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Q-ABSORBANCE RATIO METHOD FOR SIMULTANEOUS DETERMINATION OF SIMVASTATIN AND LOSARTAN POTASSIUM IN SYNTHETIC MIXTURE

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ABSTARCT

A simple and sensitive spectrophotometric method based on Q - absorbance ratio was developed for the simultaneous estimation of Simvastatin and Losartan Potassium in pharmaceutical synthetic mixture. Q - absorbance ratio method based on formation of Q-absorbance equation at two wavelengths, one is isoabsorptive point and another is the λ-max of one of the two drugs. Absorbance are measured at two selected wavelengths, one is 248.5 nm (isoabsorptive point) and another being 237 nm (λ-max of SIMVA). 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 guideline rules in terms of linearity, accuracy (recovery study), precision (repeatability, intraday, interday validation), limit of detection, 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 the binary mixture.

KEYWORDS: Simvastatin, Losartan Potassium, Q - absorbance ratio method, Binary mixture, 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]

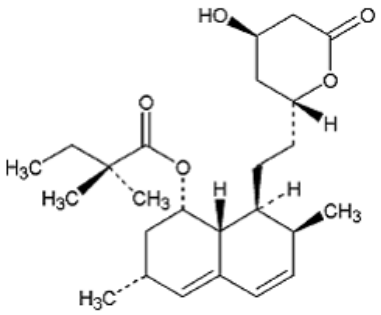

Figure 1: Structure of Simvastatin

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]

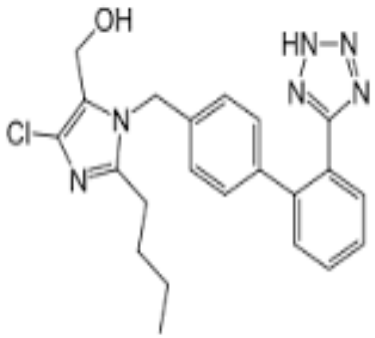

Figure 2: Losartan Potassium

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.

Determination of the analytical wavelengths

The solutions of SIMVA and LOSA were prepared separately in methanol having concentration of 10 µg/ml. These solutions were scanned separately in the range of 200-400 nm against methanol as a blank. Data were recorded at an interval of 1 nm. From the overlain spectra of the drugs, two analytical wavelengths i.e. 248.5 nm (isoabsorptive point) and 237

nm (λmax of SIMVA) were selected and absorbances were measured at these selected wavelengths for determination.

METHODOLOGY

Q - absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two drugs. From the overlay spectra of two drugs, it is observed that SIMVA and LOSA show an isoabsorptive point at 248.5 nm. The second wavelength chosen is 237 nm, which is the λ-max of SIMVA. Working standard solutions having concentration 3, 6, 9, 12 ,15, 18 and 22 µg/ml for SIMVA and LOSA were prepared in methanol and the absorbances at 248.5 nm (isoabsorptive point) and 237 nm (λ-max of SIMVA) were measured and absorptivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using following equations.

$CX = [(QM – QY) / (QX - QY)] \times A1/ax1$..... (1)

$CY = [(QM – QX) / (QY - QX)] \times A1/ay1$ …… (2)

Where, A1 and A2 are absorbances of mixture at 248.5 nm and 237 nm; ax1 and ay1 are absorptivities of SIMVA and LOSA at 248.5 nm; ax2 and ay2 are absorptivities of SIMVA and LOSA respectively at 237 nm; QM = A2 / A1, QX = ax2 / ax1 and QY = ay2 / ay1.

Validation of the proposed method

The method was validated as per the rules of International Conference on Harmonization (ICH) guidelines.[38]

Calibration curve (linearity)

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 the absorbance was measured at 248.5 nm (isoabsorptive point) and at 237 nm (λmax of SIMVA). The calibration curves were assembled by constructing absorbances versus concentrations and the regression equations were calculated.

Accuracy (% recovery)

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 prequntified sample solution of SIMVA (4 µg/ml) and LOSA (5 µg/ml). The amount of SIMVA and LOSA were calculated by putting obtained values in the equation (1) and (2). The recovery study analysis was repeated tree times and average recoveries were calculated.

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.

Intermediate precision (Reproducibility)

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 (6, 9, 12 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 quantitation 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 248.5 nm and 237 nm for determination of SIMVA and

LOSA, respectively. The amounts of the SIMVA and LOSA present in the sample solution were estimated by solving the equations which are given below:

Cx = (Qm - Qy) A1 / (Qx - Qy) ax1

Cy = A1 / ax1- Cx

RESULT AND DISCUSSION

In Q - absorbance ratio spectrophotometry method, the foremost and prime need is that both the drugs should comply with the beer's law at all the wavelength. SIMVA and LOSA obeyed linearity in the concentration range of 3-22 µg/ml in methanol at their respective λ-max and isoabsorptive point with correlation coefficient (r^2>0.99). The overlain absorption spectra of SIMVA and LOSA showing isoabsorptive point in methanol is shown in Figure 1.

Table 1: Recovery data for the proposed method.

Drug	Level	Amount taken (µg/ml)	Amount added (%)	% Mean recovery ± S.D. (n=3)
SIMVA	I	4	80	98.96 ± 1.80
	II	4	100	100.83 ± 1.44
	III	4	120	102.08 ± 1.22
LOSA	I	5	80	98.33 ± 1.44
	II	5	100	100.33 ± 0.57
	III	5	120	102.22 ± 0.38

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

Table 2: Determination of drugs in synthetic mixture by proposed method.

Sr. No.	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n =3)	
1	SIMVA	LOSA	SIMVA	LOSA	SIMVA	LOSA
	20	25	20.08	24.92	100.4 ±1.29	99.67 ±1.03

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

Table 3: Regression analysis data and summery of validation parameters for the developed method

PARAMETERS	Q-Absorbance ratio method		
	SIMVA at 237 nm	LOSA at 237 nm	SIMVA and LOSA at Isoabsorptive point (248.5 nm)
Wavelength range (nm)	237	237	248.5
Beer's law limit (µg/ml)	3-22	3-22	3-22
Regression equation (y=mx + c)	y =0.0683x+0.0126	y =0.0468x+0.007	y =0.0373x+0.0038
Slope(m)	0.0683	0.0468	0.0373

Intercept(c)	0.0126	0.007	0.0038
Correlation Coefficient (r^2)	0.9994	0.9998	0.9999
Repeatability (n=6) (%R.S.D)	1.10	0.48	0.64
Intraday (n=3) (%R.S.D)	0.36-0.77	1.11-1.70	0.27-1.04
Interday (n=3) (%R.S.D)	0.36-0.67	0.69-1.66	0.64-0.89
LOD (µg/ml)	0.75	0.37	0.22
LOQ (µg/ml)	2.27	1.13	0.67
Accuracy (n=3) (Mean % Recovery ± S.D)	100.40 ± 1.49	99.63 ± 0.80	-
%Assay ± S.D.(n=3)	100.4 % ± 1.29	99.67 % ± 1.03	-

RSD=Relative standard deviation. LOD=Limit of detection. LOQ=Limit of quantification. SD=Standard deviation.

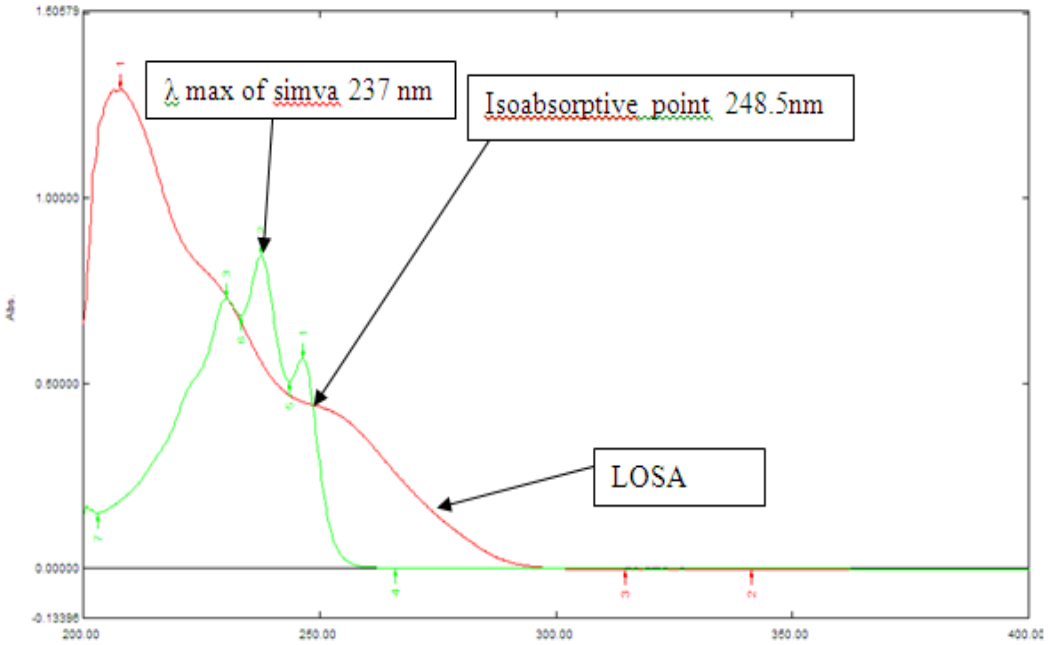

Figure 1: Overlain spectrum SIMVA (10µg/ml) and LOSA (10µg/ml) in methanol

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 98.96-102.08% for SIMVA and 98.33-102.22% for LOSA (Table 1). The precision of method was determined by repeatability, intraday and interday precision and was expressed as the % RSD which indicates good method precision (Table 3). The Limit of detection 0.75 µg/ml at 237 nm for SIMVA, 0.37 µg/ml at 237 nm for LOSA and 0.22 µg/ml at isoabsorptive point (248.5 nm). Limit of quantification for SIMVA and LOSA was found at 237 nm were 2.27 µg/ml and 1.13 µg/ml respectively and at isoabsorptive point LOQ was 0.67 µg/ml (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 100.42% and 99.67% 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.

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