

Title

Application of Bivariate Calibration Method and Derivative Spectrophotometry to Simultaneous Quantitative Resolution of Ethinylestradiol and Levonorgestrel in Pharmaceutical Formulations

Authors

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Abstract

The bivariate calibration method was applied to the quantitative resolution of ethinylestradiol (ETE) and levonorgestrel (LEV) in pharmaceutical formulations and the results were compared with those given by first derivative spectrophotometry. In the bivariate spectrophotometric method, an optimum pair of wavelengths was chosen for the determination. The determination was performed at 240.4 nm for ETE and at 257.8 nm for LEV. In the first-derivative spectrophotometry determination of ETE and LEV was realized by measurements of amplitudes of derivative spectra corresponding to zero-crossing points of LEV and ETE at 241 and 248.4 nm, respectively. The proposed procedures were validated by using various synthetic mixtures with high percentage of recovery and good precision. Mean recovery values were found 103.4% and 102.9 % for bivariate calibration method and 102.4% and 98.2 % for derivative spectrophotometry, respectively, for determination of ETE and LEV in synthetic mixtures. Also good agreement between the results obtained using the two procedures was found, which agreed also with the product label.

Key words

Ethinylestradiol; Levonorgestrel; Bivariate calibration; Derivative spectrophotometry; Quantitative resolution.

Introduction

At present there are three types of oral contraceptives available.

In the commonly used type, both an estrogen and a progestogen are present in the tablets (as either a single dosage or in three different dosages). Ethinylestradiol (ETE) is a semisynthetic estrogen female sex hormone and levonorgestrel (LEV) is a synthetic steroid with an extremely potent progestational action (Figure 1).

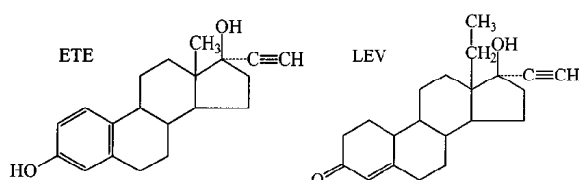


Figure 1- Structure of levonorgestrel and ethinyloestradiol.

The most commonly encountered estrogen is ETE, which is present at a very low dosage level (0.03–1.0 mg per tablet) in combination with an orally active synthetic progestin (one of the most commonly used is LEV), which is present at a level of from 5 to 30 times that of the estrogen. The formulation of these steroids in tablets of low dosage, i.e. 0.03–0.25 mg per tablet, presented a challenging analytical problem. Thus, low-dosage oral contraceptives require sensitive and accurate analytical methods for simultaneous determination of the small amounts of the estrogen in the presence of large amounts of progestogen.

Some analytical methods have been developed for quantitative determination of relatively small amounts of steroid hormones in oral contraceptives, such as gas chromatographic methods [1,2], radio immunoassay methods [3,4], reverse phase high-performance liquid chromatography (RP-HPLC) [5,6], high-performance

thin-layer chromatography (HPTLC) [7], micellar electrokinetic chromatography (MEKC) [8], liquid chromatography–mass spectrometry (LC–MS) [9–11], voltammetry [12]. Meanwhile, there are several techniques including multivariate calibration technique of partial least square (PLS) and principal component regression (PCR) [13], and first derivative spectrophotometry [14,15].

Recently, Lo'pez-de-Alba and co-workers developed the bivariate calibration method for the resolution of two-component mixtures by spectrophotometry [16–19]. This method is based on the use of the four linear regression calibration equations with two calibrations for each component at two selected wavelengths using the method of Kaiser [20].

The aim of this work is the application of bivariate calibration method to the quantitative resolution of binary mixtures containing ETE and LEV without requiring a chemical pre-treatment. As an alternative method, derivative spectrophotometry was studied for the quantitative resolution of the mixtures of the subject matter compounds. All of the developed methods were also applied to commercial contraceptives.

Experimental

Apparatus and software

A Bio-TEK kon 922 double beam UV–vis spectrophotometer with a 1 cm path length quartz cell was used. Calculations and the signal transform were obtained by using EXCEL and MATLAB 7.1.

Chemicals

Pharmaceutical grade levonorgestrel (batch no.: P16097 W from Gedeon Richter Ltd. with 99.0% purity) and ethinylestradiol (batch no.: 29401473

from Schering Co. with 99.9% purity) were used without any further purification. Analytical reagent-grade solvents were purchased from Merck (Darmstadt, Germany).

Standard Solutions

Stock standard solutions of ethinylestradiol and levonorgestrel were prepared by dissolving 10.0 mg of levonorgestrel and 10.0 mg of ethinylestradiol in absolute ethanol in a 100 ml volumetric flask and diluted to the mark with the solvent. The stock solutions were protected from direct light while keeping at room temperature. Working solutions were prepared by appropriate dilution of the stock solution in ethanol to reach concentration ranges of 2-20 and 2-30 $\mu\text{g ml}^{-1}$ for ethinylestradiol and levonorgestrel, respectively.

Preparation of real samples

Twenty tablets were finely powdered and an appropriate portion (equivalent to the median mass of seven tablets for type 1 and twelve tablets for type 2) was dissolved in 50 ml of absolute ethanol, It was mechanically shaken for a period of 20 min and filtered into a 100 ml calibrated flask. The residue was washed twice with the same solvent and diluted to the desired volume.

Procedure

Bivariate calibration method

The bivariate calibration method is a chemometric approach derived from dual Wavelengths spectrophotometry.

The linear calibration regression function for the spectrophotometric determination of an analyte A at a selected wavelength (λ_i) can be described as follows:

$$A_{Ai} = m_{Ai} \cdot C_A + e_{Ai} \quad (1)$$

where A_{Ai} is the absorbance of the analyte A at λ_i , m_{Ai} is the slope of linear regression, C_A is the concentration of analyte A, and e_{Ai} the intercept value, which reflects the differences between the model and the real system.

In case of simultaneous determination of two compounds (A and B) in

the binary mixture, the measurement of absorbance at two selected wavelengths (1 and 2) is required. The following system of equations is then obtained:

$$\begin{aligned} A_{AB1} &= m_{A1} \cdot C_A + m_{B1} \cdot C_B + e_{AB1} \\ A_{AB2} &= m_{A2} \cdot C_A + m_{B2} \cdot C_B + e_{AB2} \end{aligned} \quad (2)$$

where e_{AB1} and e_{AB2} are the sum of the intercepts of the linear calibration at two wavelengths ($e_{ABi} = e_{Ai} + e_{Bi}$). The concentration of each individual compound can be calculated as follows:

$$\begin{aligned} C_A &= \frac{A_{B1} - e_{B1} - m_{B1} C_B}{m_{A1}} \\ C_B &= \frac{m_{A2}(A_{B1} - e_{B1}) + m_{A1}(e_{B2} - A_{B2})}{m_{A2}m_{B1} - m_{A1}m_{B2}} \end{aligned} \quad (3)$$

If analytical wavelengths are selected properly, this simple algorithm allows determination of concentration of two compounds in a binary mixture with good accuracy and precision. The Kaiser method was used for the selection of the optimum wavelength set, which assured the best sensitivity and selectivity of the determination. For this purpose a series of sensitivity matrixes K were created for each binary mixture and for every pair of pre-selected wavelengths:

$$K = \begin{bmatrix} m_{A1} & m_{B1} \\ m_{A2} & m_{B2} \end{bmatrix} \quad (4)$$

where m_{A1} and m_{A2} are the slopes of regression

curves for compound A at wavelengths 1 and 2, respectively, and m_{B1} and m_{B2} the same parameters for compound B.

The determinants of these matrices were calculated and used for selection of the optimal wavelengths for absorbance measurements. These wavelengths were chosen as optimal for which the highest matrix determinant value was obtained.

Results and discussion

Absorption spectra

The zero-order spectra of ETE and LEV and their mixture in the wavelength range of 200–350 nm are shown in Figure 2. Both spectra are strongly overlapped, so the simultaneous determination of these compounds in a mixture requires special analytical techniques. The use of bivariate calibration method or derivation of spectra can help to avoid the physical separation operation.

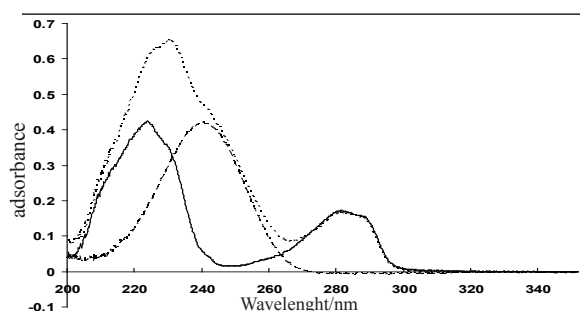


Figure 2- Absorption spectra of ethinylestradiol (—) $25 \mu\text{g ml}^{-1}$, levonorgestrel (---) $8 \mu\text{g ml}^{-1}$ and their mixture (...).

Bivariate Calibration Method

Eight wavelengths were chosen and the slope values of the linear calibration regression were estimated for each compound at these wavelengths (see Table 1). The obtained data were used for creation of the sensitivity matrix and the respective determinants were calculated. The sensitivity chart values obtained

for the mixture are shown in Table 1.

According to the data presented in Table 1, the wavelengths at 240.4 nm and 257.8 nm were selected for simultaneous determination of ETE and LEV, respectively, as they provided the highest sensitivity for each compound.

At the selected wavelengths, the single-component calibration graphs function equation for each component in the mixture and their statistical parameters are presented in Table 2.

Derivative spectrophotometry

The first- derivative spectra of ETE and LEV are shown in Figure 3.

The optimal mathematical parameters of derivation such as derivative order and derivation, window ($\Delta\lambda$) were chosen with regard to required accuracy, precision, and selectivity of determination. For this purpose, first-order derivative spectrum with $\Delta\lambda=10$ nm was chosen.

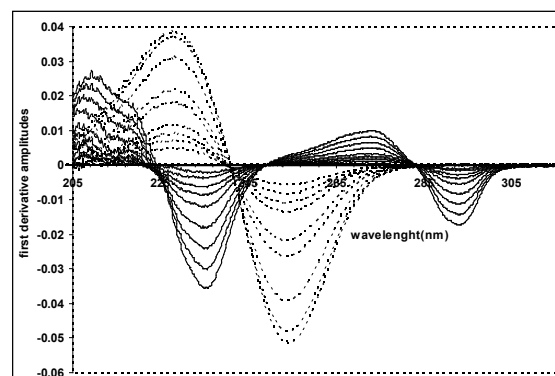


Figure 3- Derivative spectra of 2, 3, 5, 7, 10, 15, 20, 25 and $30 \mu\text{g ml}^{-1}$ ETE (—) and 2, 3, 4, 5, 8, 10, 15, 18 and $20 \mu\text{g ml}^{-1}$ LEV (...).

Linear regression functions for ETE and LEV were obtained by measuring the first derivative values at 241 and 248.4 nm corresponding to the zero-crossing point of LEV and ETE, respectively.

Table 1- Application of the method of Kaiser for the selection of the best wavelength set: the absolute values of determinants of sensitivity matrix ($K \times 10^{-4}$)

λ_1/λ_2	223.8	225.8	230.0	234.0	237.0	240.4	257.8	263.0
223.8	0	81.8	268.1	489.8	663.0	783.1	296.0	1.0
225.8		0	190.1	423.5	613.4	744.9	280.7	88.2
230.0			0	249.8	469.8	621.8	232.3	55.0
234.0				0	240.6	406.4	148.4	67.3
237.0					0	165.4	554.6	-403.5
240.4						0	836.0	-72.8
257.8							0	-26.4
263.0								0

Table 2- Linear regression analysis and its statistical results.

Method	Analyte	Linear range ($\mu\text{g/ml}$)	Slope (a)	Intercept (b)	Corr. Coeff. (r)
Derivative method $\lambda=241$	ETE	2-30	-1.32×10^{-4}	1.52×10^{-4}	0.9987
Derivative method $\lambda=248.4$	LEV	2-20	-2.35×10^{-4}	-2.35×10^{-4}	0.9991
Bivariate Method $\lambda=240.4$	ETE	2-30	3.39×10^{-2}	8.14×10^{-3}	0.9992
Bivariate Method $\lambda=240.4$	LEV	2-30	74.01×10^{-2}	2.07×10^{-3}	0.9994
Bivariate Method $\lambda=257.8$	ETE	2-30	45.16×10^{-2}	4.14×10^{-3}	0.9991
Bivariate Method $\lambda=257.8$	LEV	2-30	21.08×10^{-2}	5.82×10^{-3}	0.9993

r , correlation coefficient of the regression function; a , slope of the regression function; b , intercept of the regression function

The linear regression functions and their statistical parameters are given in Table 2.

Analysis of pharmaceutical tablet formulations

Two different types of commercial low-dosage oral contraceptives (manufactured by Abureihan Co., Iran), containing levonorgestrel and ethinylloestradiol: type I (label claim: 0.25 mg levonorgestrel and 0.05 mg ethinylloestradiol), type II (label claim: 0.15 mg levonorgestrel and 0.03 mg ethinylloestradiol), were

analyzed by bivariate calibration method and derivative spectrophotometry. Each measurement was repeated three times and relative standard deviation was also calculated. The results are shown in Table 3 (a, b).

Good coincidence was observed from the assay results of the commercial preparations by application of bivariate calibration method and derivative spectrophotometry with the declared values.

Table 3(a)- Experimental results ($\mu\text{g/ml}$) obtained by applying the proposed approaches to the commercial low-dosage oral contraceptives (Type 1).

TYPE I	ETE ($\mu\text{g ml}^{-1}$) Amount expected 3.50 ($\mu\text{g ml}^{-1}$)		LEV ($\mu\text{g ml}^{-1}$) Amount expected 17.50 ($\mu\text{g ml}^{-1}$)	
	Derivative method $\lambda=241$	Bivariate method	Derivative method $\lambda=248.4$	Bivariate method
Mean	3.48	3.52	16.97	17.11
S.D.	0.02	0.03	0.08	0.02
R.S.D.	0.57	0.75	0.48	0.15
CL(P = 0.05)	0.05	0.06	0.20	0.06
t _{calc} t Critical:4.30	1.64		1.92	
F _{calc} FCritical:39.0	2.19		1.57	

Calculated by $n = 3$. CL: confidence limit.

Table 3(b) - Experimental results ($\mu\text{g ml}^{-1}$) obtained by applying the proposed approaches to the commercial low-dosage oral contraceptives (Type II).

TYPE I	ETE ($\mu\text{g ml}^{-1}$) Amount expected 3.60 ($\mu\text{g ml}^{-1}$)		LEV ($\mu\text{g ml}^{-1}$) Amount expected 18.00 ($\mu\text{g ml}^{-1}$)	
	Derivative method $\lambda=241$	Bivariate method	Derivative method $\lambda=248.4$	Bivariate method
Mean	3.57	3.58	17.43	17.49
S.D.	0.03	0.03	0.03	0.07
R.S.D.	0.74	0.84	0.18	0.40
CL (P = 0.05)	0.07	0.07	0.14	0.08
t _{calc} t Critical:4.30	1.73		2.71	
F _{calc} FCritical:39.0	1.29		5.25	

Calculated by $n = 3$. CL: confidence limit.

A comparative statistical study (paired-t-test and F-test) of these two methods was carried out (Table 3 (a,b)). By comparing the results it can be observed that there is no evidence for systematic differences between the two methods.

Method validation

Quantitative resolution of the synthetic mixtures containing various concentrations of ETE and LEV was carried out by two methods. The means recoveries and the relative standard deviations of the methods were computed.

Results are summarized in Table 4. The results also confirm the accuracy and precision of the proposed methods.

Table 4- Recovery results obtained from the binary mixtures by the proposed analytical approaches

Mixture ($\mu\text{g/mL}$)		ETE Recovery (%)		LEV Recovery (%)	
ETE	LEV	Derivative method $\lambda=241$	Bivariate method	Derivative method $\lambda=248.4$	Bivariate method
5	3	103.8	104.5	98.6	102.9
10	3	102.8	103.5	97.5	102.7
15	3	102.5	103.1	99.3	103.6
20	3	104.4	104.1	97.7	102.8
25	3	101.7	103.5	100.2	104.5
5	5	102.4	103.4	98.1	102.7
5	8	102.9	103.9	97.4	98.6
5	10	98.7	99.1	98.3	103.6
5	15	102.1	103.5	96.5	104.7
5	18	104.8	105.7	98.3	103.2
Mean		102.4	103.4	98.2	102.9
R.S.D.*		1.62	1.64	1.06	1.63

*R.S.D.: relative standard deviation

Also the proposed methods were validated as to linearity (evaluated by regression equations) (Table 2).

Conclusions

Results obtained by Bivariate calibration method are good, compared with those of obtained by derivative spectrophotometry.

The proposed method provides a simple, rapid, low cost, accurate, and reproducible quantitative analysis for the determination of ETE and LEV in pharmaceutical formulations, without need of any chemical pretreatment such as separation. So it could be applied to routine analysis and quality control of

pharmaceutical formulations.

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