL, respectively, demonstrating a linear approach with a correlation coefficient (R^2) of 0.999. All three methods were used to analyse the synthetic mixture and the percentage mean assay for simvastatin and candesartan was 100±2%. The statistical validation of the method revealed a lower percentage RSD, demonstrating its precision, accuracy and robustness. **Conclusion:** For the simultaneous estimation of candesartan and simvastatin, all of the approaches were found to be accurate, simple, precise and repeatable. Candesartan and simvastatin in pharmaceutical dose forms can be quantified using these new analytical techniques.

Keywords: Analytical method validation, Candesartan, First order derivative method, Q-ratio method, Simultaneous equation method, Simvastatin, UV-visible spectroscopy.

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INTRODUCTION

Hypertension is one of the major rising problems in today's world. According to WHO nearly 1.28 billon adults aged from 30-79 are suffering from this disease. Nearly 46% of adults are unaware of hypertension. Hypertension is a condition where blood pressure is 140/90 mmHg or higher.[1] Rise in cholesterol level is another serious problem that can cause number of health issues. Cholesterol can also increase in blood pressure that can result in hypertension. During the early and rate-limiting stage of cholesterol production, HMG-CoA reductase catalyses the conversion of HMG CoA to mevalonate. Statins, a class of pharmaceuticals that lower cholesterol and are the most often prescribed and effective medications for treating hypercholesterolemia and lowering the morbidity and mortality linked to coronary heart disease.[2] Simvastatin (SIM) is [(2R,4R)-8-~2-[(1S,3R,7S,8S and 8aR)][ethyl}-4-hydroxy-6-oxotetrahydro -2H-pyran-2-yl] Figure 1(a) illustrates -3,7-dimethyl 1,2,3,7,8,8 a hexahydro naphthalen-1-yl 2,2-dimethylbutanoate1. It has a molecular weight of 418.566 g/mol and the formula $C_{25}H_{38}O$.[3] Chemically, it is derived from lovastatin; however, the structure is different because the ester side chain has an extra methyl group. Simvastatin is a lipophilic statin that is mostly metabolized by CYP450 3A4 and has a brief half-life. Simvastatin decreases LDL cholesterol by 25-50% at doses ranging from 5-80 mg. Over a

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five-year period, simvastatin has been demonstrated to lower overall mortality by up to 30% and the risk of cardiovascular disease by 35%.[4] Angiotensin II receptor antagonist of the AT1 subtype, Candesartan (CAN), is selective in nature. It is a non-peptide with the chemical formula (±). 2-ethoxy-1-hydroxyethyl-1-[benzyl p-(o-1H-tetrazol-5-ylphenyl)] cyclohexyl carbonate, -7-benzimidazolecarboxylate (ester) (Figure 1(b)).[5] With a molecular weight of 610.7 g/mol and an intrinsic solubility of 0.0595 mg/L, candesartan cilexetil is a BCS class II medication (low solubility, high permeability). It acts as a weak acid (pka1: 3.50 and pka2: 5.85). When the pH ranges from 1.2 to 6.8, the dose number is greater than 1.[6] Different approaches were reported for the assessment of Simvastatin in human plasma by LCMS/MS along with their pharmacokinetic study,[7-12] in combination with other drugs.[13-15] Estimation of candesartan were also reported along with other drugs in human plasma by LCMS/MS.[16-19] Determination and stability of simvastatin along with other statin or with other anti-hypertensive agents by HPLC in different formulations were reported.[20-25] Estimation of candesartan or salt of candesartan or combination along with other drugs including their stability and impurity profiling by RP-HPLC in dosage form.[26-31] Several spectroscopic techniques were found for the estimation of candesartan[32-34] and simvastatin[35-39] single or in combination with other drugs[40-42] during the literature survey.

In the present study estimation of simvastatin and candesartan were performed by UV-VIS spectroscopy since other analytical methods like HPLC, LC-MS/MS, etc are costly, tedious and require more solvent resources. In contrast UV-vis method is cost effective, simple and rapid. Moreover, in this study three different methods (1st derivative, Simultaneous Estimation Method and Q Value/ Absorbance ratio Method) were utilized for the estimation of simvastatin and candesartan in combination as no reported study were found upon literature survey that uses these approaches. The studied methods were sensitive, robust and rugged and inter-related to each other. Furthermore, we have tried to provide a comparative study among all the three methods and utilise the approaches for further future researches. Even statistical analysis was also performed to find out the level of significance among these three methods.

MATERIALS AND METHODS

Chemicals and reagents

Simvastatin and Candesartan cilexetil were collected as gift sample from Abott Healthcare Pvt Ltd. Sodium dihydrogen phosphate was purchased from Qualichems, Gujarat, Vadodara. The solvent used for the dilution was double distilled water prepared in the DM plant with in the campus of Birbhum Pharmacy School, Birbhum.

Equipment

UV-probe software was loaded onto a computer connected to a double beam UV-visible spectrophotometer (SHIMADZU 1900i). A pair of quartz cuvettes with 1cm width was utilized for analysis. Weighing of the samples was carried out in a Mettler balance. Whatman filter paper no.41 was utilized for study.

Method Development

Preparation of standard stock solution

Both Candesartan and Simvastatin is BCS class II drug having low aqueous solubility that's why phosphate buffer of pH 7 was used as solvent. Accurately 25 mg of both pure drugs (SIM and CAN) were weighed and transferred in 25 mL of each volumetric flask. To the volumetric flask 15 mL of the Phosphate buffer of pH 7 was added and sonicated for 10min to dissolve the drug completely. Once the drugs have been fully dissolved, add the phosphate buffer to get the volume up to 25 mL, which will yield a 1000 µg/mL concentration. This was marked as standard stock solution. From this stock solution suitable dilutions were made to attend a concentration ranging from 1-15 µg/mL for both SIM and CAN as working standard for all the methods.

Method I: Simultaneous equation method

Suitable dilutions were made from the stock solutions of both the drugs and scanned in UV against the blank from 400-200 nm. The λ_{max} of both the drugs were recorded (239 and 250 nm) for SIM and CAN. Simultaneous estimation of both the drugs was performed taking overlay spectra of both the drugs in zero order. Isosbestic point (218.5 µg/mL) was also noted from the overlay spectra of both the drugs. Simultaneous estimation was performed taking simultaneous equation in consideration. The absorbance was noted for SIM and CAN at different concentrations. For SIM (2-10 µg/mL) and CAN (1-5 µg/mL) at varying concentration levels, the calibration curve was presented having absorbance on the Y-axis and concentration on the X-axis. The absorptivity (A1%, 1cm) of both the drug at different wavelengths was calculated. In the simultaneous equation, absorbance and absorptivity were calculated and substituted to obtain the concentration.[17]

Method II: 1º derivative method

Following an effective sequential dilution of the stock solution, the working solution of SIM and CAN was scanned in the 200-400 nm range against blank solvent. The zero order spectra (Figure 2) of both the above drugs were recorded and overlain. First order (1º) derivative of both SIM and CAN were generated from the UV-Prove software and overlain for analysis. Estimation of both drugs in the synthetic mixture was performed after selecting the zero-crossing point of SIM (251.5 nm) and CAN (263 nm) of both the drugs from the overlain of 1st derivative spectra. It should be noted that no interference of one drug with the other drug should be there. The above standard stock solution was

diluted to prepare the concentration ranging from 1-15 µg/mL for SIM and CAN in phosphate buffer (pH 7).[43]

Method III: Absorption ratio method

In the absorbance ratio method, the analysis of the drugs was carried out based on the absorbance of the wavelength. Two wavelengths were selected out of which one point is the isosbestic point and another is the λ_{max} of any one among the two. From the overlain spectra isosbestic point (218.5 nm) and λ_{max} of SIM (239 nm) were selected for the formation of Q absorbance equation. The absorptivity values were calculated for SIM are 0.0302 (ax1), 0.0487 (ax2) and for CAN are 0.06 (ay1), 0.0374 (ay2) at 218.5 nm and 239 nm, respectively.

$C_{SIM}=(Q_M - QY)\times A_1/(QX-QY)\times ax1$,

$C_{CAN}=(Q_M - QX)\times A_1/(QY-QX)\times ax2$,

Where,

$C_{SIM=}$concentration of simvastatin,

$C_{CAN=}$concentration of candesartan,

A_1=absorbance of drug at 218.5 nm,

$Q_{M=}$absorbance of sample at 218.5 nm/absorbance of sample at 239 nm,

QX=ax1/ax2

QY=ay1/ay2

ax_1=absorptivity of Simvastatin at 218.5 nm,

ay_1=absorptivity of Candesartan at 218.5 nm,

ax_2=absorptivity of Simvastatin at 239 nm,

ay_2=absorptivity of Candesartan at 239 nm.

Method validation

ICH Q2 (R1) guideline was followed for the validation of the developed method. Linearity, Specificity, Robustness, Ruggedness, Limit of Detection (LOD), Limit of Quantitation (LOQ), Precision and Assay were calculated as the validation parameters.[44,45]

Linearity

The linearity was established for the proposed method for CAN and SIM in the range of 1-5 µg/mL and 2-10 µg/mL respectively. The calibration curve was plotted for CAN and SIM taking concentration on X-axis and absorbance on Y-axis and was evaluated by the least-squares method in Microsoft Excel® for three different methods. The coefficient of determination (r^2) was also determined for three different methods.

Limit of Detection and Limit of Quantitation

The LOD and LOQ was calculated from the calibration curve using the given formula:

LOD=3.3×σ/s

LOQ=10×σ/s

Where σ denotes standard error of y-intercept of regression line and s denotes the slope of the calibration curve.

Precision

Repeatability, intraday precision and interday precision were used to measure the method's precision for the three distinct approaches. The repeatability study was carried out as replicate analysis of the middle concentration, intraday was considered within the day at morning, afternoon and evening whereas interday precision was considered for three consecutive days. Percentage recoveries were calculated using the recorded absorbance for each reading and the straight-line equation obtained from linearity studies. Further the average percentage recoveries were calculated and the results were expressed in % RSD.

Specificity

The specificity of the method was measured by analysing the standard along with an excipient (magnesium stearate) to find out if any interference of the excipient is there with the standard or not. To carryout specificity each 10 mg of the both the standard drug were spiked with magnesium stearate at 5 mg (50%), 10 mg (100%) and 15 mg (150%) was analysed and % recovery was determined.

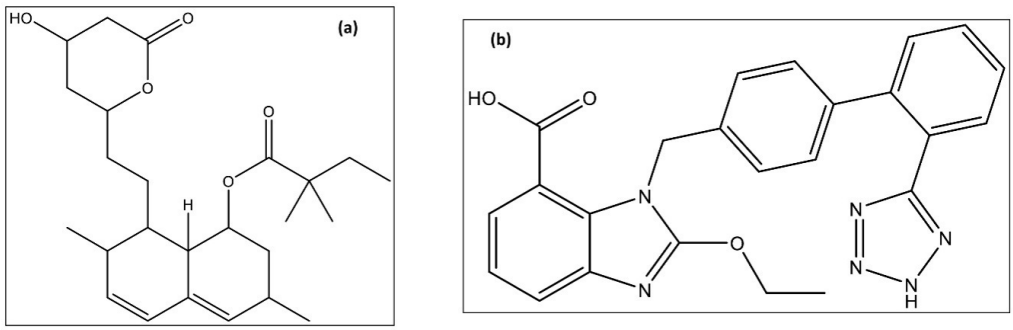

Figure 1: Chemical structure of (a) Simvastatin and (b) Candesartan.

Robustness

The robustness of the method was performed by varying the temperature and the pH of the solvent. Two different temperatures were taken into consideration i.e. room temperature and 18°C along with two different solvents phosphate buffer pH 7 and phosphate buffer pH 7.2. The % recovery and % RSD for both the drugs were calculated.

Ruggedness

The ruggedness of the method was evaluated taking two different analysts and the result was reflected as % recovery and % RSD.

Assay

The synthetic mixture was prepared in the ratio 1:2 for CAN: SIM. The CAN and SIM were taken in the ratio of 10 mg and 20 mg respectively. Both the drugs were effectively mixed and poured into a 100 mL volumetric flask. Phosphate buffer (pH 7) was poured up to 50 mL and sonicated till all the particles were effectively dissolved. Once dissolved the volume was made up to 100 mL. Serial dilutions were made to achieve a concentration ratio of 5 µg/mL and 10 µg/mL for CAN and SIM respectively. The synthetic mixture was analysed and quantified with the help of the three different methods and % recovery was calculated.

Statistical Analysis

Validation parameter output of all the methods were compared using ONE way ANOVA (Analysis of variance). All calculations for the One-way ANOVA were carried out using GraphPad Prism 10, ver. 8.0.1.[46] The test was performed concerning repeatability, precision, robustness, ruggedness and assay. The result was considered significant if the result lies within 95% confidence interval (i.e. $p<0.05$).

RESULTS

Determination of λ_{max}, isosbestic point and zero crossing point

Both the drugs SIM and CAN was scanned in UV from 400-200 nm and the absorbance maxima of SIM (239 nm) and CAN (250 nm) were recorded for simultaneous equation method (Figure 2). The zero-crossing point was noted for the 1° derivative method (Figure 3) and the isosbestic point was noted from the spectra given in Figure 4.

Calibration Curve

The calibration curve was plotted for all the three methods using concentration on x-axis and absorbance on y-axis. For the simultaneous estimation method (method I), the calibration curve was plotted in two different wavelengths i.e. 239 nm for SIM and 250 nm for CAN. Correlation coefficient R^2 obtained for both the calibration curves were 0.9995 and 0.9956 for SIM and CAN respectively. In method II i.e. 1st order derivative, calibration curve was plotted in two different wavelengths having zero crossing point i.e. 251.5 nm for SIM and 263 nm for CAN. A straight line was obtained having a correlation coefficient R^2 of 0.9981 and 0.9972 for SIM and CAN respectively. Lastly in method III, calibration curve was plotted in two different wavelengths taking isosbestic point (218.5 nm) and λ_{max} of SIM i.e. 239 nm in Q ratio method. The correlation coefficient (R^2) obtained for the straight-line calibration curve were 0.9991 and 0.9995 at isosbestic point and for SIM respectively. Calibration

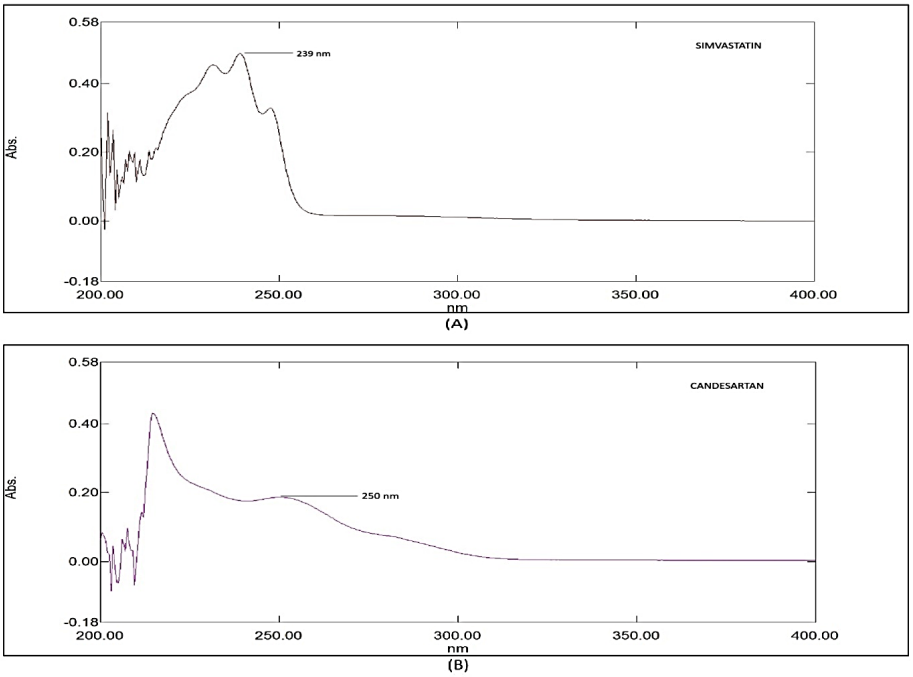

Figure 2: (A) UV spectra of Simvastatin (B) UV spectra of Candesartan.

curve of candesartan and simvastatin at different wavelength was shown in Figure 5.

Validation

Linearity

Linear calibration curve was obtained after plotting graph with concentration in x axis and absorbance in y axis. The coefficient of determination (R^2) was found to be in limits as per ICH guidelines for all three methods and the data are summarized in Table 1. This shows an excellent correlation of the linearity for all the three methods mentioned above.

Limit of detection and limit of quantitation

LOD of Simvastatin was 0.27 µg/mL, 0.52 µg/mL and 0.27 µg/mL, respectively and its LOQ was 0.80 µg/mL, 1.57 µg/mL and 0.80 µg/mL, respectively, as determined by the three techniques.

LOD of Candesartan using the three methods was 0.40 µg/mL, 0.32 µg/mL and 0.18 µg/mL respectively and LOQ of Candesartan using the three methods were 1.20 µg/mL, 0.96 µg/mL and 0.53 µg/mL respectively (Table 1).

Precision

The precision of all the above method was recorded for repeatability, intraday and interday. The result was expressed in %RSD (Relative Standard Deviation). The %RSD was found to be <2 for all the method (Table 2). The results were within the limit of ICH guideline.

Robustness

The robustness of all the methods was performed taking variation of temperature and solvent system. The results were expressed in %RSD (Relative standard deviation). The % RSD of all the method was found to be <2 (Table 3). The results were within the limit of ICH guideline.

Ruggedness

The ruggedness of all the methods was performed taking two different analysts and the results were expressed in the form of %RSD. The %RSD of all the method was found to be <2 (Table 3). The results were within the limit of ICH guideline.

Specificity

The specificity of the method was observed taking magnesium stearate as an excipient and recording any interference of the excipient on recovery of the drug. The recovery of the drug for all the three methods was found to be 98-100, 100-102 and 99-101. The result of the recovery was well into the limit according to the ICH guidelines.

Assay

The assay was performed taking synthetic mixture of CAN and SIM in 1:2 ratio. The assay was performed for all the three methods and the recovery of Simvastatin and Candesartan were recorded in Table 4. The recovery was well into the limit of 100±2 %. This shows that all the methods developed were effectively applied and quantified the amount of Simvastatin and Candesartan.

Statistical Analysis

One-way ANOVA was used to compare the outcomes of the simultaneous estimate approach, first order derivative method and Q ratio method for the estimation of Simvastatin and Candesartan. The findings demonstrated that there was no statistically significant difference between the values, with the computed ANOVA test values being higher than the tabulated ones (with a probability of 0.05). The results are presented in Table 5. This indicates that there is no significant difference between the results of all the three methods

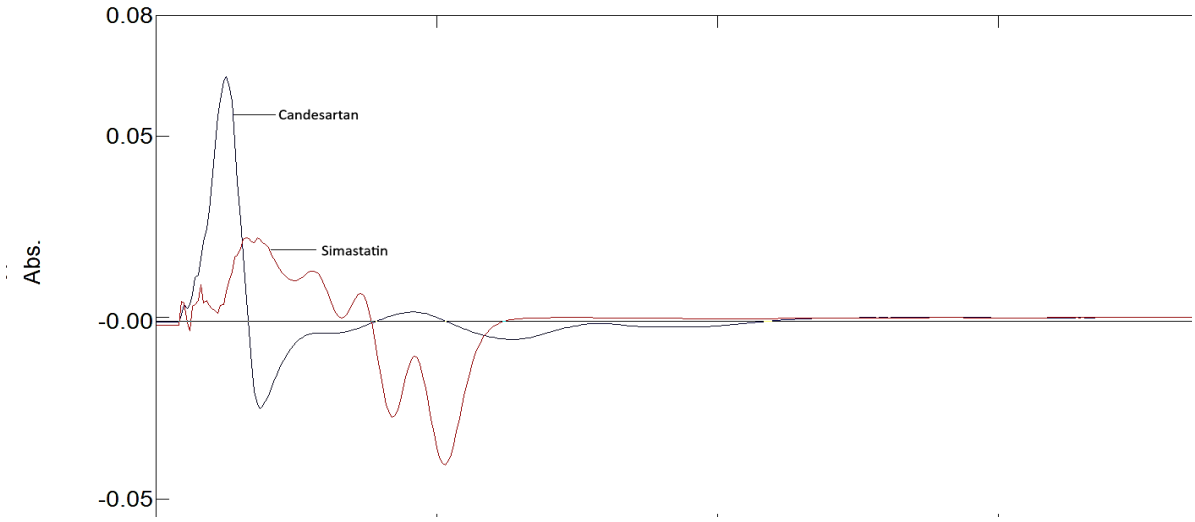

Figure 3: Overlay of first order derivative spectra of Simvastatin and Candesartan.

Table 1: Linearity and range data of candesartan and simvastatin.

	Method I		Method II		Method III	
Parameters	**SIM**	**CAN**	**SIM**	**CAN**	**SIM**	**Isobestic point**
Linearity-range (µg/mL)	2-10	1-5	2-10	1-5	2-10	1-5
Slope	0.0466	0.0347	0.004	0.001	0.0272	0.0466
Intercept	0.0172	0.0149	0.0006	0.0011	0.0292	0.0172
Correlation coefficient	0.9995	0.9956	0.9981	0.9972	0.9991	0.9995
LOD (µg/mL)	0.27	0.40	0.52	0.32	0.27	0.18
LOQ (µg/mL)	0.80	1.20	1.57	0.96	0.80	0.53

Table 2: Repeatability, intraday and interday precision data of candesartan and simvastatin.

	Method I		Method II			Method III	
	% Recovery	**% RSD**	**% Recovery**	**% RSD**		**% Recovery**	**% RSD**
Repeatability (*n*=6)							
SIM	100.3	0.37	100.4	1.98	Isosbestic point	99.2	0.46
CAN	101.9	0.84	101.7	1.80	SIM	100.3	0.37
Intraday (*n*=3)							
SIM	100.5	0.21	101.3	0.41	Isosbestic point	99.6	0.36
CAN	102.2	0.54	100.8	0.83	SIM	100.1	0.36
Interday (*n*=3)							
SIM	100.5	0.21	101.4	0.24	Isosbestic point	100.5	0.35
CAN	101.3	0.55	101.1	1.90	SIM	99.8	0.55

Table 3: Robustness and ruggedness result for candesartan and simvastatin.

	Method I		Method II			Method III	
	% Recovery	**% RSD**	**% Recovery**	**% RSD**		**% Recovery**	**% RSD**
Robustness							
SIM	99.5	0.86	100.5	1.09	Isobestic point	99.3	0.59
CAN	101.5	0.5	99.2	1.68	SIM	100.8	0.29
Ruggedness							
SIM	101.3	0.24	100.3	0.70	Isobestic point	100.8	0.42
CAN	101.0	0.54	100.2	0.16	SIM	100.6	0.37

Table 4: Results of the assay.

Conc. (µg/mL)		% Purity					
		Method I		Method II		Method III	
CAN	SIM	CAN	SIM	CAN	SIM	CAN	SIM
5	10	100.5	100.8	99.6	99.4	100.3	100.1
5	10	100.2	100.6	99.8	99.9	100.2	99.9
5	10	99.9	100.1	99.5	99.6	100.9	100.2
5	10	100.9	100.9	99.2	99.4	100.9	99.8
5	10	99.8	100.8	99.8	99.7	99.8	99.9
Mean		100.3	100.6	99.6	99.6	100.4	100.0

Table 5: Statistical Analysis of the simultaneous estimation, first order derivative method and Q ratio method

	Method I	Method II	Method III	p-value by One-way ANOVA
	Average			
Repeatability				
CAN	101.9	101.7	99.2	0.0042
SIM	100.3	100.4	100.3	0.0001
Intraday precision				
CAN	102.2	100.8	99.6	0.0098
SIM	100.5	101.3	100.1	0.01
Interday precision				
CAN	101.3	101.1	100.5	0.04
SIM	100.5	101.4	99.8	0.0058
Robustness				
CAN	101.5	99.2	99.3	0.0205
SIM	99.5	100.5	100.8	0.0002
Ruggedness				
CAN	101.0	100.2	100.8	0.007
SIM	101.3	100.3	100.6	0.0001
Assay				
CAN	100.3	99.6	100.4	0.0151
SIM	100.6	99.6	100.0	0.0001

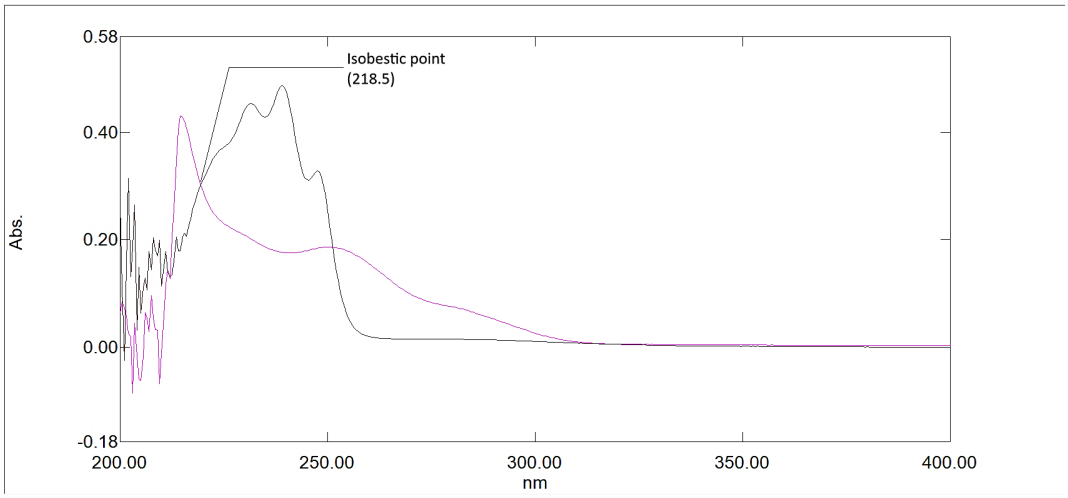

Figure 4: Overlay spectra of Simvastatin and Candesartan showing isobestic point.

DISCUSSION

Stock solution of CAN and SIM were prepared using phosphate buffer of pH 7 and working standards were prepared after further serial dilutions. The λ_{max} for simvastatin and candesartan was determined from the individual UV spectra and found at 239 nm and 250 nm respectively. The isobestic point was determined at 218.5 nm. ICH Q2(R1) guidelines were followed for the validation of the developed method and parameters like linearity, precision, robustness, ruggedness etc., were determined. Linearity was established within the concentration range of 1-5 µg/mL for Candesartan and 2-10 µg/mL for Simvastatin having correlation coefficient greater than 0.99 for all the methods. The percentage RSD determined for accuracy, precision and robustness was less than 2%. The findings of the % recoveries validate the method's accuracy. The LOD and LOQ study approach was used and it was found to be sensitive. It has been demonstrated that the suggested approach is accurate, precise, resilient and sensitive.

Assay of the synthetic mixture was performed using all the three methods and results are within limits as per ICH guidelines. All of the techniques were sufficiently sensitive to identify and quantify trace levels of candesartan and simvastatin in a sample. One way

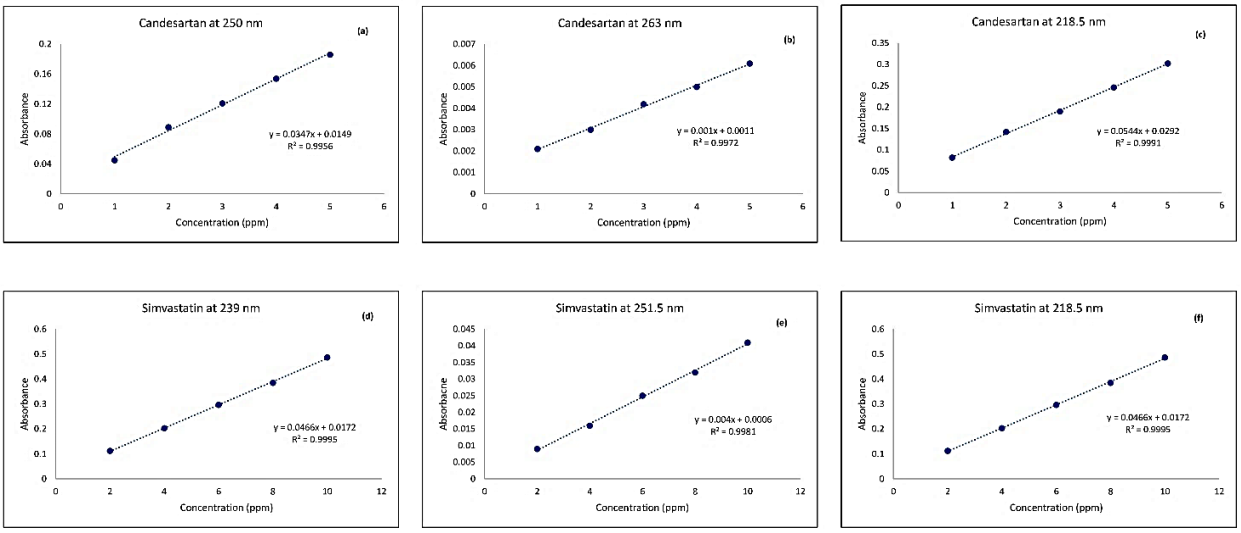

Figure 5: Calibration curve of (a) Candesartan at 250 nm, (b) Candesartan at 263 nm, (c) Candesartan at 218.5 nm, (d) Simvastatin at 239 nm, (e) Simvastatin at 251.5 nm, (f) Simvastatin at 218.5 nm.

ANOVA was applied to compare the results obtained from all the methods and for all the instance the *p*-value are greater than 0.05.

CONCLUSION

All of the variables together suggest that the Simultaneous Equation Method, First Derivative Method and Q-Ratio Method, are quick, easy, accurate and precise ways to estimate the simultaneous presence of simvastatin and candesartan in a mixture. Therefore, in routine quality control analysis, the proposed approach might be recommended for the simultaneous assessment of simvastatin and candesartan.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CAN: Candesartan; **SIM:** Simvastatin; **UV:** Ultraviolet; **SD:** Standard Deviation; **RSD:** Relative Standard Deviation; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification.

SUMMARY

The goal of the current study was to develop and validate three UV-spectrophotometric techniques for the simultaneous measurement of candesartan and simvastatin in the mixture. The Simultaneous Equation Method was the initial approach, with λ1 (the λ_{max} of Simvastatin) being 239 nm and λ2 (the λ_{max} of Candesartan) being 250 nm. The second technique was the first order derivative method, which used two distinct wavelengths with zero crossing points-263 nm for candesartan and 251.5 nm for simvastatin. The third approach, the Q-Absorbance ratio method, had λ1 (the isosbestic point) of 218.5 nm and λ2 (the simvastatin λ_{max}) of 239 nm. According to ICH criteria, the methods were validated. R2 values were found to be about 1. The techniques are sensitive enough to identify and measure trace levels of candesartan and simvastatin in the sample. The results showed that the methods were accurate (the percentage RSD was less than 2%). It was discovered that both approaches' recovery percentages fell between 98 and 101%. Therefore, it was determined that all three approaches were accurate, precise, quick, economical and easy to use. They may be applied to routine analysis for the simultaneous estimate of simvastatin and candesartan in the mixture.

REFERENCES

1. World health organization. Hypertension. 2023 [internet]. Available from: https://www.who.int/news-room/fact-sheets/detail/hypertension#:~:text=Overview,get%20your%20blood%20pressure%20checked
2. Mauro VF, MacDonald JL. Simvastatin: a review of its pharmacology and clinical use. Ann Pharmacother. 1991;25(3):257-64. https://doi.org/10.1177/106002809102500309
3. Drugbank Online. Simvastsatin. 2024 [internet]. Available from: https://go.drugbank.com/drugs/DB00641
4. Robinson JG. Simvastatin: present and future perspectives. Expert Opin Pharmacother. 2007;8(13):2159-27. https://doi.org/10.1517/14656566.8.13.2159
5. Husain A, Azim MS, Mitra M, Bhasin PS. A review on candesartan: pharmacological and pharmaceutical profile. J Appl Pharm Sci. 2011;30:12-7.
6. Figueroa-Campos A, Sánchez-Dengra B, Merino V, Dahan A, González-Álvarez I, García-Arieta A, *et al.* Candesartan cilexetil *in vitro-in vivo* correlation: predictive dissolution as a development tool. Pharmaceutics. 2020;12(7):633. https://doi.org/10.3390/pharmaceutics12070633
7. Alakhali KM. Method validation for analysis of simvastatin in human plasma using liquid chromatography tandem mass spectrometry (LC-MS-MS). J Clin Diagn Res. 2013;7(12):2739. https://doi.org/10.7860/JCDR/2013/6261.3748
8. Yang H, Feng Y, Luan Y. Determination of Simvastatin in human plasma by liquid chromatography-mass spectrometry. J Chromatogr B. 2003;785(2):369-75. https://doi.org/10.1016/S1570-0232(02)00800-0

9. Silva SC, Rezende GR, Boralli VB. Quick and simple LC-MS/MS method for the determination of simvastatin in human plasma: application to pharmacokinetics and bioequivalence studies. Braz J Pharm Sci. 2014;50:543-50. https://doi.org/10.1590/S1984-82502014000300013
10. Barrett B, Huclova J, Bořek-Dohalský V, Němec B, Jelinek I. Validated HPLC-MS/MS method for simultaneous determination of simvastatin and simvastatin hydroxy acid in human plasma. J Pharm Biomed Anal. 2006;41(2):517-26. https://doi.org/10.1016/j.jpba.2005.11.020
11. Partani P, Verma SM, Monif T. Development and validation of an LC-MS-MS method for determination of simvastatin and simvastatin acid in human plasma: application to a pharmacokinetic study. J Chromatogr Sci. 2016;54(8):1385-96. https://doi.org/10.1093/chromsci/bmw087
12. Moon SJ, Lee S, Jang K, Yu KS, Yim SV, Kim BH. Comparative pharmacokinetic and tolerability evaluation of two simvastatin 20 mg formulations in healthy Korean male volunteers. Transl clin pharmacol. 2017;25(1):10-4. https://doi.org/10.12793/tcp.2017.25.1.10
13. Karanam SR, Katakam P, Chandu BR, Hwisa NT, Adiki SK. Simultaneous determination of ezetimibe and simvastatin in rat plasma by stable-isotope dilution LC-ESI-MS/MS and its application to a pharmacokinetic study. J Pharm Anal. 2014;4(4):286-94. https://doi.org/10.1016/j.jpha.2013.08.002
14. GK D, RL S. Simple bioanalytical quantification method for simultaneous estimation of simvastatin and ezetimibe in human plasma by reverse-phase high-performance liquid chromatography technique. Asian J Pharm Clin Res. 2020;13(1):160-5. http://dx.doi.org/10.22159/ajpcr.2020.v13i1.36111
15. Elawady T, Ibrahim F, Khedr A, Belal F. Simultaneous determination of ezetimibe, atorvastatin and simvastatin using quadrupole LC-MS: Application to combined tablets and plasma after SPE. Acta Chromatogr. 2021;33(3):245-52. https://doi.org/10.1556/1326.2020.00752
16. Jeong HC, Seo YH, Gu N, Rhee MY, Shin KH. Determination of candesartan or olmesartan in hypertensive patient plasma using UPLC-MS/MS. Transl clin pharmacol. 2021;29(4):226. https://doi.org/10.12793/tcp.2021.29.e21
17. Karra VK, Pilli NR, Inamadugu JK, Rao JS. Simultaneous determination of pioglitazone and candesartan in human plasma by LC-MS/MS and its application to a human pharmacokinetic study. J Pharm Anal. 2012;2(3):167-73. https://doi.org/10.1016/j.jpha.2012.01.002
18. Bonthu MG, Atmakuri LR, Jangala VR. Simultaneous determination of candesartan and hydrochlorothiazide in human plasma by LC-MS/MS. Brazilian Journal of Pharmaceutical Braz J Pharm Sci. 2018;54(01):e17381. https://doi.org/10.1590/s2175-97902018000117381
19. Singh B, Lokhandae RS, Dwivedi A, Sharma S, Dubey N. Improved simultaneous quantitation of candesartan and hydrochlorthiazide in human plasma by UPLC-MS/MS and its application in bioequivalence studies. J Pharm Anal. 2014;4(2):144-52. https://doi.org/10.1016/j.jpha.2013.05.003
20. Dixit RP, Barhate CR, Padhye SG, Viswanathan CL, Nagarsenker MS. Stability indicating RP-HPLC method for simultaneous determination of simvastatin and ezetimibe from tablet dosage form. Indian J Pharm Sci. 2010;72(2):204. https://doi.org/10.4103/0250-474X.65028
21. Alshitari W, Al-Shehri F, Abd El-Hady D, Albishri HM. A simple HPLC method containing greener modifier and slighter temperature elevated for simultaneous determination of three statin drugs in tablets. Acta Chromatogr. 2021;34(2):210-5. https://doi.org/10.1556/1326.2021.00896
22. Jain N, Jain R, Swami H, Jain DK. RP-HPLC Method for Simultaneous Estimation of Simvastatin and Ezetimibe in Bulk Drug and its Combined Dosage Form. Asian J Res Chem. 2008;1(1):29-31.
23. Alhazmi HA, Alnami AM, Arishi MA, Alameer RK, Al Bratty M, Rehman ZU, Javed SA, Arbab IA. A fast and validated reversed-phase HPLC method for simultaneous determination of simvastatin, atorvastatin, telmisartan and irbesartan in bulk drugs and tablet formulations. Sci Pharm. 2018;86(1):1. https://doi.org/10.3390/scipharm86010001
24. Nováková L, Šatínský D, Solich P. HPLC methods for the determination of simvastatin and atorvastatin. Trends Anal Chem. 2008;27(4):352-67. https://doi.org/10.1016/j.trac.2008.01.013
25. Ashfaq M, Ullahkhan I, SHANAZ QUTAB SY, Naeemrazzaq S. HPLC determination of ezetimibe and simvastatin in pharmaceutical formulations. J Chil Chem Soc. 2007;52(3):1220-3. http://dx.doi.org/10.4067/S0717-97072007000300005
26. Rao A, Surya T. Development and validation of a RP-HPLC method for estimation of hydrochlorothiazide and candesartan cilexetil in pharmaceutical dosage form. Int J Pharm. 2013;3:166-9.
27. Revathi R, Saravanan VS, Ethiraj T, Marreddy JL. RP-HPLC analysis for quantitation of candesartan cilexetil in solid dosage forms. Asian J Pharm Anal. 2013;3(4):115-8.
28. Ranganath MK, Kalyani Arikatla K, Deka P. RP-HPLC method for simultaneous estimation of Candesartan and Amlodipine in bulk and pharmaceutical dosage forms. Int J Med Pharm Res. 2020;4(5):133-8.
29. Kulsum S, Sagar GV, Padmalatha M. Validated RP-HPLC Method for the Quantitation of Candesartan in Bulk and Pharmaceutical Dosage Forms. Res J Pharm Technol. 2011;4(6):997-8.
30. Babu KS, Kumar ND, Gosada U, Sharma N. A validated ultra high-pressure liquid chromatography method for separation of candesartan cilexetil impurities and its degradents in drug product. Pharm Methods. 2012;3(1):31-9. https://doi.org/10.4103/2229-4708.97718
31. Kola H, Shanmugasundaram S. Analytical quality by design-based RP-HPLC method for quantification of pioglitazone and candesartan cilexetil in bilayer tablet and its forced degradation studies. Pharmacia (0428-0296). 2023;70(1). https://doi.org/10.3897/pharmacia.70.e95099
32. Pradhan KK, Mishra US, Pattnaik S, Panda CK, Sahu KC. Development and validation of a stability-indicating UV spectroscopic method for candesartan in bulk and formulations. Indian J Pharm Sci. 2011;73(6):693. https://doi.org/10.4103/0250-474X.100254
33. Ravisankar P, Vidya VS, Manjusha K, Mounika YN, Babu PS. Validated UV spectrophotometric method for quantitative determination of candesartan cilexetil in bulk and pharmaceutical dosage form. Der Pharmacia Lettre. 2016;8(9):252-7.
34. Ahmad Charoo N, Bashir M, Abdalla E, Ali KI. Determination of candesartan cilexetil in tablet dosage forms and dissolution testing samples by first derivative UV spectrophotometric method. Anal Lett. 2009;42(14):2232-43. https://doi.org/10.1080/00032710903137434
35. Dey S, Pradhan PK, Upadhayay UM, Patel C, Lad B. Method development and validation of simvastatin by UV spectrophotometric method. J Pharm Res. 2012;5(12):5380-2.
36. Bandgar SA, Jadhav NR. Validated UV Spectrophotometric method for estimation of Simvastatin in Bulk and Pharmaceutical Formulation. Res J Pharm Technol. 2019;12(12):5745-8.
37. Wang L, Asgharnejad M. Second-derivative UV spectrometric determination of simvastatin in its tablet dosage form. J Pharm Biomed Anal. 2000;21(6):1243-8. https://doi.org/10.1016/S0731-7085(99)00231-9
38. Chavhan V, Reddy K, Ahhirao K. Development of UV spectrophotometric methods and validation for estimation of simvastatin in bulk and tablet dosage form by absorbance maxima and area under the curve method. J Appl Pharm. 2014;6:55-64.
39. Birari AE. Development and Validation of UV-Spectrophotometric Method for Estimation of Simvastatin in Bulk and Solid Dosage Form. Int J Pharm Sci Res. 2015;6(1):185-9.
40. Singla V, Bhaskar R, Bhaskar R. Simultaneous estimation of simvastatin and metformin hydrochloride in bulk and solid dosage forms. Rasayan J Chem. 2010;3(3):507-13.
41. Rajput G, Singh S, Kurmi BD. Simultaneous estimation of simvastatin and fenofibrate from their combined dosage form by ultraviolet-visible spectroscopy using simultaneous equation method. Pharmaspire. 2021;13:117-21.
42. Sunkara N, Rajeswari BU, Swathi B, Arunkumar S, Ratnakar N. UV spectrophotometric method development and validation of ezetimibe and simvastatin in bulk and pharmaceutical dosage form. Int J Pharma Chem Res. 2017;3(3):581-5.
43. Shah SR, Pradhan P, Dey S. Quantitative estimation of cefixime and moxifloxacin in pharmaceutical preparation by UV spectrophotometric method. Int J Pharm Tech Res. 2013;5(1):198-204.
44. ICH: Guideline on the Validation of Analytical Procedures: Methodology, 62 (96), 19th May 1997.
45. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use, ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Method Q2 (R1), Step 4, 2005.
46. Bose P, Dey S, Basak S, Shah S, De A. Simultaneous RP-HPLC and UV Spectroscopic Method Development and Validation for Estimation of Ibandronate Sodium in Bulk and Pharmaceutical Dosage Form. Anal Bioanal Chem. 2015;2(2):99-112. https://doi.org/10.22036/abcr.2015.10758

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