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Simultaneous Estimation of Ezetimibe and Lovastatin by Derivative Spectroscopy

S.J.RAJPUT[1] AND H.A.RAJ[2]*

**[1]Pharmaceutical Quality Assurance Laboratory.
Pharmacy Department, Faculty of Technology & Engineering M. S. University of Baroda, Vadodara – 390001, India.**

[2*] Department of Quality Assurance, Shri Sarvajanik Pharmacy College, Near Arvind Baug, Mehsana - 384 001,India.

**[*]E mail: contacthasu@yahoo.co.in
Ph. No: 0932777619**

Abstract: Derivative spectroscopy offers a useful approach for the analysis of drugs in multi-component mixtures. In this study a first-derivative spectroscopic method was used for simultaneous determination of ezetimibe and lovastatin using the zero-crossing technique. The measurements were carried out at wavelengths of 265.20 and 245.4 nm for ezetimibe and lovastatin respectively. The method was found to be linear (r^2>0.9993) in the range of 1- 40 µg/ml for ezetimibe at 265.20 nm. The linear correlation was obtained (r^2>0.9950) in the range of 1-40 µg/ml for lovastatin at 245.4 nm. The limit of determination was 0.39 and 0.12 µg/ml for ezetimibe and lovastatin respectively. The limit of quantification was1.3 and 0.41 µg/ml. The method was successfully applied for simultaneous determination of ezetimibe and lovastatin in binary mixture.
Key words: *Ezetimibe; Lovastatin; simultaneous determination; first Derivative zerocrossing; Spectroscopic determination*.

INTRODUCTION:

Ezetimibe is a new anti-hyperlipidemic agent and chemically it is, 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-hydroxyprppyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone[1]. It is a selective cholesterol absorption inhibitor that effectively blocks intestinal absorption of dietary and biliary cholestrol[2]. LDL lowering and statins drug a combination was reduced LDL by >50% of baseline often cannot be achieved [3-5]. With new evidence for benefit of LDL reduction <70 mg % in high risk CAD patients, there is a need of a newer antihyperlipidemic drug combination. Ezetimibe in combination with statins is found to be more efficacious in reducing LDL levels. When high doses of statins are required for therapeutic goals or there is side effect with high statin doses, a combination of ezetimibe with low statin doses is a safe and effective alternative in dyslipidemia management. There is additional 15 – 20 % reduction in LDL with same statin doses, if combined with ezetimibe[6]. One of the statin from that is lovastatin, a potent statin presently available in the market. It inhibits the enzyme 3-hydroxy-3-methyl glutaryl coenzyme A (HMG CoA) reductase. Lovastatin when combined in low doses i.e. 10-20 mg/day; with ezetimibe can be a most potent and safe combination for reduction of LDL-cholestrol[7]. So the combination formulation might be in offing. Lovastatin is official in B.P and the official method is a LC methosd[8]. Other methods reported in literature are TLC [9,10] and HPTLC[11]. One the HPLC[12] method and a GC[13] method are available for simultaneous estimation of lovastatin and simvastatin. Similarly for ezetimibe, RP-HPLC[14], HPLC[15], LCMSMS[16] methods are reported. But no official or reported procedure is present for simultaneous determination of ezetimibe and lovastatin in pharmaceutical preparations. The reported procedures are time consuming, expensive and relatively complicated. Derivative spectroscopy provides a greater selectivity than common spectroscopy and offers a powerful approach for resolution of band overlapping quantitative analysis of multicomponent mixture[15, 16]. The aim of this study was to develop a simple, fast and sensitive derivative spectroscopic method for simultaneous determination of ezetimibe and lovastatin in binary mixtures on the basis of zero-crossing measurement. This

method could be applied for determination of both drugs in the presence of each other.

MATERIALS AND METHODS:

CHEMICALS AND REAGENTS

Ezetimibe and lovastatin were obtained as a gift sample from Sun pharmaceutical Ltd. Baroda. Methanol used was of analytical grades and obtained form S.D. fine chemicals. Commercial pharmaceutical formulations of the drugs are not yet available in market so the binary mixture was made by mixing tablets of both the individual drug, containing 10 mg of ezetimibe (Ezetib, Unisearch) and 10 mg of lovastatin (Statin, Unisearch), which was analyzed by the proposed technique.

INSTRUMENTS

A shimadzu UV-1700 double beam UV-Visible spectrophotometer with software of uvprobe was used for all measurements. The zero order absorption spectra were recorded over the wavelength range of 200 –380 nm, against a solvent blank, in quartz cuvettes with 1 cm diameter. For all solutions, the derivative spectra were obtained over 200-380 nm range at 2nm slit width ($\Delta\lambda$). The ordinate maximum and minimum were adjusted to the magnitude of derivative values.

STANDARD AND CALIBRATION SOLUTIONS

Standard stock solution of ezetimibe and lovastatin were prepared by seperately dissolving 10 mg of ezetimibe and lovastatin respectively in 100 ml methanol. Accurate volumes were transferred into two sets of 10 ml calibrated flask. The first series contained varying concentrations of ezetimibe (1– 40 µg/ml). The second series contained varying concentration of lovastatin (1–40 µg/ml). The calibration curves for derivative spectroscopy were constructed by plotting drug concentration versus the absorbance values of the first derivate spectrum (D_1) at 265.20 nm for ezetimibe and at 245.4 nm for lovastatin and the regression equation was computed.

SPECTROSCOPIC MEASUREMENTS

The difference between spectra of standard solutions of ezetimibe and lovastatin versus their solvent blanks were recorded in the rang of 200 – 380 nm. The first order derivative spectra of the standard solutions of each drug and those containing mixtures of both drugs were obtained in the same range of wavelength (200 – 380 nm) against blanks. The values of D_1 amplitudes for ezetimibe in the presence of lovastatin and vice versa measured at 265.20 nm (zero-crossing of lovastatin) and 245.4 nm (zero crossing of ezetimibe) respectively.

ACCURACY AND PRECISION

To establish the reliability of the proposed method, two series of solutions containing 10, 20, 30 and 40 µg/ml of ezetimibe plus 10 µg/ml of lovastatin and 10, 20, 30 and 40 µg/ml of lovastatin plus 10 µg/ml ezetimibe were prepared respectively and analyzed as discussed above. Precision of the procedure was calculated by within – day and between-day variations. Accuracy of the method was measured as percentage of deviation between added and measured concentrations (recovery study).

ANALYSIS OF TABLETS

Ten tablets each of lovastatin (Statin, 10 mg Unisearch) and ezetimibe (Ezetib, 10mg Unisearch) were weighed accurately and powdered. The powder equivalent to 10 mg of lovastatin and 10 mg of ezetimibe was weighed accurately and transferred to 100 ml volumetric flask. 20 ml methanol was added to it and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with menthol. This solution is expected to contain 100 µg/ml lovastatin and 100 µg/ml ezetimibe. From the stock solution, 1ml was taken in to a 10 ml volumetric flask and the volume make up to the mark with methanol to get a final concentration of lovastatin (10 µg/ml) and ezetimibe (10 µg/ml). The concentration of ezetimibe and lovastatin in tablets were calculated using the corresponding calibrated curve.

RESULTS AND DISCUSSION:

DERIVATE SPECTROSCOPIC METHOD

Zero-order absorption spectra of ezetimibe and lovastatin showed overlapping peaks that interfere with the simultaneous determination of this formulation (fig 1) Development of a method for simultaneous determination of two or more compounds in a sample without previous separation is always of interest. Derivative spectroscopy, based on a mathematical transformation of the spectra zero-order curve into the derivative spectra, allows a fast, sensitive and precise resolution of a multicomponent mixture and overcomes the problem of overlapping of a multi-component system. Derivative spectroscopy on the basis of zero-crossing measurements involves measurement of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero-crossing wavelength of the derivative spectra of individual components, which should be only a function of the concentration of other componet. The spectroscopic parameters including derivative order, wavelength and $\Delta\lambda$ values should be optimized to obtain maximum resolution, sensitivity and reproducibility. In this study first-derivative technique (D_1) traced with $\Delta\lambda$ = 2 nm was used to resolve the spectral overlapping. Zero – crossing points of 200-380 nm is presented in fig 2. The optimum D_1 values without interference for ezetimibe and lovastatin were 265.20 and 245.4 nm respectively (fig 2).

CALIBRATION CURVES AND STATISTICAL ANALYSIS

The linearity of the method was established form first-derivative spectra by measurement of the absorbance of standard solutions containing varying concentrations of each compound in the presence of constant concentration of the other one. The calibration curves were constructed by plotting the D_1 value against ezetimibe or lovastatin concentration at the zero-crossing wavelength of lovastatin (265.20 nm) or ezetimibe (245.4 nm) respectively. The obtained results are summarized in

Table 1. The linearity of the calibration curves and the adherence of the method to Beer's law are validated by the high value of the correlation coefficient and the value of intercept on ordinate which is close to zero.

VALIDATION

The limit of detection that was found to be 0.39 µg/ml and 0.12 µg/ml for ezetimibe and lovastatin. The accuracy and precision were determined by using synthetic mixture of ezetimibe and lovastatin in the laboratory. The mean recoveries and SD are illustrated in Tables 2 and 3. Data of these tables showed a good accuracy and precision over the entire concentration range. The within-day and between-day variations showed co-efficient of variation (CV%) values less than 1% for both ezetimibe and lovastatin respectively in all four selected concentrations. The data indicate that the proposed derivative spectroscopic method is highly precise during one analysis and between different runs.

The percentage of recovery in each case was calculated. The results obtained from the recoveries of both drugs (Tables 2,3) showed excellent accuracy. The influence of excipients was studied by mixing two formulation containing 10 µg/ml of ezetimibe and 10 µg/ml of lovastatin. No interference was observed from the presence of excipient in the amounts, which are commonly present in tablet dosage forms. Study of stability of ezetimibe and lovastatin in the solutions during analysis showed that analytes were stable at least for 72 hr in solutions.

The proposed method was successfully applied to analyze preparation containing ezetimibe and lovastatin. The results are summarized in Table 5. The results obtained are in good agreement with the labeled content.

From the results of this study it may be concluded that the proposed first-derivative spectroscopic method for simultaneous determination of ezetimibe and lovastatin is a simple, rapid, practical, reliable and inexpensive method that may be used for routine analysis. Furthermore, no preliminary separation, as well as expensive and unavailable instrument is required.

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TABLE 1: STATISTICAL DATA OF CALIBRATION CURVES OF EZETIMIBEAND LOVASTATIN USING FIRST-DERIVATIVE SPECTRA.

Parameters	Ezetimibe	Lovastatin
Wavelength (nm)	265.20	245.4
Linearity (µg/ml)	1-40	1-40
Regression equation *	Y=0.00018X ±0.00001	Y= 0.0012x ±0.0032
Correlation coefficient	0.9993	0.9950
Limit of detection (µg/ml)	0.39	0.12
Limit of quantification (µg/ml)	1.3	0.41

*Y=bx + a, where x is the concentration of drug in µg/ml Y is the amplitude at the specified wavelength, b is slope and a is intercept.

TABLE 2: ACCURACY AND PRECISION DATA FOR DETERMINATION OFEZETIMIBE IN THE PRESENCE OF LOVASTATIN (10 MCG/ML) BYFIRST DERIVATIVE SPECTROSCOPY.

Added amount of ezetimibe (µg/ml)	Found (µg/ml) SD	
	Within day *	Between day *
10	9.98±0.12	10.02± 0.17
20	19.99± 0.36	20.10 ±0.25
30	30.01± 0.14	29.98 ±0.19
40	39.90 ±0.39	40.19± 0.20

* Mean of six determinations

TABLE 3: ACCURACY AND PRECISION DATA FOR DETERMINATION OF LOVASTATIN IN THE PRESENCE OF EZETIMIBE (10 MCG/ML) BY FIRST DERIVATIVE SPECTROSCOPY.

Added amount of lovastatin (µg/ml)	Found (µg/ml) SD	
	Within day *	Between day *
10	10.02±0.10	9.99±0.11
20	20.10± 0.17	20.10±0.27
30	30.11±0.11	29.98± 0.39
40	39.98±0.39	39.99± 0.40

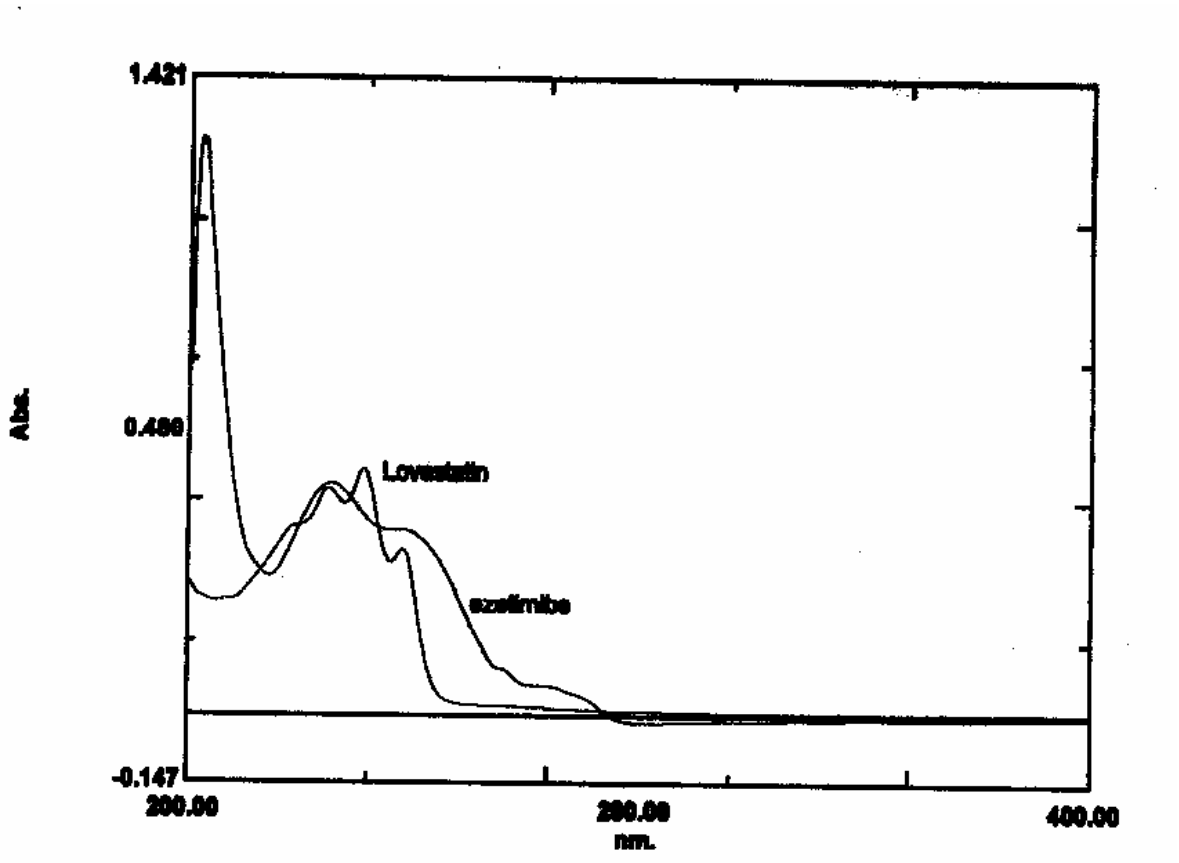
* Mean of six determinations

TABLE 4: RESULTS OF THE ANALYSIS OF BINARY MIXTURE.

Formulation	Lovastatin % Found ± S.D. (n=4)*	Ezetimibe % Found ± S.D. (n=4)*
Mixture 1	99.8 ± 0.39	100.38 ± 0.22
Mixture 2	101.2 ± 0.20	99.9 ± 0.12

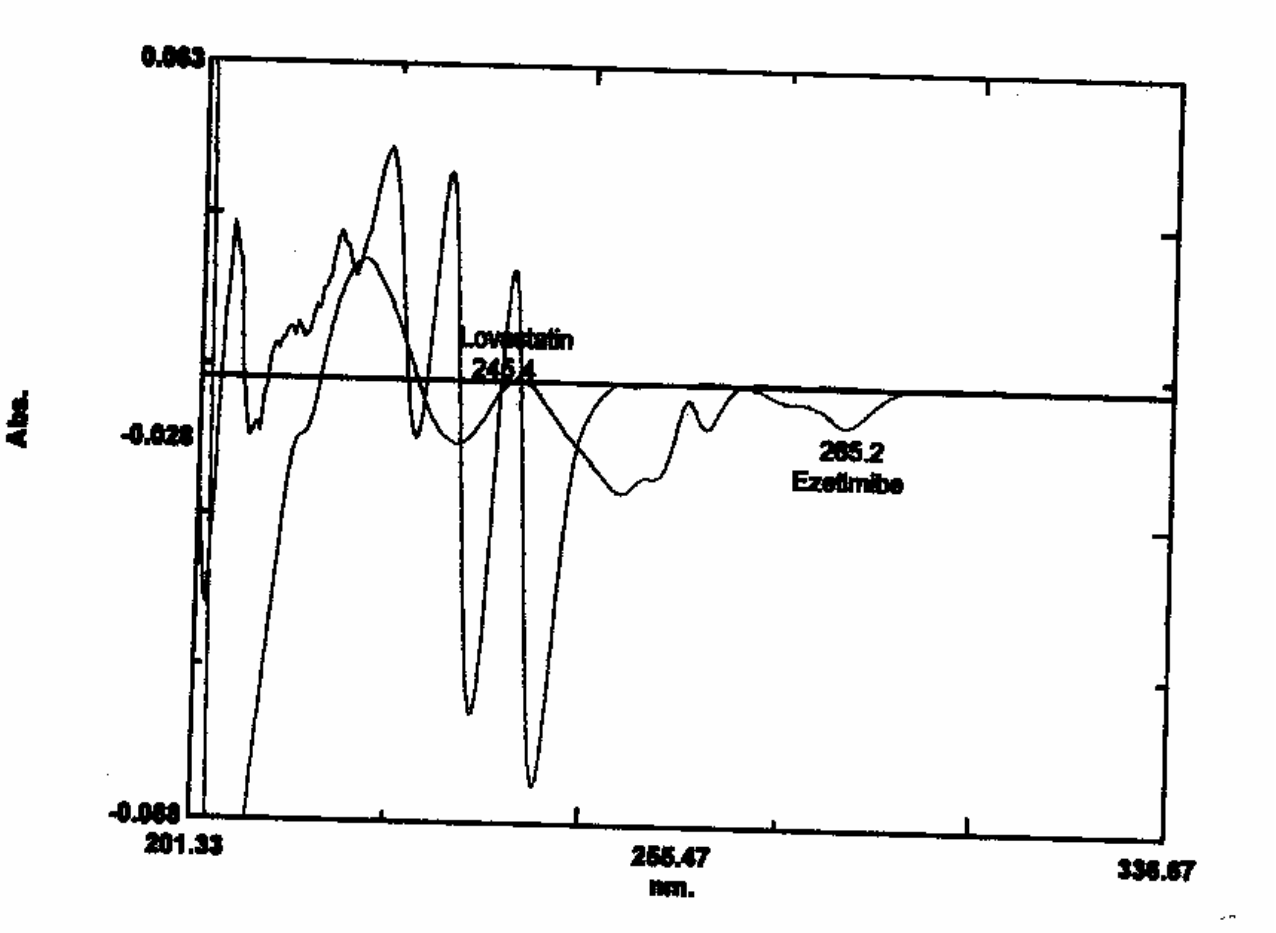
* Mean of four determinations. Mixture 1 is powder of 10 tablet of Statin (10 mg lovastatin, Unisearch) and 10 tablet of Ezetib (10 mg ezetimibe, Ranbaxy). Mixture 2 is powder of standard 10 mg of lovastatin and 10 mg of ezetimibe. SD is the standard deviation.

Figure 1: Zero order spectra



Zero order spectra of (a) ezetimibe (10 µg/ml) and (b) lovastatin (10 µg/ml).

Figure 2: First derivative spectra



First derivative spectra of (a) ezetimibe (10 µg/ml) and (b) lovastatin (10 µg/ml).

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