Research Article

Development and Validation of Second Order Derivative Spectrophotometric Method for Simultaneous Estimation of Simvastatin and Losartan Potassium in Synthetic Mixture

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ABSTRACT

A simple and sensitive second order derivative spectrophotometric method was developed for the simultaneous estimation of Simvastatin and Losartan Potassium in synthetic mixture. The derivative spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP). The second order derivative spectra was obtained in methanol and the determinations were made at 220.2 nm (ZCP of Simvastatin) for Losartan Potassium and 234.9 nm (ZCP of Losartan Potassium) for Simvastatin. The two drugs comply with beer lambert's law over the linearity range of 3-22 µg/ml. The method was validated as per ICH guidelines in terms of linearity, accuracy (recovery study), precision (repeatability, intraday, interday precision), limit of detection and limit of quantification. All the validation parameters were found to be within acceptable limits. The method was found to be simple, sensitive, rapid, cost effective, accurate, and precise for the routine analysis of both the drugs in pharmaceutical dosage form.

Keywords: Simvastatin, Losartan Potassium, Second order derivative spectrophotometric method, Zero crossing point, Pharmaceutical dosage form, Validation.

INTRODUCTION

Simvastatin (SIMVA) (Figure 1) is chemically, [(1S,3R,7S,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl]2,2-dimethylbutanoate, The empirical formula is $C_{25}H_{38}O_5$[1], Simvastatin is a White to off-white crystalline powder. It is very slightly soluble in methanol. Simvastatin is soluble in n-hexanel, sparingly soluble in ethanol. Simvastatin is a lipid-lowering agent. Simvastatin is a prodrug in which the 6-membered lactone ring of simvastatin is hydrolyzed in vivo to generate the beta,delta-dihydroxy acid, an active metabolite structurally similar to HMG-CoA (hydroxymethylglutaryl CoA). Once hydrolyzed, simvastatin competes with HMG-CoA for HMG-CoA reductase, a hepatic microsomal enzyme. Interference with the activity of this enzyme reduces the quantity of mevalonic acid, a precursor of cholesterol[2].

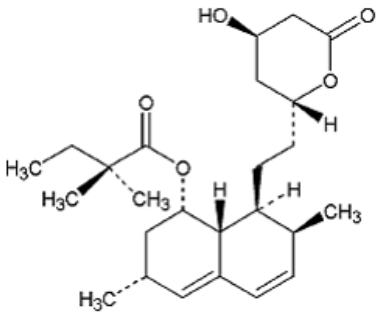

Fig. 1: Structure of Simvastatin

Losartan Potassium (LOSA) (Figure 2**)** is chemically, potassium;[2-butyl-5-chloro-3-[[4-[2-(1,2,3-triaza-4azanidacyclopenta-2,5-dien-5-yl)phenyl]phenyl]methyl]imidazol-4yl]methanol The empirical formula is $C_{22}H_{22}ClKN_6O$. Losartan Potassium is a white to yellowish substance. Soluble in Water, Slightly soluble in ethanol and methanol.[11] Losartan is an angiotensin-receptor blocker and Inhibition of angiotensin II binding to AT1 inhibits its AT1-mediated vasoconstrictive and aldosterone secreting effects and results in decreased vascular resistance and blood pressure[12]

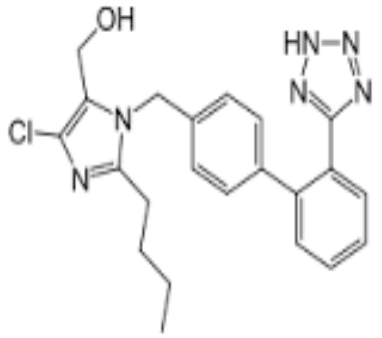

Fig. 2: Structure of Losartan Potassium

MATERIAL AND METHODS

Instruments

A Shimadzu double beam UV/Visible spectrophotometer instrument 1600 (Japan) with spectral width of 2 nm, wavelength accuracy of ± 0.5 nm and a pair of 10 mm equated quartz cell was used to measure absorbance of all the solutions. Spectra were certainly obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 5, Mumbai, India) was used in the study.

Materials and Reagents

Pure sample of SIMVA was provided as a gift sample from Emcure pharmaceuticals limited and LOSA was obtained from Intas pharmaceuticals limited. Synthetic mixture (500 mg) of SIMVA (20 mg) and LOSA (25 mg) was prepared in laboratory using generally used excipients (455 mg) like lactose, talc, magnesium stearate. Methanol AR Grade was received form S.D fine Chemicals Ltd, Mumbai, India. Whatman filter paper no 41. All the chemicals used were of analytical grade.

Preparation of Standard Stock Solution

An precisely weighed quantity of SIMVA (10 mg) and LOSA (10 mg) were transferred to a separate 100 ml volumetric flask and dissolved them and diluted to the mark with methanol to obtain standard solution having concentration of SIMVA (100 µg/ml) and LOSA (100 µg/ml).

Preparation of sample solution

A quantity of the synthetic mixture equivalent to 20 mg of SIMVA and 25 mg of LOSA was transferred to a 100 ml volumetric flask. The content was combined with methanol (50 ml), sonicated for 20 minute to dissolve the drug as perfectly as desirable. The solution was filtered through a Whatman filter paper No. 41. The volume was fixed up to the mark with methanol. An aliquot of this solution (1 ml) was transferred in to a 10 ml volumetric flask and the volume was fixed up to mark with methanol.

Methodology

The standard solution of SIMVA (10 µg/ml) and LOSA (10 µg/ml) were scanned separately in the UV range of 200-400 nm. The zero-order spectra thus obtained was then processed to obtain second-derivative spectra. The two spectra were overlain and it appeared that SIMVA showed zero crossing at 220.2 nm, while LOSA showed zero crossing at 234.9 nm. At the zero crossing point (ZCP) of SIMVA (220.2 nm), LOSA showed a second-derivative absorbance, whereas at the ZCP of LOSA (234.9 nm), SIMVA showed a second-derivative absorbance. Hence 234.9 and 220.2 nm was selected as analytical wavelengths for determination of SIMVA and LOSA, respectively. These two wavelengths can be employed for the determination of SIMVA and LOSA without any interference from the other additives in their synthetic mixture.

VALIDATION OF THE DEVELOPED METHOD

The method was validated as per the International Conference on Harmonization (ICH) guidelines.

Linearity (Calibration curve)

The calibration curves were constructed over a concentration range of 3 – 22 µg/ml for both drugs. Accurately measured standard working solutions of SIMVA (0.3, 0.6, 0.9, 1.2, 1.5, 1.8, and 2.2 ml) and LOSA (0.3, 0.6, 0.9, 1.2, 1.5, 1.8, and 2.2 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol and Second-derivative absorbance was measured at 234.9 nm for SIMVA and 220.2 nm for LOSA. The calibration curves were assembled by constructing absorbances versus concentrations and the regression equations were calculated.

Accuracy (recovery study)

The accuracy of an analytical procedure is the closeness of agreement between the value which is accepted as true value and the value found. The recovery experiment were carried out by adding known amount of standard solution of SIMVA and LOSA at 80%, 100%, and 120% level to prequantified sample solution of SIMVA (4 µg/ml) and LOSA (5 µg/ml). The amount of SIMVA and LOSA were analyzed by proposed method.

Method precision

Repeatability

Repeatability of the method was determined by analyzing standard solution of SIMVA and LOSA at (9 µg/ml for SIMVA and LOSA) six times without changing the parameters of measurement and % RSD was calculated.

Intraday and Interday precision

The intraday and interday precision of the suggested method was determined by examining the corresponding responses 3 times on the same day and on 3 different days for a period of 1 week for 3 different concentrations of standard solutions of SIMVA and LOSA (9, 12, 15 µg/ml for both). The result was reported in terms of relative standard deviation (% RSD).

Limit of detection and limit of quantification

ICH guideline describes several approaches to determine the detection and quantification limits. These include visual evaluation, signal-to-noise ratio by the use of standard deviation of the response and the slope of the calibration curve. The limit of detection (LOD) and limit of quantification (LOQ) were calculated using signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated:

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response,

S = slope of the calibration curve.

Determination of SIMVA and LOSA in synthetic mixture

Synthetic mixture was prepared by mixing generally used excipients in the pure drugs in our laboratory. Sample solution was prepared as described previously. The responses of the sample solution were measured at 234.9 nm and 220.2 nm for determination of SIMVA and LOSA, respectively. The amounts of the SIMVA and LOSA present in the sample solution were estimated by proposed methods.

RESULT AND DISCUSSION

In second derivative spectrophotometric method, the foremost and prime need is that the drugs should comply with the beer's law at selected wavelengths. Linear correlation was obtained between absorbance and concentration of SIMVA and LOSA in the concentration ranges of 3-22 µg/ml. The standard solutions of SIMVA and LOSA were scanned separately in the UV range, and zero-order spectra (Figure 3) thus obtained was then processed to obtain second-derivative spectra. The two derivative spectra showed maximum absorbance at 220.2 nm (ZCP of SIMVA) for LOSA and 234.9 nm (ZCP of LOSA) for SIMVA. Second-derivative absorbances (D1) were recorded 234.9 nm for SIMVA and 220.2 nm for LOSA (Figure 4). Second derivative spectra give good quantitative determination of both the drugs at their respective wavelengths without any interference from the excipients in their synthetic mixture.

Table 1: Recovery Data of SIMVA and LOSA by first order derivative spectrophotometric Method

% Level (n=3)	Amount of drug taken (µg/ml)		Amount standard added (µg/ml)		% Recovery ± S.D	
	SIMVA	LOSA	SIMVA	LOSA	% SIMVA ± S.D	%LOSA ± S.D
80%	4	5	3.2	4	101.0 ± 1.78	100.7 ± 1.21
100%	4	5	4	5	99.62 ± 0.65	99.8 ± 0.34
120%	4	5	4.8	6	100.52 ± 1.36	101.1 ± 1.92

S.D. is standard deviation and n is number of replicate.

Table 2: Analysis of SIMVA and LOSA in synthetic mixture in by developed method

Sr. No.	Label claim (mg)		Amount found (mg)		% Label claim ± S.D (n=3)	
1	SIMVA	LOSA	SIMVA	LOSA	SIMVA	LOSA
	20	25	19.90	25.02	99.48 ± 2.43	100.1 ± 3.32

S.D is standard deviation and n is number of replicate.

Table 3: Regression analysis data and summary of validation parameters by proposed second order derivative spectrophotometric method

Parameter	Second derivative spectrophotometric Method	
	SIMVA	LOSA
Wavelength	234.9 nm	220.2 nm
Beer's Law Linearity Range (µg/ml)	3-22	3-22
Regression equation (y=mx + c) Slope(m) Intercept(c)	y =0.0016x+0.0003 0.0016 0.0003	y =0.0004x+0.0004 0.0004 0.0004
Correlation Coefficient (r^2)	0.9994	0.9996
Repeatability (% RSD , n= 6)	1.69	1.70
Intraday Precision %RSD (n = 3)	1.02 – 1.82	1.21 - 1.63
Interday Precision %RSD (n = 3)	1.63 - 1.76	1.57 - 1.80
LOD (µg/ml)	0.51	0.90
LOQ (µg/ml)	1.56	2.75
Accuracy (n=3) (Mean % Recovery ± S.D)	100.39 ± 1.27	100.05 ± 1.16
%Assay ± S.D.(n=3)	99.48 ± 2.43	100.1 ± 3.32

LOD = Limit of detection, LOQ = Limit of quantification, RSD = Relative standard deviation, S. D. = Standard deviation, n = number of replicates

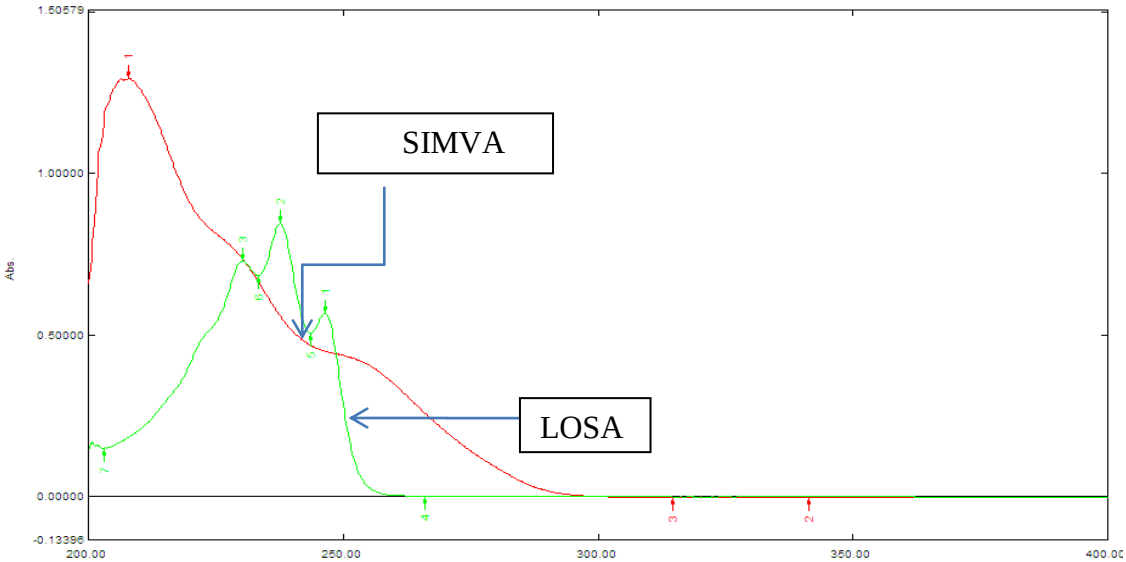

Fig. 3: Overlain zero order spectra of SIMVA (10 µg/ml) and LOSA (10 µg/ml)

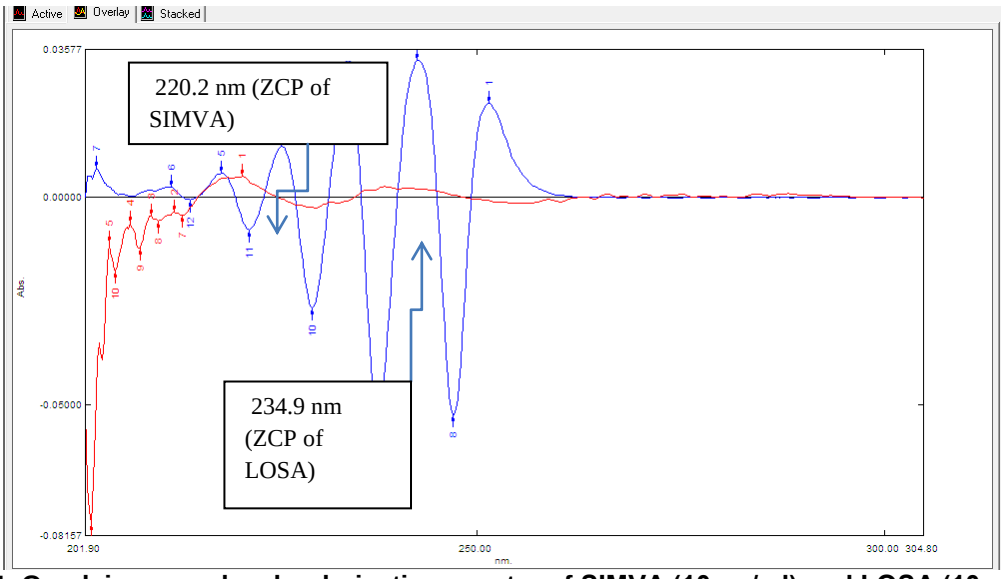

Fig. 4: Overlain second-order derivative spectra of SIMVA (10 µg/ml) and LOSA (10 µg/ml)

The validation parameters were studied at all the selected wavelengths for the developed method. All the validation parameters were found to be within acceptable limits. The % recoveries were found to be in the range of 99.62 – 101.00 % for SIMVA and 99.8 – 101.1 % for LOSA (Table 1). The precision of method was determination by repeatability, intraday, interday precision and was expressed as the % RSD which indicates good method precision (Table 3), The LOD and LOQ for SIMVA at 234.9 nm were found to be 0.51 µg/ml and 1.56 µg/ml, respectively. The LOD and LOQ for LOSA at 220.2 nm were found to be 0.90 µg/ml and 2.75 µg/ml, respectively. All the regression and validation parameters are summarized in Table 3. The proposed spectrophotometric method was successfully applied to SIMVA and LOSA in synthetic mixture. SIMVA and LOSA content in synthetic mixture were found to be 99.48 % and 100.1 % respectively (Table 2).

CONCLUSION

The result of the analysis of synthetic mixture by the suggested method is highly reproducible and reliable and it is in good agreement with the label claim of drug. The method can be used for the regular analysis of the SIMVA and LOSA in synthetic mixture without any intervention of the excipients.

ACKNOWLEDGEMENT

The authors wish to thank Emcure pharmaceuticals limited and Intas pharmaceuticals limited for providing SIMVA and LOSA pure drug powder as gift sample for research work. The authors are highly thankful to Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Kherva, Mehsana, Gujarat, India for providing all the facilities to carry out the work.

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