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SIMULTANEOUS ESTIMATION OF EZETIMIBE AND ROSUVASTATIN IN DRUG MIXTURE BY FIRST DERIVATIVE SPECTROSCOPIC METHOD

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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 rosuvastatin using the zero-crossing technique. The measurements were carried out at wavelengths of 290 and 245.6 nm for ezetimibe and rosuvastatin, respectively. The method was found to be linear ($r^2 > 0.9994$) in the range of 5- 40 µg/mL for ezetimibe at 290 nm. The linear correlation was obtained ($r^2 > 0.9935$) in the range of 5- 80 µg/mL for rosuvastatin at 245.6 nm. The limit of determination was 0.43 and 0.69 µg/mL for ezetimibe and rosuvastatin, respectively. The limit of quantification was 1.44 and 2.89 µg/mL for ezetimibe and rosuvastatin, respectively. The method was successfully applied for simultaneous determination of ezetimibe and rosuvastatin in drug mixture.

Key words: Ezetimibe, Rosuvastatin, Simultaneous determination, First derivative zero crossing, 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-azet idinone[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

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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 is rosuvastatin, a potent statin presently available in the market. It inhibits the enzyme 3-hydroxy-3-methyl glutaryl coenzyme A (HMG CoA) reductase[7]. Rosuvastatin 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[8]. So the combination formulation might be in offing. For estimation of rosuvastatin, various methods were reported in literature survey like estimation in dosage form by RP-HPLC[9] and in human plasma by LCMS[10]. Methods reported for ezetimibe are RP-HPLC[11], HPLC[12], LCMSMS[13] and TLC[14]. But no official or reported procedure is present for simultaneous determination of ezetimibe and rosuvastatin 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 rosuvastatin in binary mixture on the basis of zero-crossing measurement. This method could be applied for determination of both drugs in the presence of each other.

EXPERIMENTAL

Chemicals and reagents

Ezetimibe and rosuvastatin were obtained as a gift sample from Sun Pharmaceutical Ltd., Baroda. Methanol used was of analytical grade 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 and 10 mg of rosuvastatin, 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 pathlength. For all solutions, the derivative spectra were obtained over 200-380 nm range

at 2 nm slit width (Δλ). The ordinate maximum and minimum were adjusted to the magnitude of derivative values.

Standard and calibration solutions

Standard stock solution of ezetimibe and rosuvastatin were prepared by separately dissolving 10 mg of ezetimibe and rosuvastatin, 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 rosuvastatin (1–80 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 290 nm for ezetimibe and at 245.6 nm for rosuvastatin and the regression equation was computed.

Spectroscopic measurements

The difference between spectra of standard solutions of ezetimibe and rosuvastatin versus their solvent blanks were recorded in range 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 rosuvastatin and vice versa measured at 290 nm (zero-crossing of rosuvastatin), and 245.6 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 rosuvastatin and 10, 20, 30 and 40 μg/mL of rosuvastatin plus 10 μg/mL ezetimibe were prepared, respectively and analyzed as discussed above. Precision of the procedure was calculated by intra–day and inter-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 rosuvastatin (Rosuva, 10 mg Ranbaxy) and ezetimibe (Ezetib, 10 mg Unisearch) were weighed accurately and powdered. The powder equivalent to 10 mg of rosuvastatin 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 rosuvastatin and 100 μg/mL ezetimibe. From the stock solution, 1 mL was taken into a 10 mL volumetric flask and the volume was made up to the mark with methanol to get a final concentration of rosuvastatin (10 μg/mL) and ezetimibe (10 μg/mL). The concentration of ezetimibe and rosuvastatin in tablets were calculated using the corresponding calibrated curve.

RESULTS AND DISCUSSION

Derivate spectroscopic method

Zero-order absorption spectra of ezetimibe and rosuvastatin 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[15,16]. 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[17]. The spectroscopic parameters including derivative order, wavelength and $\Delta\lambda$ values should be optimized to obtain maximum resolution, sensitivity and reproducibility[18-20]. 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 rosuvastatin were 290 and 245.6 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 rosuvastatin concentration at the zero-crossing wavelength of rosuvastatin (290 nm) or ezetimibe (245.6 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.

Table 1: Statistical data of calibration curves of ezetimibe and rosuvastatin using first-derivative spectra

Parameters	Ezetimibe	Rosuvastatin
Wavelength (nm)	290	245.6
Linearity (μg/mL)	5 – 40	5- 80
Regression equation*	y = 0.0008 x + 0.0011	y= -0.0005 x – 0.0004
Correlation coefficient	0.9994	0.9935
Limit of detection (μg/mL)	0.43	0.69
Limit of quantification (μg/mL)	1.44	2.89

*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.

Validation

The limit of detection was found to be 0.43 μg/mL and 0.69 μg/mL for ezetimibe and rosuvastatin, respectively. The accuracy and precision were determined by using synthetic mixture of ezetimibe and rosuvastatin 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 rosuvastatin, respectively in all the 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 and 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 rosuvastatin. 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 rosuvastatin in the solutions during analysis showed that analytes were stable at least for 72 hr in solutions.

Table 2: Accuracy and precision data for determination of ezetimibe in the presence of rosuvastatin (10 µg/mL) by first derivative spectroscopy

Added amount of ezetimibe (µg/mL)	Found (µg/mL) SD	
	Within day*	Between day*
10	10.42 ± 0.12	10.01 ± 0.21
20	19.99 ± 0.11	20.10 ± 0.17
30	30.03 ± 0.12	30.11 ± 0.21
40	39.98 ± 0.17	39.89 ± 0.22

*Mean of six determinations

Table 3: Accuracy and precision data for determination of rosuvastatin in the presence of ezetimibe (10 µg/mL) by first derivative spectroscopy

Added amount of rosuvastatin (µg/mL)	Found (µg/mL) SD	
	Within day*	Between day*
10	9.98 ± 0.37	10.01 ± 0.65
20	19.99 ± 0.12	19.89 ± 0.12
30	30.19 ± 0.03	30.20 ± 0.08
40	40.02 ± 0.16	39.65 ± 0.55

*Mean of six determinations

The proposed method was successfully applied to analyze preparation containing ezetimibe and rosuvastatin. The results are summaries in Table 4. 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 rosuvastatin 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.

Table 4: Results of the analysis of commercial product

Formulation	Rosuvastatin % Found ± S.D. (n = 4)*	Ezetimibe % Found ± S.D. (n = 4)*
Mixture 1	98.8 ± 0.59	99.9 ± 0.11
Mixture 2	99.3 ± 0.74	98.4 ± 0.37

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

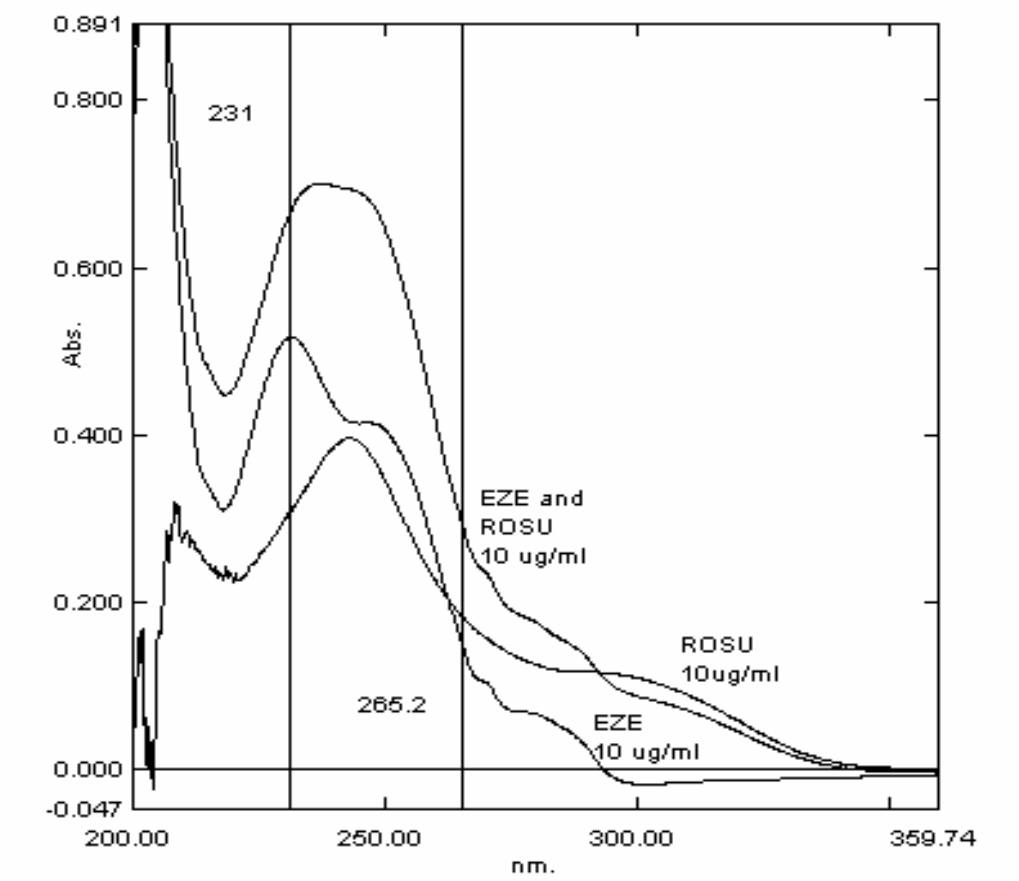

Fig. 1: Zero order spectra of ezetimibe (10 µg/mL) and rosuvastatin (10 µg/mL)

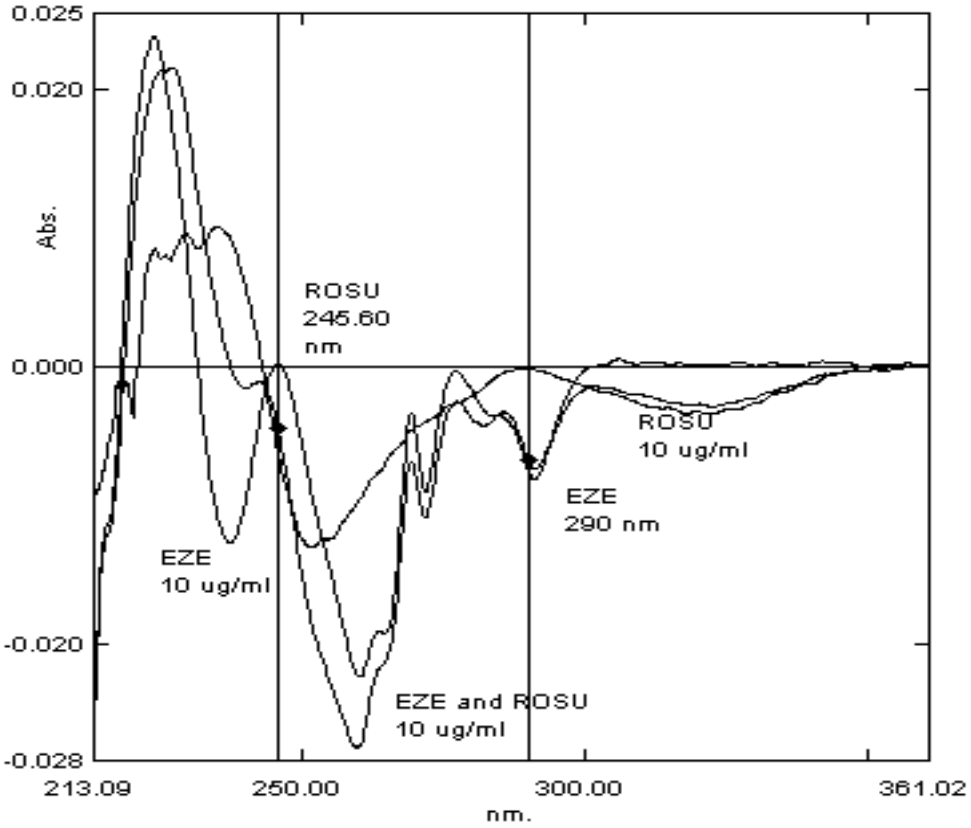

Fig. 2: First derivative spectra of ezetimibe (10 µg/mL) and rosuvastatin (10 µg/mL)

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