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A Rapid Derivative Spectrophotometric Method for Simultaneous Determination of Ethinylestradiol and Drospirenone in Dosage Forms

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Original Research Article

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Abstract: A combination of ethinylestradiol and drospirenone is used as an oral contraceptive and also for the treatment of premenstrual dysphoric disorders, acne and hirsutism. In this study, a derivative spectrophotometric method has been developed and validated for the simultaneous determination of drospirenone and ethinylestradiol. First order derivative spectrum was used for the determination of ethinylestradiol at 211 nm and drospirenone at 298 and 302 nm. The developed method was linear over the concentration range of 0.25-2.5 µg/mL and 20-200 µg/mL for ethinylestradiol and drospirenone, respectively. The within-day and between-day precision and accuracy were acceptable for both compounds (CV<2.5% and error<2.4%). The proposed method was used for simultaneous determination of ethinylestradiol and drospirenone without any separation before analysis.

Keywords: Ethinylestradiol, Drospirenone, Derivative spectrophotometry, Simultaneous.

INTRODUCTION

Drospirenone, (6R, 7R, 8R, 9S, 10R, 13S, 14S, 15S, 16S, 17S)-1,3',4',6,6a,7S,9,10,11,12,13,14,15,15a,16-hexadecahydro-10,13-dimethyspiro-[17H-dicyclopropa-6,7:15,16]cyclopenta[a]phenanthrene-17,2'(5H)-furan]-3,5'(2H)-dione) (Fig-1), which is an analogue of spironolactone shows progestronic, anti-mineralocorticoid and anti-androgenic activity [1]. Ethinylestradiol, 19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17diol (Fig-2), is an estrogenic compound which is used in oral contraceptive formulations in combination with a progestin drug.

A combination of ethinyestradiol and drospirenone is orally used as an effective and safe contraceptive and also for the treatment of acne, hirsutism and premenstrual dysphoric disorder [1-3].

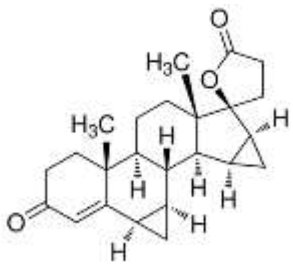

Fig-1: Chemical structure of drospirenone

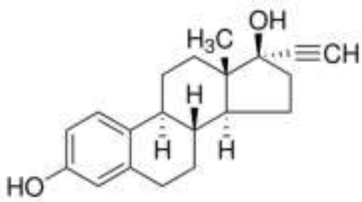

Fig-2: Chemical structure of ethinylestradiol

Several HPLC [4, 5] or HPLC/MS methods [6-9] have been reported for the determination of ethinylestradiol alone or in combination with other drug substances. Literature survey also showed few reported

HPLC methods for the determination of drospirenone in combination dosage forms with ethinylestradiol [10-13]. Spectrophotometric methods are preferred and commonly used techniques for routine analysis due to their simplicity and economical advantages. No spectrophotometric method has been reported for simultaneous determination of these drugs. Because of the zero order spectral overlapping of ethinylestradiol and drospirenone, conventional spectrophotometry could not be used for simultaneous determination of these drugs. Derivative spectrophotometry is a useful technique for simultaneous determination of two or more active compounds in a mixture. In this study, derivative spectrophotometric method with zero-crossing technique has been used for determination of these drugs in a combination dosage form to solve the spectral overlapping problem.

MATERIALS AND METHODS

Chemicals

Ethinylestradiol (Batch No. 18901105001) was from Beijing Zizhu Pharmaceutical Co. Ltd, China. Drospirenone (Batch No. 1106-1) was from Shanghai Modern Pharmaceutical Co. Ltd., China. Both drugs were kindly provided by Abouraihan Pharmaceutical Company, Tehran, Iran. Methanol and other compounds were of analytical grade and purchased from Merck (Darmstadt, Germany).

Instrumentation

A UV-Visible spectrophotometer from Shimadzu (Model 160A, Kyoto, Japan) with a fixed band width of 2 nm was used for spectrophotometric measurements.

Spectrophotometric Measurements

The zero-order spectra of ethinylestradiol and drospirenone were recorded in the range of 200-400 nm against methanol as blank. The first to fourth order derivative spectra of these solutions were obtained at different $\Delta\lambda$ values at the same wavelength range. The zero-crossing points of these spectra were assigned to find out the appropriate wavelengths for determination of these drugs.

Calibration

Synthetic mixtures containing 0.25, 0.5, 1, 1.5, 2, and 2.5 μg/mL ethinylestradiol and fixed concentration of drospirenone (120 μg/mL) were prepared. The proposed method was applied and the derivative value at 211 nm using the first order ($\Delta\lambda$=4.0) spectra was measured. The derivative value was constructed over the ethinylestradiol concentration.

The same procedure was performed and synthetic mixtures of of drospirenone at 20, 40, 80, 120, 160, and 200 μg/mL and fixed concentration of ethinylestradiol (1.5 μg/mL) were prepared. The first order derivative value was measured at 298 ($\Delta\lambda$=8.0) and 302 nm ($\Delta\lambda$=24.0) and the calibration curves were constructed. Six series of these solutions were prepared.

Accuracy and Precision

Three synthetic standard solutions of drospirenone at 20, 80 and 200 μg/mL in the presence of a fixed concentration of ethinylestradiol (1.5 μg/mL) were prepared and analyzed using the proposed spectrophotometric method. The concentration of the solutions was calculated using the calibration curves. This procedure was repeated for three times to find out the within-day accuracy and precision. The same procedure was also performed in three consecutive days to evaluate the between-day accuracy and precision. The same procedure was performed for synthetic solutions of ethinylestradiol at 0.5, 1.5, and 2.5 μg/mL in the presence of fixed concentration of drospirenone (120 μg/mL).

Application of the Method

Twenty Yasmin tablets containing 0.03 mg ethinylestradiol and 3.00 mg drospirenone was accurately weighed and finely powdered by a mortar and pestle. A sample equivalent to one tablet was accurately weighed and transferred to a 10 mL volumetric flask. After addition of 7 mL of methanol and sonication for 20 min, the flask made up to volume. The mixture was centrifuged and a portion of the supernatant was diluted two times and subjected to the proposed spectrophotometric method. The derivative values for ethinylestradiol and drospirenone were compared with a standard solution at the same concentration value.

Relative Recovery

Relative recovery was studied by spiking the powdered tablets with appropriate concentrations of standard solutions of drospirenone and ethinylestradiol. The derivative value for this solution at the specified wavelengths was compared with a standard solution at the same concentration value to calculate the recovery.

RESULTS AND DISCUSSION

Spectrophotometric Measurements

The zero-order spectrum of drospirenone and ethinylestradiol showed a marked overlapping in the wavelength range of 200-400 nm (Fig-3). First to fourth order derivative spectra of ethinylestradiol and drospirenone were studied at different $\Delta\lambda$ values to find out the suitable zero-crossing points.

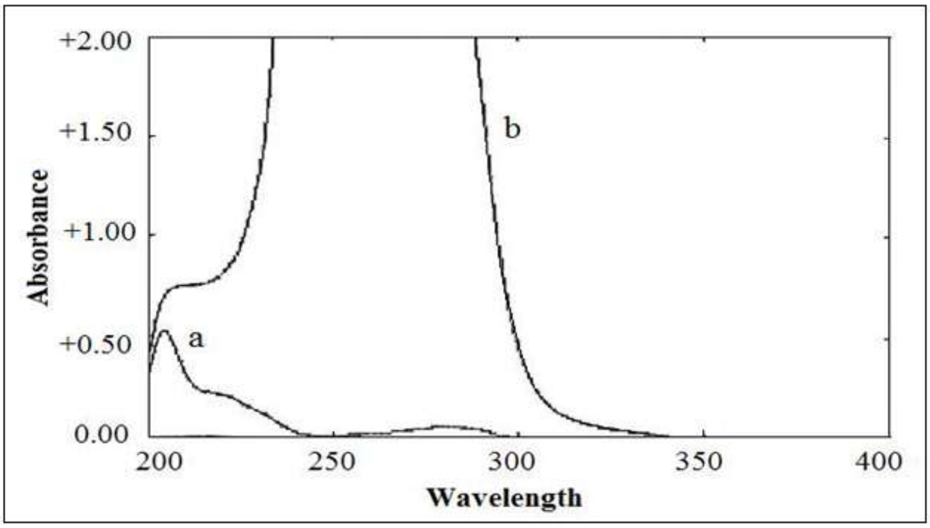

Fig-3: Zero order spectra of (a) ethinylestradiol (6 μg/mL) and (b) drospirenone (150 μg/mL)

Using the first order derivative spectrum of drugs, the zero-crossing points were assigned. At zero-crossing points, the derivative value of one of the components would be proportional to its concentration, where the value of the other compound is zero. The ^{1}D ($\Delta\lambda$=4.0) zero-crossing wavelengths of drospirenone was at 211 nm which is suitable for determination of ethinylestradiol (Fig-4).

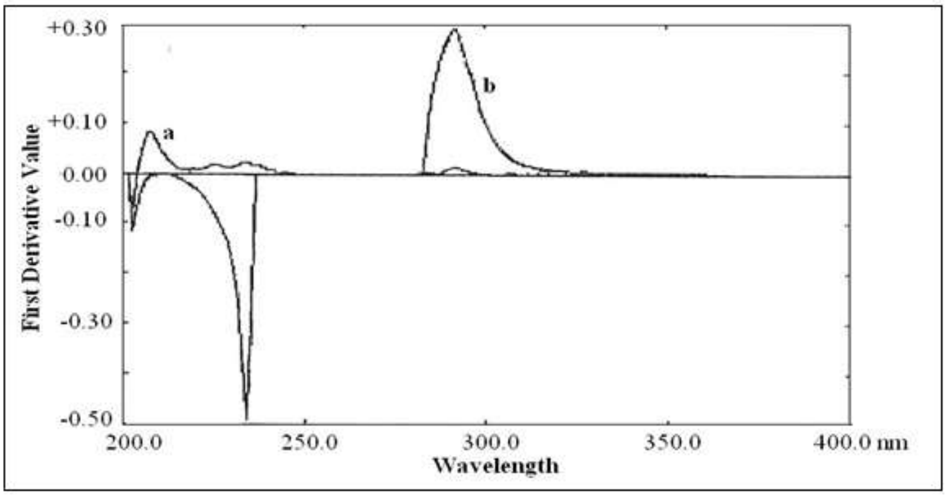

Fig-4: First order ($\Delta\lambda$=4.0) spectra of (a) ethinylestradiol (6 μg/mL) and (b) drospirenone (150 μg/mL)

The ^{1}D zero-crossing wavelengths of ethinylestradiol at 298 nm ($\Delta\lambda$=8.0) (Fig-5) and 302 nm ($\Delta\lambda$=24.0) (Fig-6) could also be used for determination of drospirenone. Acceptable linearity for the calibration curve of drospirenone and ethinylestradiol was observed. Therefore, these wavelengths were used for further determinations.

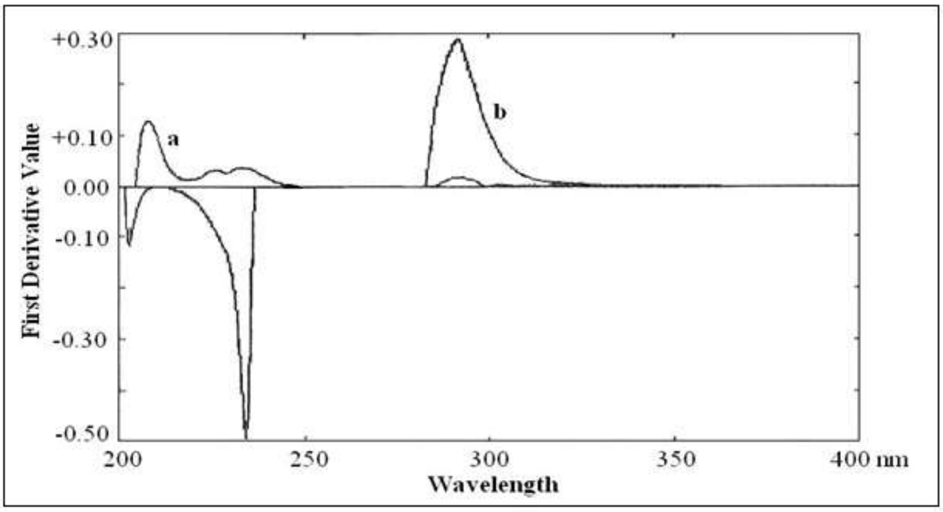

Fig-5: First order (Δλ=8.0) spectra of (a) ethinylestradiol (6 μg/mL) and (b) drospirenone (150 μg/mL)

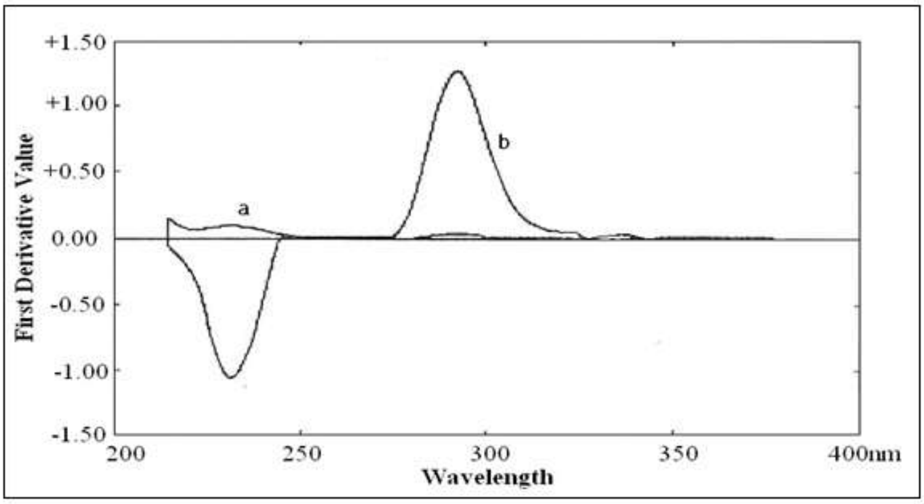

Fig-6: First order (Δλ=24.0) spectra of (a) ethinylestradiol (6 μg/mL) and (b) drospirenone (150 μg/mL).

Linearity

Regression analysis was carried out for six series of synthetic calibration solutions of ethinylestradiol and drospirenone. The calibration curves were linear with high value of correlation coefficient (r^2>0.995). Table-1 shows the statistical analysis of the experimental data. The quantification limit and detection limit was calculated using the following equations [14]:

$$LOQ = 10\sigma/s \quad \text{and} \quad LOD = 3.3\sigma/s$$

Where,

σ is the standard deviation of intercept and s is the slope of the calibration graph.

Table-1: Statistical data for calibration curves of ethinylestradiol and drospirenone in mixtures with different concentrations using first order derivative spectra.

Parameters	Ethinylestradiol[a] $^1D_{211}$ (Δλ=4.0)	Drospirenone[b] $^1D_{298}$ (Δλ=8.0)	Drospirenone[b] $^1D_{302}$ (Δλ=24.0)
Linearity range	0.25-2.5 μg/mL	20-200 μg/mL	20-200 μg/mL
Regression equation	Y=0.0076X+0.0014	Y=0.0017 X+0.0082	Y=0.0032 X+0.0207
SD of slope	0.00011	5.5×10^{-5}	5.2×10^{-5}
RSD of slope (%)	1.51	3.22	1.61
SD of intercept	0.0003	0.0012	0.0022
Correlation coefficient	0.996	0.995	0.998
LOQ	0.39	7.06	6.88
LOD	0.13	2.33	2.27

[a]In the presence of drospirenone (120 μg/mL); [b]In the presence of ethinylestradiol (1.5 μg/mL)

Accuracy and Precision

The within-day and between-day accuracy and precision are shown in table-2. The CV values less than 2.5% indicated reasonable repeatability of the proposed spectrophotometric method.

Table-2: Accuracy and precision data of determination of ethinylestradiol and drospirenone using first order derivative spectra.

Added (μg/mL)	Within-day (n = 3)			Between-day (n = 9)		
	Found (μg/mL)	CV (%)	Error (%)	Found (μg/mL)	CV (%)	Error (%)
Ethinylestradiol[a] $^{1}D_{211}$ (Δλ=4.0)						
0.500	0.489±0.012	2.45	-2.20	0.492±0.010	2.03	-1.60
1.500	1.504±0.006	0.40	0.27	1.498±0.014	0.93	-0.30
2.500	2.510±0.031	1.24	0.40	2.503±0.018	0.72	0.12
Drospirenone[b] $^{1}D_{298}$ (Δλ=8.0)						
20.00	19.64±0.31	1.58	-1.80	19.87±0.25	1.26	-0.65
80.00	80.57±0.77	0.96	0.71	80.29±0.76	0.95	0.36
200.00	198.46±0.71	0.36	-0.77	199.84±1.49	0.75	-0.08
Drospirenone[b] $^{1}D_{302}$ (Δλ=24.0)						
20.00	19.53±0.36	1.84	-2.35	19.71±0.28	1.42	-1.45
80.00	80.17±0.30	0.37	0.21	79.95±0.60	0.75	-0.06
200.00	200.07±1.41	0.70	0.04	199.83±1.31	0.66	-0.09

[a]In the presence of drospirenone (120 μg/mL); [b]In the presence of ethinylestradiol (1.5 μg/mL)

Application

The content of drospirenone and ethinylestradiol in Yasmin tablets were analyzed by the developed spectrophotometric method and also a previously reported HPLC method. The results are shown in Table-3. Paired t-test at 95% confidence interval did not show significant difference between two methods.

Table-3: Comparison of the developed method with the reference method for the determination of Yasmin tablets

Compound	Label claimed (mg)	Found (mean ± SD)		Statistical Tests*
		Proposed method	HPLC method	
Drospirenone	3.000	3.078±0.038	3.084±0.010	t = 0.762 F = 0.052
Ethinylestradiol	0.0300	0.0296±0.0014	0.0291±0.0022	t = 0.734 F = 0.433

*Theoretical values of t and F at p = 0.05 are 3.182 and 9.277, respectively.

Relative Recovery

The recovery was 98.2±0.2% and 99.3±0.1% for drospirenone and ethinylestrediol, respectively. The high recovery value confirmed the suitability of the proposed method for determination of drugs in dosage forms.

CONCLUSION

In this study, first order derivative spectrophotometry was used for the simultaneous determination of drospirenone and ethinylestradiol. This method is simple, rapid and sensitive, and could be used without any pretreatment procedure for routine quality control studies.

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