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Simultaneous estimation of ezetimibe and simvastatin by vierodt's method

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Abstract

A simple, fast, and precise simultaneous spectrometric method for the estimation of Ezetimibe and Simvastatin in API and in synthetic mixture was developed. The proposed method is based on the formation and solving of simultaneous equations using 230 and 247.6nm as two analytical wavelength. Ezetimibe shows absortion maximum at 230nm and simvastatin shows absorbance at 247.6 nm in methanol. Beer's law was obeyed in the concentration range of 2-20µg/ml for Ezetimibe and 2-16 µg/ml for simvastatin. The molar absorptivity and sandell's sensitivity were found to be 1.703 $x10^4$ and 2.4 x 10^{-2} respectively for Ezetimibe and for simvastatin 2.23 x $10^4$and 1.87 x 10^{-2} respectively. The method allow rapid analysis of binary pharmaceutical formulation with accuracy. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method. The developed method was found to be very precise as % C.V calculated came out to be less than 2%.

Key-words: Ezetimibe, simvastatin, simultaneous spectrometric analysis, method validation

Introduction

Ezetimibe (EZ), chemically1- (4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone, is in class of lipid lowering compound that selectively inhibits the intestinal absorption of cholesterol and related phytosterols. The drug is not official in any pharmacopoeia. Only HPLC (in bulk and formulations)[1] and LC/MS (in plasma)[2] methods have been reported for the estimation of Ezetimibe. Simvastatin, chemically Butanoic acid, 2,2-dimethyl-1, 2,3,7,8,8a-hexahydro-3, 7-dimethyl-8- [2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)-ethyl]-1-naphthalenyl ester is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzymeA (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol biosynthesis. The drug is official in USP-2003 [3] and B.P-2004 [4]. Several methods such as LC-MS/MS [5], HPLC [6] and second derivative spectroscopy [7] have been reported for estimation of this drug.

Fixed dosage combination containing Atorvastatin and Ezetimibe is available only in tablet form by the brand name 'Vytorin' in US market. Even though various methods reported in the literature for estimation of Ezetimibe and Simvastatin individual or in combination with other drugs no method had been reported for simultaneous estimation of these two drugs using simultaneous equations in synthetic mixture.The present study was aimed at the simultaneous estimation of Ezetimibe and Simvastatin by simultaneous equations method. This method was validated according to the ICH guidelines.

Material and methods

Instrument: Double beam UV - visible spectrophotometer (Shimadzu UV-1800) with 1 cm matched quartz cells, band pass 1.8 nm, Shimadzu -AX -200 electronic weighing balance and Ultrasonicator, Enertech electronics Pvt. Ltd.

Drug Sample: Ezetimibe and Simvastatin were obtained as gift sample from M/s Ind-Swift Laboratories Ltd., Patiala (Punjab).

Chemicals and Reagent: Methanol A.R. Grade (Loba Chemie, Mumbai).

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Preparation of standard solutions

Accurately weighed Ezetimibe (10 mg) and Simvastatin (10 mg) were transferred in 100 ml volumetric flask separately, dissolved and diluted upto the mark with methanol. The final solutions contained 100 µg per ml of the solutions.

Preparation of API mixture and synthetic mixture of Ezetimibe and Simvastatin

The API mixture and synthetic mixture of Ezetimibe and Simvastatin was prepared in ratio of 1:1. For API mixture, accurately weighed 10 mg each of Ezetimibe and Simvastatin were transferred to a 100 ml volumetric flask, dissolved and diluted upto the mark with methanol. The synthetic mixture was prepared by addition of different excipients along with accurately weighed 10 mg each of Ezetimibe and Simvastatin to 100 ml volumetric flask. The mixture was dissolved in 25 ml methanol and sonicated for 20 minutes. The solution was diluted upto the mark with methanol and filtered through Whatman filter paper No. 40.

Determination of wavelength of maximum absorbance

Standard Ezetimibe solution (2.5 ml) and Simvastatin (2.5 ml) were transferred into 25 ml volumetric flask. The volume was adjusted to 25 ml with methanol. The absorbance of the final solution was scanned in the range 200 to 400 nm against methanol as blank. Maximum absorbance was obtained at 230 nm and 247.6 nm for Ezetimibe and Simvastatin respectively.

Preparation of calibration curve for Ezetimibe and Simvastatin

Standard solutions of Ezetimibe (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 ml) and standard solutions of Simvastatin (0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 ml) were transferred in to a series of 25 ml volumetric flask. The volume was adjusted with methanol and mixed. The absorbances of the solutions were measured at 230 nm and 247.6 nm against methanol as blank.

Estimation of Ezetimibe and Simvastatin in API mixture and synthetic mixture

The API mixture and synthetic mixture (1, 2 and 3 ml) were transferred and diluted to mark with methanol. The absorbances of these solutions were measured at 230 nm and 247.6 nm. Amount of Ezetimibe and Simvastatin was determined by solving the simultaneous equations**.** Two simultaneous equations were formed using these absorptivity coefficient values.

$$A_1 = 41.62 \times C_1 + 75.58\ C_2 \text{------- (1)}$$

$$A_2 = 39.37 \times C_1 + 53.46\ C_2 \text{------- (2)}$$

Where C_1 and C_2 are concentration of Ezetimibe and Simvastatin respectively in gm/liter in the sample solution. A_1 and A_2 are the absorbances of the mixture at 230 nm and 247.6 nm respectively.

Recovery Studies and Validation of the Method according to I.C.H Guidelines [8]

To study the accuracy, reproducibility and precision of the above-proposed method, recovery studies were carried out by addition of standard drug solution to pre-analyzed samples taking into consideration percentage purity of added bulk drug sample. Precision of the method was studied by carrying out interday, intraday analysis and expressed as % C.V. Limit of Detection (LOD) and Limit of Quantitation (LOQ) were studied based on standard deviation of the response and the slope.

Results and discussion

The optical characteristics such as Beer's law limits, molar extinction coefficient, Sandell's sensitivity, correlation coefficient, slope and intercept of regression equation are summarized in Table 1.The values obtained for determination of Ezetimibe and Simvastatin in synthetic mixture by developed method are summarized in Table 3.To evaluate the validity and reproducibility of the method, known amounts of pure drug was added to pre-analyzed synthetic mixture and mixture were analyzed by developed method and percent recoveries are given in Table 2. Interference studies reveal that the common excipients and other additives usually present in the dosage form do not interfere in the developed method. The low value of standard deviation and % C.V (less than 2% at each step of validation) as given in Table II confirms the precision of the method. The Limit of detection (L.O.D) and Limit of quantitation (L.O.Q) for Simvastatin and Ezetimibe are shown in Table II. In conclusion, the developed spectrophotometric method is simple, sensitive, accurate and reproducible and can be used for routine simultaneous determination of Ezetimibe and Simvastatin in bulk as well as in synthetic mixture.

TableNo. 1: Optical Parameters and Regression Characteristics of Ezetimibe and Simvastatin in Methanol

Parameters	Ezetimibe		Simvastatin	
	230 nm	247.6 nm	230 nm	247.6 nm
Beers's law limit (µg/ml)	2-20	2-20	2-10	2-16
Molar absorptivity ($l\ mole^{-1}cm^{-1}$)	1.703×10^4	1.61×10^4	3.16×10^4	2.23×10^4
Sandell's sensitivity (mg/cm^2/.001absorbance unit)	2.4×10^{-2}	2.54×10^{-2}	1.32×10^{-2}	1.87×10^{-2}
Regression equation (y= a + bc)				
slope (b)	0.0437	0.048	0.1122	0.0626
intercept (a)	-0.081	-0.0112	-0.1583	-0.0514
Correlation coefficient	0.9992	0.9995	0.9994	0.9993

Table No. 2: Summary of Validation Parameters for Combination of Ezetimibe and Simvastatin

Parameters	Observations	
	SIM	EZ
Precision (% CV)		
A. Repeatability (n= 6)	0.98	0.65
B. Intra-day (n=9)	1.33	1.05
C. Inter-day (n=9)	1.45	1.078
% Recovery	98.42- 100.418	99.12- 100.324
Specificity	No interference found	No interference found
Limit of detection (µg / ml)	0.279	0.370
Limit of quantitation (µg / ml)	1.09	1.25

Table No. 3: Summary of Results of Simultaneous Estimation of Ezetimibe and Simvastatin in Synthetic Mixture

Mixture	Amount (mg)		Amount found[a] (mg)		%Recovery found [a]		% CV	
	SIM	EZ	SIM	EZ	SIM	EZ	SIM	EZ
A	10	10	9.92 ±0.111	10.09 ±0.045	99.22 ±1.01	100.75 ±0.75	1.09	0.98
B	10	10	9.85 ±0.169	10.12 ±0.05	98.96 ±1.15	101.85 ±0.50	1.43	0.789

a: average of four readings

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