

**SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF
ESTRADIOL VALERATE AND CYPROTERONE ACETATE**
**ESTRADIOLVALERATVESİPROTERONASETAT'INBİRARADA
SPEKTROFOTOMETRİK TAYİNİ**

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ABSTRACT

Binary combinations of estradiol valerate (EV) and cyproterone acetate (CA) were determined by Vierordt's and Absorbance ratio methods. Solutions of standards and samples were prepared in 0.1 N methanolic NaOH. Absorbance-values at 242.0 nm (λ_{max} of EV), 255.0 nm (λ_{iso}) and 282.8 nm (λ_{max} of CA) were read against blank. The recoveries of the drugs from standard mixtures were in both methods between 98.1% and 101.9 %. The precision of the methods for EV and CA were better than 1.03 % and 0.97 % as relative standard deviation, respectively.

Key Words : Estradiol valerate, cyproterone acetate, simultaneous determination, Vierordt's and Absorbance ratio methods

ÖZET

Estradiol valerat (EV) ve siproteron asetatin (CA) ikili karışımları Vierordt ve Absorbans Oranları metodlarıyla tayin edildi. Standart ve numune çözeltileri 0.1 N metanollü NaOH ile hazırlandılar. Çözeltilerin absorbans değerleri, 242.0 nm (EV nin λ_{max} 'i), 255.0 nm (λ_{iso}) ve 282.8 nm de (CA nın λ_{max} 'i) köre karşı okundu. Bileşenlerin standart karışımlarından geri kazanımları -her iki metod için- % 98.1 ile % 101.9 arasındadır. EV ve CA için metodların tekraredilebilirliği bağıl standart sapma olarak % 1.03 ve % 0.97 den iyidir.

Anahtar Kelimeler : Estradiol valerat, siproteron asetat, bir arada tayin, Vierordt ve Absorbans Oranları metodu

INTRODUCTION

The combination of estradiol valerate (Estra-1,3,5(10)-triene-3,17-diol(17 β)-,17-pentanoate) and cyproterone acetate (6-chloro-17-hydroxy-1 α ,2 α -mefhylenepregna-4,6-diene-3,20-dione acetate) in the ratio 2:1 as m/m are used in hormon replacement therapy by climacterium. Estradiol valerate (EV) is official in USP (1) and cyproterone acetate (CA) in Eur .Ph. (2). They were determined by LC and spectrophotometry, respectively. Various methods have been reported on the determination of estradiol derivatives and cyproterone acetate. These are colorimetry (3), fluorometry (4,5), GC (6), GC-MS (7), RIA (8), HPLC (9-12). But no references were cited for the simultaneous determination of these drugs.

In this study, Vierordt's and absorbance ratio methods were applied for their simultaneous determination from dosage forms. Vierordt's method was proposed by Heilmeyer (13) and succesfully applied to binary mixtures (14-16). Absorbance ratio method was introduced by Pernarowski for simultaneous determination of binary and ternary drug combinations (17-21). The advantage of the methods proposed is that the calculations are based on direct UV measurements.

MATERIALS AND METHODS

Apparatus

A Shimadzu 1601 UV visible spectrophotometer connected to an IBM-PC and a Lexmark 1020 printer was used for the absorbance measurements. The measurements were made with 1-cm quartz cells. Raw data was processed by Shimadzu-UVPC software. Operating conditions : Slit-width 2 nm, scan range 225-350 nm, scan speed 2 nm min⁻¹ equipped with 1-cm quartz cells.

Chemicals and materials

Estradiol valerate, Cyproterone acetate and Climen® dragees were supplied from Schering İlaç San.A.S., Istanbul, Turkey.

Climen's ® declared content was as follows : pink dragees white dragees

estradiol valerate. 2 mg 2 mg

cyproterone acetate. 1 mg

Solutions were filtered by Schleicher & Schuell FB 030/2 disposable filters (porewidth 0.45 µm). Methanol and NaOH (Merck) were analytical reagent grade.

Solutions

Stock solutions of EV (100 µgml⁻¹) and CA (50 µgml⁻¹) were prepared in methanol. Standard solutions of the drugs (n=5) were prepared as follows : 0.8 - 4.0 ml of stock EV and 1.0 - 5.0 ml of stock CA were transferred to 10-ml calibrated flasks, separately. 1.0 ml 1 N NaOH was added to each flask and they were diluted to volume with methanol (EV : 8-40 µgml⁻¹ and CA : 5-25 µg ml⁻¹). The UV measurements were made against 1N NaOH : methanol (1:9) as blank.

Sample preparation

Pink and white dragees were powdered and treated separately. Powder equivalent to about 10 mg of EV were accurately weighed and transferred into a 100-ml calibrated flask with about 50 ml methanol. The mixture was shaken mechanically for 15 minutes and diluted to volume with the same solvent. The solution was filtered and 2.5 ml of the filtrate was pipetted to a 10-ml calibrated flask. After addition of 1 ml of 1N NaOH, the solutions were diluted to volume with methanol. The absorbance-values of standard and sample solutions at 242.0 nm, 255.0 nm and 282.8 nm were measured against blank. Concentration of EV and CA were calculated using the formulas given below.

Calculations in Vierordt's method :

$$C_1 = (A_1 / \alpha) (b - m / b - a) \qquad C_2 = (A_2 / \beta) (b - (m-a) / m (b - a))$$

C_1 and C_2 are the concentrations of EV and CA as g/100 ml.

A_1 and A_2 are absorbance values of the mixture at 242.0 nm (λ_{max} of EV) and 282.8 nm (λ_{max} of CA).

α_1 and α_2 are the absorptivities ($A_{1\%}^{1\text{cm}}$) of EV at the relevant wavelengths ($a_1 = A_{242}/C$ and $\alpha_2 = A_{282.8}/C$)

β_1 and β_2 are the absorptivities ($A_{1\%}^{1\text{cm}}$) of CA ($\beta_1 = A_{242}/C$ and $\beta_2 = A_{282.8}/C$)

The abbreviations used are $a = \alpha_2/\alpha_1$, $b = \beta_2/\beta_1$ and $m = A_2/A_1$.

Calculations in Absorbance Ratio method :

The determination of EV and CA were performed by using following equations :

$$C_1 = (Q_1 - b_1 / a_1) (A_{\text{iso}} / a_{\text{iso}}) \times 10^3, \quad C_2 = (Q_2 - b_2 / a_2) (A_{\text{iso}} / a_{\text{iso}}) \times 10^3$$

C_1 and C_2 are concentrations of EV and CA as $\mu\text{g/ml}$, respectively.

Where : $Q_1 = A_1/A_{\text{iso}}$ for EV and $Q_2 = A_2/A_{\text{iso}}$ for CA.

A_1 and A_2 denotes A_{max} values of EV at 242.0 nm and CA at 282.8 nm.

A_{iso} = absorbance at isoabsorptive point ($\lambda_{\text{iso}} = 255.0$ nm)

b_1 and b_2 are intercept values of the regression equations.

a_1 = slope of regression equation (Q_1 versus $C_1 / C_1 + C_2$)

a_2 = slope of regression equation (Q_2 versus $C_2 / C_1 + C_2$)

a_{iso} = absorptivity ($A_{1\%}^{1\text{cm}}$) at isoabsorptive point ($= A_{\text{iso}} / C_1 + C_2$)

RESULTS AND DISCUSSION

Since EV and CA have overlapping spectras between 225-350 nm, they cannot be determined in binary combinations by direct UV measurements (Fig.1). But the proposed Vierordt's and Absorbance ratio methods enable the calculation of the concentration of the drugs using their UV absorptions. In both methods, factors affecting the results are the accuracy

of the absorbance measurements, the relative concentrations of the active ingredients, the nature of the pharmaceuticals and the spectral characteristics of the components of the mixture.

For calculations in these methods, it is necessary to select two points on the wavelength scale where the absorptivities are at a maximum. The wavelengths so chosen for either of the substances should not coincide with a sharply sloping part of the spectral curve of the other compound. The suitable pair of wavelengths for the mixture of EV and CA were 242.0 nm

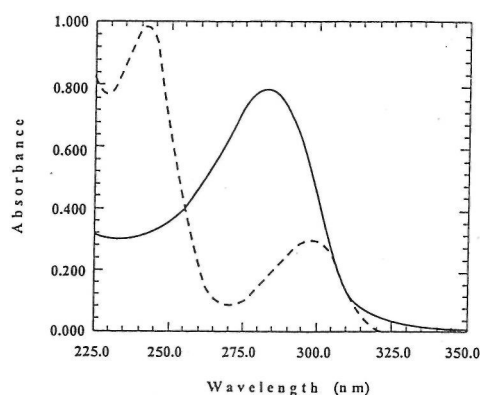


Figure 1. UV absorption spectra of EV ($40 \mu\text{g ml}^{-1}$) (.....) and CA ($20 \mu\text{g ml}^{-1}$) (—)

(λ_{max} of EV) and 282.8 nm (λ_{max} of CA). The absorptivities of EV and CA at these wavelengths, on which calculations of Vierordt's method are based, were given in Table 1.

Table 1. Absorptivities ($A^{1\text{cm}}_{1\%}$) of estradiol valerate (EV) and cyproterone acetate (CA) in Vierordt's method

EV		CA	
α_1	α_2	β_1	β_2
253.08	45.82	155.25	410.65

* α_1 and β_1 are the absorptivities at 242.0 nm and α_2 and β_2 at 282.8 nm, respectively.

For the calculations of absorbance ratio method, beside 242.0 nm and 282.8 nm, isoabsorptive wavelength was used. The isoabsorptive point of the overlapped spectra of EV and CA were at 255.0 nm. Calibration data of EV and CA for absorbance ratio method were in

Table 2. They obey Beer's law between the concentrations of 8 - 40 $\mu\text{g/ml}$ and 5-25 $\mu\text{g/ml}$ for EV and CA, respectively.

Table 2. Calibration data of EV and CA in Absorbance ratio method

Drug	Calibration equation*	correlation coefficient	concentration range ($\mu\text{g/ml}$)
EV	$y = 1.7822 x + 0.4549$	0.9959	8-40
CA	$y = 1.5884 x + 0.6782$	0.9894	5-25

* $y = A_{\text{max}}/A_{\text{iso}}$ and $x = C_1/C_1 + C_2$

The accuracy of the methods was investigated by assaying a number of standard mixtures, in which the ratio of EV and CA varies from 4:1 to 1:1. Recoveries between 98.1 - 101.9 % were obtained (Table 3). The results show the general applicability of the methods to mixtures of EV and CA composed other than 2:1.

Table 3. Recovery of EV and CA from standard mixtures by Vierordt's and Absorbance ratio methods

Added (mg)		Found(%)			
EV	CA	EV		CA	
		VM*	AR*	VM	AR
1.6	1.0	99.3	100.9	99.5	101.0
2.0**	1.0**	100.2	97.9	100.4	101.9
2.4	1.0	100.9	98.3	101.3	99.1
3.2	1.0	99.2	98.4	98.8	98.6
4.0	1.0	100.9	99.1	100.6	99.6
2.0	0.8	98.1	101.2	99.1	101.1
2.0	1.2	99.5	99.7	98.2	98.8
2.0	1.6	100.1	100.4	101.6	101.5
2.0	2.0	100.8	100.9	100.9	101.8
Mean		99.9	99.6	100.5	100.4
SD		0.95	1.27	1.19	1.34
%RSD		0.95	1.28	1.19	1.33

*VM = Vierordt's method, AR = Absorbance ratio method

**m/m ratio of EV and CA in the commercial formulation

Table 4. Assay results for EV in pink dragees* (Climen®)

Label claim of EV	Found (mean \pm SD) %		
	VM	AR	DI(283.1)
2 mg (=100 %)	100.3+1.03	99.3 \pm 0.96	100.4 \pm 0.86
statistics for**	VM - AR	VM - D1	AR - D1
t-values	1.77	0.13	2.09
F-values	1.20	1.46	1.22

*Content of pink dragees : EV (2mg) + CA (1mg)

** $t_{te0} = 2.23$ and $F_{te0} = 5.05$ for $p = 0.05$ and $n = 6$

Table S. Assay results for CA in pink dragees* (Climen®)

Label claim of CA	Found (mean \pm SD) %		
	VM	AR	DI(297.7)
1 mg (=100 %)	99.8 \pm 0.87	100.3 \pm 0.97	99.0 \pm 1.41
statistics for**	VM - AR	VM-D1	AR-D1
t-values	0.79	1.23	1.80
F-values	1.24	2.59	2.08

*Content of pink dragees : EV (2mg) + CA (1mg)

** $t_{\text{teo}} = 2.23$ and $F_{\text{teo}} = 5.05$ for $p = 0.05$ and $n = 6$

Six samples of combination drugs (pink dragées) were assayed by the proposed methods and the assay results were given in Table 4 and 5. The concentrations found show good agreement with the stated contents of the dosage units and the results obtained by derivative UV spectrophotometric method (In preparation for submission). Table 4 and 5 represent also precision data for the assays . The relative standard deviation of the concentrations of EV and CA were better than 1.03 % and 0.97 % , respectively.

F- and t-tests show that the difference between the results obtained by either the methods proposed and the derivative UV spectrophotometric methods are not significant for $p = 0.05$ and $n = 6$.

White dragées, which contain EV only were determined by direct UV measurements at 298.2 nm (2nd λ_{max} of EV), since absorption at shorter wavelengths might be more subject to matrix interference.

The calibration equation for direct UV method was $A = 0.0079 C + 0.0010$ ($r = 0.9985$). The results obtained were also compared with ones obtained by Derivative UV method (Table 6). According to statistical data, EV, as single compound, can be determined by direct UV method.

Table 6. Assay results for EV in white dragées* (Climen®)

Label claim of EV	Found (mean \pm SD) %	
	UV (298.2)	DI(283.1)
1 mg (=100 %)	99.9 \pm 0.84	100.4 \pm 0.62
statistics (for p = 0.05 and n = 6)		
t-values ($t_{te0} = 2.23$)	1.15	
F-values ($F_{te0} = 5.05$)	1.78	

White dragées contain 1 mg of EV only

In conclusion, Vierordt's and absorbance ratio method can be applied successfully to the determination of EV and C A in 2 : 1 combination. The advantage of these methods is that the calculations are based on direct UV measurements, which can be performed with a simple UV spectrophotometer. Vierordt's method should be preferred to absorbance ratio method, since calculations are relative simpler.

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