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Short communication

Simultaneous spectrophotometric determination of cyproterone acetate and estradiol valerate in pharmaceutical preparations by ratio spectra derivative and chemometric methods

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Abstract

Ratio spectra derivative spectrophotometry and two chemometric methods (classical least squares, CLS and inverse least squares, ILS, were proposed for the simultaneous quantitative analysis of a binary mixture consists of cyproterone acetate (CA) and estradiol valerate (EV) in the commercial pharmaceutical preparations. In the ratio spectra derivative method, linear regression equations for both drugs were obtained by measuring the analytical signals at the wavelenghts corresponding to either maximums and minimums in the first derivative spectra of the ratio spectra. In the chemometric techniques, the concentration matrix was prepared by using the synthetic mixtures containing these drugs. The absorbance matrix corresponding to the concentration matrix was obtained by measuring the absorbances at 14 wavelengths in the range 220–290 nm for the zero-order spectra. Two chemometric calibrations were constructed by using the absorbance matrix and concentration matrix for the prediction of the unknown concentrations of CA and EV in their mixture. The numerical values were calculated by using 'MAPLE V' software. The accuracy and the precision of the methods have been determined and they have been validated by analyzing synthetic mixtures containing these two drugs. The proposed methods were successfully applied to a pharmaceutical formulation, sugar-coated tablet, and the results were compared with each other.

Keywords: Cyproterone acetate; Estradiol valerate; Ratio spectra derivative spectrophotometry; Chemometric methods; Pharmaceutical preparation

1. Introduction

Combination of estradiol valerate (EV) with cyproterone acetate (CA) is used in hormone replacement therapy during climacterium. Various methods including spectrophotometry [1–3],

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HPLC [4,5], fluorimetry [6] and radioimmunoassay [7] have been used for the determination of EV and CA in pharmaceutical preparations and biological liquids. Yücesoy and Erol [2,3] used different spectrophotometric methods for the simultaneous analysis of EV and CA in a pharmaceutical preparation.

In the method of Salinas et al. the concentrations of active compounds were determined by measuring the amplitudes of the minimum or maximum at points corresponding to the selected wavelengths [8]. The same method was already applied to determine the active compounds in different mixtures and many pharmaceutical preparations [9–16].

Chemometric calibration techniques can be summarized as multiple linear regression (MLR) (classical least square, CLS and inverse least squares, ILS calibrations), principal component regression (PCR) and partial least regression (PLS) techniques [17–22]. Chemometric calibration techniques in spectral analysis is gaining importance in the quality control of drugs in mixtures and pharmaceutical formulations containing two or more drugs with overlapping spectra due to not need any separation procedure in the drug determinations. Dinç and Onur used these techniques for the simultaneous analysis of a binary and a ternary mixture [23,24].

In this paper, the numerical parameters of CLS and ILS methods were optimized for the determination of title drugs in their binary mixture and the proposed calibration techniques were validated with synthetic mixtures of CA and EV. First derivative of the ratio spectra was also proposed for the simultaneous determination of CA and EV in their mixture. All the methods proposed were applied to a pharmaceutical preparation marketed in Turkey and the results were compared with each other.

2. Experimental

2.1. Apparatus

Shimadzu 1601 PC double beam spectrophotometer with a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC was used for all the spectrophotometric measurements.

2.2. Materials

CA and EV were kindly donated by Schering Pharm. Ind., Turkey and used without further purification.

All the solvents used in spectrophotometric analysis were of analytical reagent grade.

2.3. Standard solutions

Solutions of 50 mg/100 ml of CA and 100 mg/100 ml EV were prepared, separately, with a solvent mixture 1 M NaOH: methanol (1:9).

2.4. Sample preparation

20 sugar-coated tablets were accurately weighed and powdered in a mortar. An amount of the tablet mass equivalent to one tablet content was dissolved in 60 ml of 1 M NaOH: methanol (1:9). After 30 min of mechanically shaking the solution was filtered in a 100 ml volumetric flask. The residue was washed three times with 10 ml solvent then the volume was completed to 100 ml with the same solvent. All the spectrophotometric methods were applied to the latest diluted solution.

2.5. Methods

2.5.1. (a) CLS method

the CLS method is involved in the application of MLR to the classical expression of the Beer–Lambert law of spectroscopy:

$$A = K \times C$$

This equation is a matrix equation and it can be written as a linear equation system:

$$\begin{aligned} A_1 &= K_{11}C_1 + K_{12}C_2 + \cdots\cdots K_{1c}C_c \\ A_2 &= K_{21}C_1 + K_{22}C_2 + \cdots\cdots K_{2c}C_c \\ &\cdots\cdots \\ A_w &= K_{w1}C_1 + K_{w2}C_2 + \cdots\cdots K_{wc}C_c \end{aligned} \quad (1)$$

where A_w is the absorbance at the w^{th} wavelength, K_{wc} is the calibration coefficient for the c^{th}

component at the wth wavelength, C_c is the concentration of the cth component.

2.5.2. (b) ILS method

The ILS method is also involved the application of MLR to the inverse expression of the Beer–Lambert law of spectroscopy:

$$C = P \times A$$

This equation can be written as a linear equation system:

$$\begin{aligned} C_1 &= P_{11}A_1 + P_{12}A_2 + \cdots\cdots P_{1w}A_w \\ &\cdots\cdots \\ C_2 &= P_{21}A_1 + P_{22}A_2 + \cdots\cdots P_{2w}A_w \\ C_c &= P_{c1}A_1 + P_{w2}A_2 + \cdots\cdots P_{cw}A_w \end{aligned} \quad (2)$$

where, A_w is the absorbance at the w^{th} wavelength, P_{cw} is the calibration coefficient for the c^{th} component at the w^{th} wavelength, C_c is the concentration of the c^{th} component.

3. Results and discussion

3.1. Ratio spectra first derivative spectrophotometry

The ratio spectra of different EV standards at increasing concentrations in 1 M NaOH: methanol (1:9) were obtained by dividing each with the standard spectrum of the solution of CA (see Fig. 2a). The first derivative of these spectra (^{1}DD) was traced with the interval of $\Delta\lambda = 4$ nm using the scaling factor (SF) = 10 (see Fig. 2b). As seen in Fig. 2b, there exist one maxima (235.4 nm) and one minima (249.5 nm) and we found that two of them are suitable for the determination of EV in EV+CA mixture. We selected 249.5 nm for the determination of this compound in the assay of synthetically prepared pharmaceutical preparation, tablet, due to its lower R.S.D. value and higher mean recovery among the wavelengths mentioned (Table 1). The ratio and ratio derivative spectra of the solutions of CA in different concentrations in 1 M NaOH: methanol (1:9), traced with the interval of $\Delta\lambda = 4$ nm and SF = 10 by using the standard spectrum of EV as divisor by computer aid was demonstrated In Fig. 3a and b, respectively. In these spectra, one maxima (264.1 nm) and one minima (277.1 nm) were found suitable for the quantification of CA in EV+CA. Measured analytical signals at these wavelenghts are proportional to the concentrations of the drugs. We selected 264.1 nm for the determination of this compound in the assay of pharmaceutical preparation, sugar-coated tablet, due to its lower R.S.D. value and suitable mean recovery among the wavelengths mentioned (Table 1).

Calibration graphs were established from analytical signals measured at 235.4 and 249.5 nm for standards containing 16–48 μg/ml of EV and at 264.1 and 277.1 nm for standards containing 4–32 μg/ml CA corresponding to maxima and minima in the absence of each other.

In the method, the mean recoveries and relative standard deviations calculated for synthetic mixtures prepared in our laboratory are illustrated in Table 1. Also, Beer's law compliance for both compounds, the regression equations and correlation coefficients were summarized in Table 2. Mean recoveries and relative standard deviations of the method were found satisfactory.

Divisor concentration is main instrumental parameter. The standard spectra of 10 μg/ml of CA and 20 μg/ml of EV was considered as suitable for the determination of EV and CA, respectively, as divisor. The $\Delta\lambda$ found as optimum for the first derivative of their ratio spectra was 4 nm (SF = 10).

A critical evaluation of all the proposed methods was performed by statistical analysis of the data, where slopes, intercepts and correlation coefficients were shown in Table 2.

Comparison of the spectra of CA and EV in standard and drug formulation solutions showed that the wavelengths of maximum absorbances in the zero-order spectra did not change. It has been decided that excipients placed in the commercial preparations selected (lactose, starch, avicel, povidon, sodium dodecylsulfate, aerosil, magnesium stearate and titan dioxide) did not interfere the quantitation of CA and EV in these methods.

Summary of the assay results for commercial preparation were shown in Table 5. The results of two chemometric methods and ratio spectra derivative spectrophotometry developed by us for the

Table 1
Recovery results for EV and CA in synthetic mixtures by ratio spectra first derivative method

Mixture number	Added (μg)	EV recovery (%)		Added (μg)	CA recovery (%)	
		235.4 nm	249.5 nm		264.1 nm	277.1 nm
1	16	102.3	102.5	10	100.7	100.1
2	24	100.0	99.8	10	101.4	103.2
3	32	104.8	100.2	10	98.4	99.1
4	40	96.0	101.0	10	98.5	100.3
5	48	100.1	101.2	10	101.9	95.6
6	20	100.1	101.1	4	99.3	100.0
7	20	101.4	101.8	12	100.9	97.3
8	20	99.8	99.8	16	98.5	99.8
9	20	103.8	102.1	24	98.5	103.1
10	20	99.7	101.7	32	101.0	101.5
n = 10	$\bar{x} = 100.8$	100.8			100.3	100.0
R.S.D.		2.44	1.18		1.49	2.35

R.S.D., relative standard deviation.

same commercial formulation were compared by Student's *t*-test. The calculated (experimental) *t*-values did not exceed the tabulated (theoretical) values in the test, indicating that there was no significant difference between the methods compared.

3.2. Chemometric methods

Fig. 1 shows the zero-order absorption spectra for EV and CA and their binary mixture in 1 M NaOH and methanol. For two techniques, the absorbance data matrix for the training set in different compositions (in the working range of 16–48 μg/ml for EV and 4–32 μg/ml for CA in both chemometric methods) were obtained by the measurements of absorbances between 230 and 320 nm in the intervals with $\Delta\lambda = 5$ nm at 14 wavelengths in the zero-order absorption spectra. In the techniques, calibration or regression was obtained by using the absorbance matrix and concentration matrix for prediction of the unknown concentrations of EV and CA in their binary mixtures and pharmaceutical formulations.

The predictive ability of a model can be defined in various ways. The most general expression is the standard error of calibration (SEC) in the calibration step and the standard error of prediction (SEP) in the prediction step which is given the following equation:

$$\mathrm{SEP(or\ SEC)} = \sqrt{\sqrt{\frac{\sum_{i=1}^{N}(C_i^{Added} - C_i^{Found})^2}{n}}} \qquad (3)$$

where C_i^{Added} is the added concentration of drug,

Table 2
Beer's law data and statistical analysis for the calibration graphs of EV and CA using ratio spectra derivative spectrophotometric procedures

Compound	λ (nm)	Regression equations a (S.E.) b (S.E.)		r	Concentration range (μg/ml)
EV	235.4	1.86×10^{-2} (6.00×10^{-4})	3.70×10^{-3} (2.00×10^{-4})	0.9984	16.0–48.0
EV	249.5	-6.64×10^{-2} (9.60×10^{-4})	-7.28×10^{-3} (3.26×10^{-4})	0.9997	16.0–48.0
CA	264.1	4.94×10^{-1} (1.80×10^{-2})	-2.80×10^{-1} (3.60×10^{-2})	0.9995	4.0–32.0
CA	277.1	-2.50×10^{-1} (4.90×10^{-3})	1.78×10^{-1} (9.90×10^{-3})	0.9994	4.0–32.0

a, Slope; b, intercept; r, correlation coefficient; S.E., standard error.

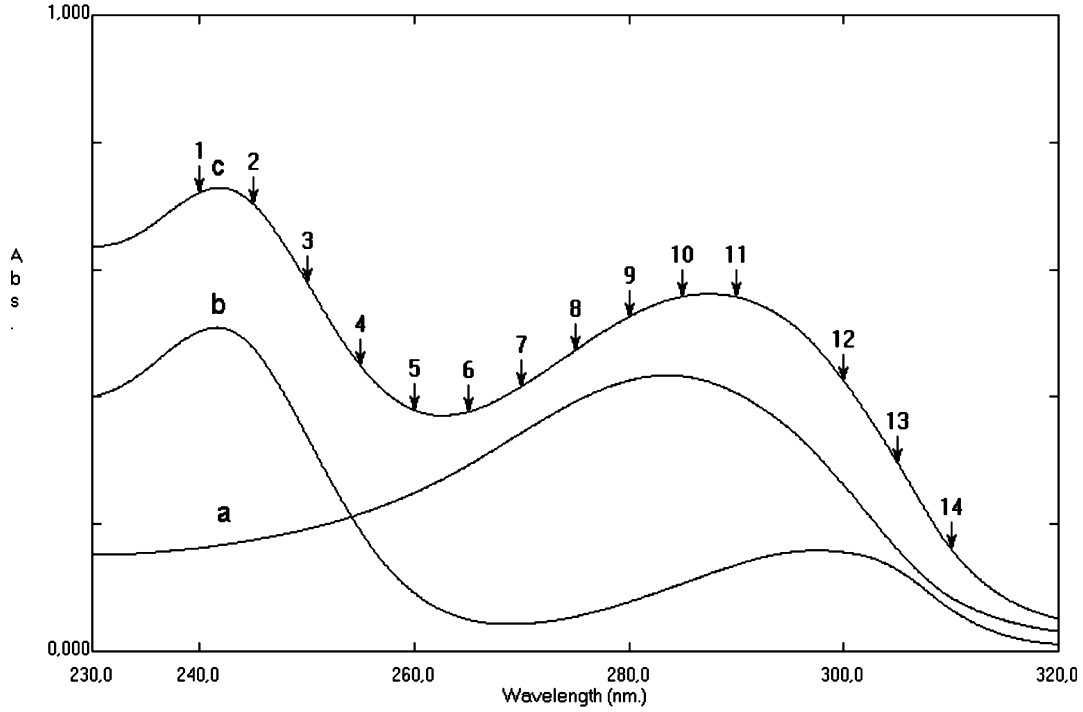


Fig. 1. Zero-order absorption spectra of (a) 20 μg/ml solution of EV, (b) 10 μg/ml solution of CA in 1 M NaOH:methanol (1:9).

C_i^{Found} is the predicted concentration of drug and n is the total number of samples.

In order to test the proposed techniques, the sets of synthetic mixtures containing the two drugs in variable composition were prepared. The results obtained in the application of CLS and ILS methods to the same binary mixture are indicated in Tables 3 and 4. The SEP values were completely acceptable (0.49 and 0.35 for EV and 0.07 and 0.10 for CA, respectively, for CLS and ILS methods) (Table 4).

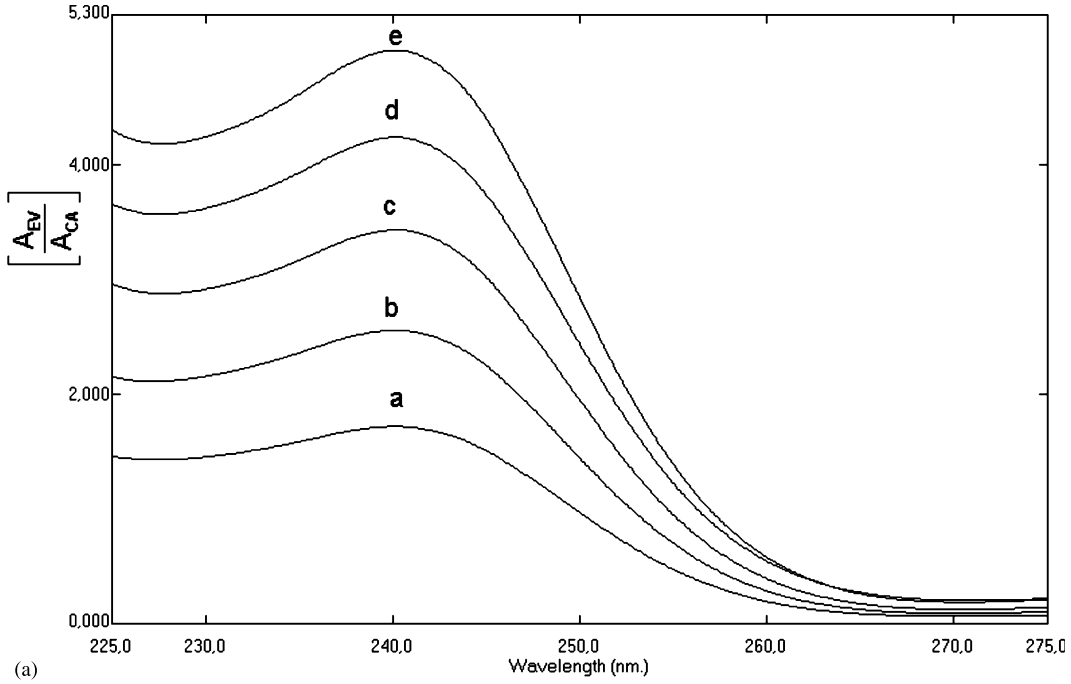
In Table 5, r is defined as the correlation between constituent concentrations and shows the absorbance effects relating to the constituent of interest. r values obtained in the methods close to 1 mean no interference was coming from the other constituents in this set of synthetic mixtures.

The SEC values were found acceptable in CLS and ILS methods (0.63 and 0.42 for EV and 0.08 and 0.11 for CA), respectively (Table 5), in the calibration step.

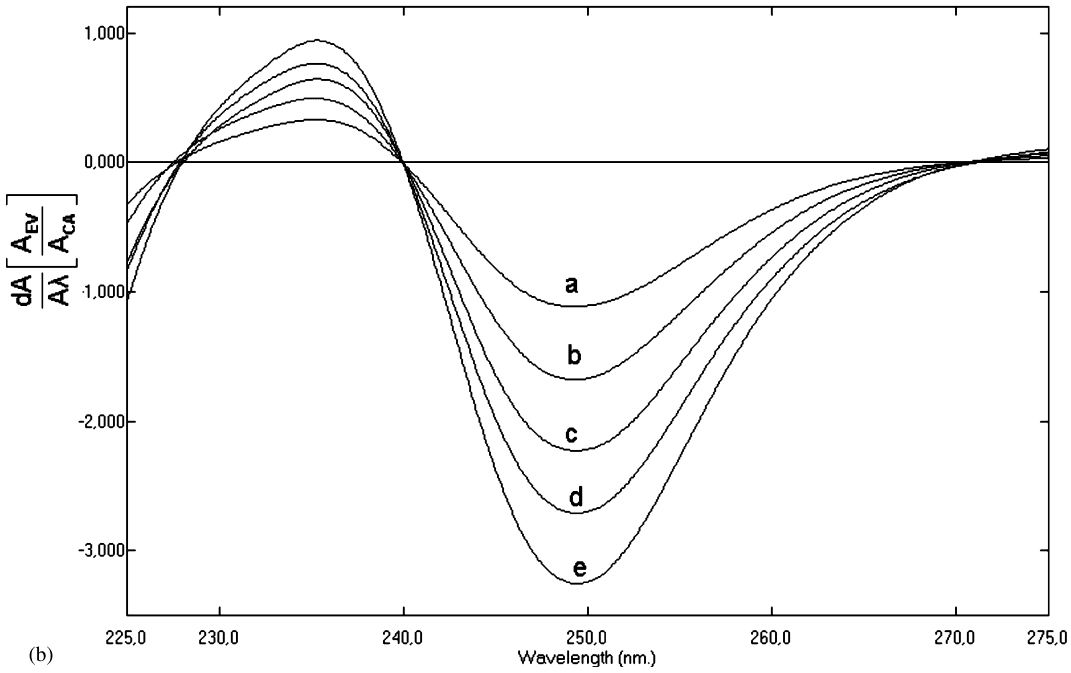
The standard addition method was applied to the commercial preparation. The obtained results for ratio spectra derivative, ILS and PCR methods were found to be 2.09 ± 0.11, 2.04 ± 0.15 and 2.02 ± 0.08 for EV, and 1.03 ± 0.07, 1.04 ± 0.09 and 1.07 ± 0.05 (mg/tablet $\pm$ S.D.) for CA, respectively. The results were obtained in the average of 5 replicate for each drug. We observed that excipients did not interfere in the quantitative analysis of CA and EV in samples.

Mean recoveries and relative standard deviations for the CLS and ILS techniques were found as 101.3 and 1.19%, 100.8 and 0.71%, for EV and 100.1 and 0.54%, 100.2 and 0.69% for CA, respectively, in the synthetic mixtures of both drugs (Table 3).

Comparison of the spectra of EV and CA in standard and drug formulation solutions showed that the wavelength of maximum absorbances in the zero-order spectra did not change. It has been decided that excipients placed in the commercial preparations selected (lactose, starch, avicel, povi-

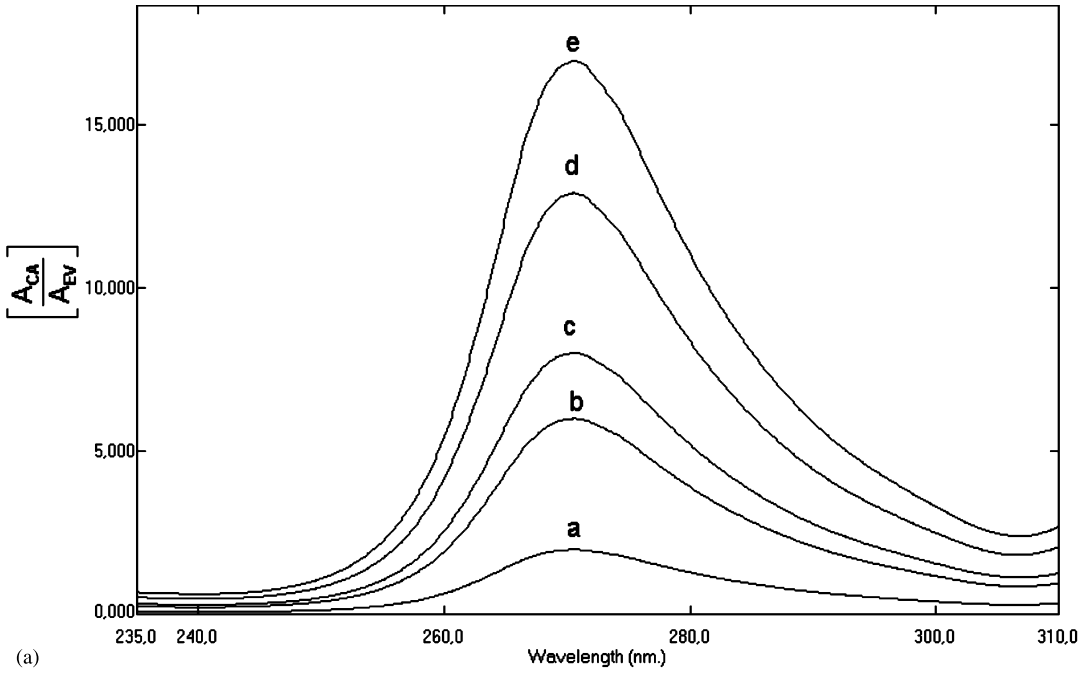


(a)



(b)

Fig. 2. Ratio spectra (a) and first derivative of the ratio spectra (b) of (a) 16 μg/ml, (b) 24 μg/ml, (c) 32 μg/ml, (d) 40 μg/ml, (e) 48 μg/ml solution of EV in 1 M NaOH:methanol (1:9) when 16 μg/ml solution of CA in 1 M NaOH:methanol (1:9) used as divisor ($\Delta\lambda = 4$ nm, scaling factor = 10).



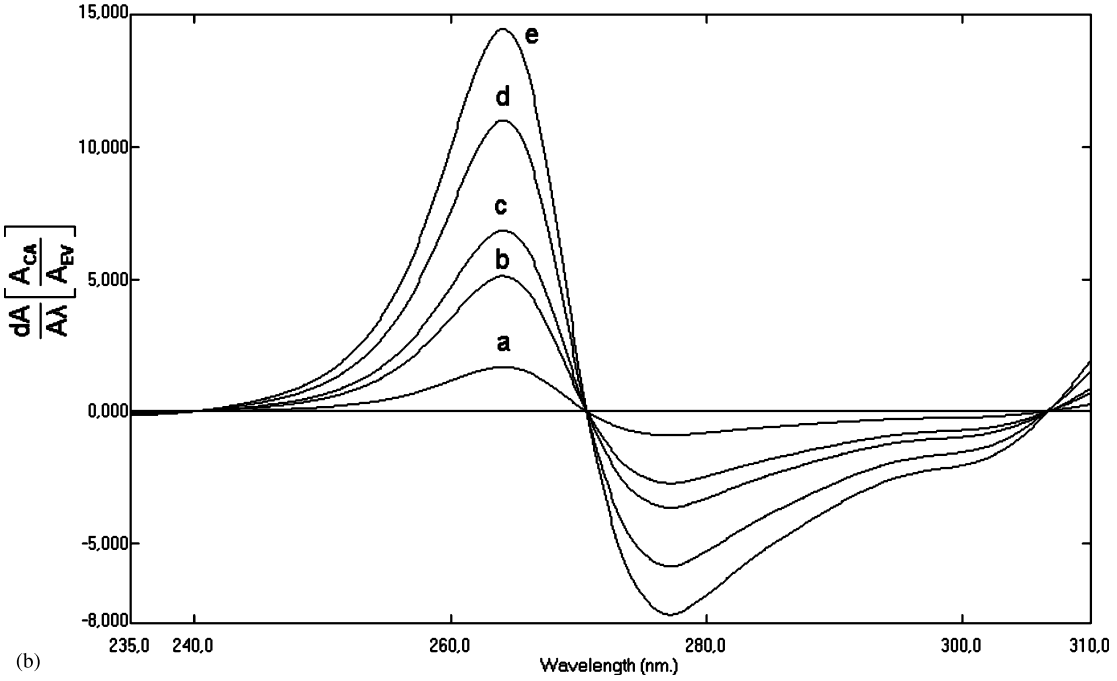


Fig. 3. Ratio spectra (a) and first derivative of the ratio spectra (b) of (a) 4 μg/ml, (b) 12 μg/ml, (c) 16 μg/ml, (d) 24 μg/ml, (e) 32 μg/ml solution of CA in 1 M NaOH:methanol (1:9) when 32 μg/ml solution of EV in 1 M NaOH:methanol (1:9) used as divisor ($\Delta\lambda = 4$ nm, scaling factor = 10).

Table 3
Results obtained for the determination of EV and CA in synthetic mixtures by using the classical least squares and inverse least squares techniques

Mixture composition		Recovery (%)			
Added (μg)		CLS		ILS	
EV	CA	EV	CA	EV	CA
16.0	10.0	101.3	101.0	100.6	101.0
24.0	10.0	102.1	100.0	100.8	100.0
32.0	10.0	102.5	101.0	102.2	101.0
40.0	10.0	100.3	99.0	100.5	99.5
48.0	10.0	101.7	100.0	101.5	100.0
20.0	4.0	103.0	100.0	101.0	100.0
20.0	12.0	102.5	100.8	100.5	100.8
20.0	16.0	100.5	100.0	100.5	98.8
20.0	24.0	100.0	100.0	100.5	100.4
20.0	32.0	99.5	100.0	99.5	100.3
$\bar{x}$		101.3	100.1	100.8	100.2
R.S.D.		1.21	0.54	0.71	0.69

don, sodium dodecylsulfate, aerosil, magnesium stearate and titan dioxide) did not interfere the quantitation of EV and CA in these methods. All the results obtained by using the methods described above were compared with each other and no significant difference was observed between the amount of drugs found as theoretical values for t at $P = 0.05$ level for commercial formulation (Table 5).

4. Conclusion

In this study, the ratio spectra derivative, CLS and ILS methods could be applied with great success for the simultaneous determination of EV and CA in mixtures and the pharmaceutical formulation selected containing its mixture without interference of each other. Easy measurements on the separate peaks, higher values of analytical signals and no need to work only at zero-crossing points (sometimes co-existing compounds have no maximum or minimum at these wavelengths) is an advantage for ratio spectra derivative spectrophotometry in comparison with the derivative spectrophotometry [2]). This method is also an advantageous method by not needing any additional mathematical calculations and, working in different mediums and measurements in comparison with the methods explained in literatures [2,3] (difference spectrophotometry, absorbancy ratio and Vierordt's methods). Satisfactory results were obtained by using chemometric methods but they need a software for the mathematical calculations. Using only zero-order spectra in the procedures and not need any other mode, such as derivative mode, in the instruments are an advantage for the chemometric methods. These three methods were found suitable for simple and precise routine analysis of the pharmaceutical preparation se-

Table 5
Assay results of commercial preparation (1 mg CA and 2 mg EV/sugar-coated tablet) (mg/sugar-coated tablet)

Methods	EV mean ± S.D.[a]	CA mean ± S.D.
Classical least squares	2.10 ± 0.24	1.05 ± 0.15
Inverse least squares	2.10 ± 0.24	1.05 ± 0.15
Ratio spectra derivative method	2.00 ± 0.45	1.10 ± 0.24

*Obtained results are average of ten tablets for three techniques.
[a] S.D., standard deviation.

Table 4
Summary of statistics in CLS and ILS methods for EV and CA in the mixture

	SEP		SEC		r		Intercept		Slope	
	CLS	ILS	CLS	ILS	CLS	ILS	CLS	ILS	PCR	ILS
EV	0.49	0.35	0.63	0.42	0.9996	0.9998	−0.020	−0.25	1.01	1.02
CA	0.07	0.10	0.08	0.11	0.9999	0.9999	0.003	0.012	0.99	1.00

lected. Good agreement was seen in the assay results of pharmaceutical preparation, sugar-coated tablet, for all the methods proposed.

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