DETERMINATION OF ESTRADIOL VALERATE IN PHARMACEUTICAL PREPARATIONS BY ZERO - AND FIRST-ORDER DERIVATIVE SPECTROPHOTOMETRIC METHODS

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ABSTRACT

This paper describes zero- and first-order derivative spectrophotometric methods for determination of estradiol valerate in pharmaceutical preparations. The solutions of the standard and pharmaceutical samples were prepared in methanol. Absorbances of estradiol valerate were measured at 280 nm for zero-order by measuring height of peak from zero, at 292 nm for first-order derivative spectrophotometric method by measuring peak to peak height. The linearity ranges were found to be 1.25-12.5 μg mL^{-1} for the zero and first-order derivative spectrophotometric methods. The developed methods in this study are accurate, sensitive, precise, and reproducible and can be directly and easily applied to the pharmaceutical preparation. Also, the results obtained from two spectrophotometric methods were compared and no significant difference was found statistically.

Keywords: Estradiol valerate, Zero-, First-order derivative spectrophotometric method, Pharmaceutical preparation.

INTRODUCTION

Estradiol valerate (Figure 1) is a synthetic estrogen that is very significant in clinical medicine. Estradiol valerate can be used to treat menopause syndrome and prostate cancer, and can be used together with progestogen for the inhibition of ovulation [1].

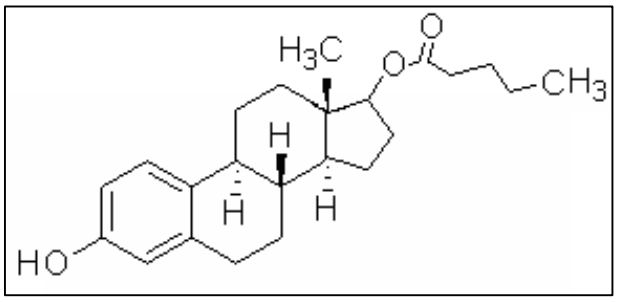

Figure 1: Chemical structure of estradiol valerate

Several analytical methods have been reported for determination of estradiol valerate including colorimetry [2], fluorimetry [3, 4], gas chromatography [5] gas chromatography-mass spectrometry (GC-MS) [6] and high performance liquid chromatography (HPLC) [7, 8].

To our knowledge, there is no derivative spectroscopic method for determination of estradiol valerate in pharmaceutical preparation in literature. Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands, and for eliminating the effect of baseline shifts and baseline tilts. It consists of calculating and plotting one of the mathematical derivatives of a spectral curve [9]. In the last year, this technique has been rapidly gained its application in the analysis of pharmaceutical preparations.

We wanted to develop new spectrophotmetric methods for determination of estradiol valerate in pharmaceutical preparation without the necessity of sample pre-treatment. After developing zero- and first-order derivative spectrophotometric methods were also carried out and all optimization parameters were also considered. Also, the developed methods were applied to commercial preparation as dragee.

MATERIALS AND METHODS

Chemicals and reagents

Estradiol valerate was obtained from Sigma-Aldrich (St. Louis, MO, USA). Climen dragee containing 2 mg estradiol valerate (Schering Pharmaceutical Industry, Istanbul, Turkey) was used for analysis.

Instrumentation

A Thermospectronic double-beam UV-Visible spectrophotometer (HEλIOSβ) with the local control software was used. Zero- and first-order derivative spectra of reference and sample solutions were recorded in 1 cm quartz cells at a scan speed of 600 nm min^{-1}, a scan range of 250-310 nm and fixed slit width of 2 nm.

Preparations of the standard and quality control solutions

The stock standard solution of estradiol valerate was prepared in methanol to a concentration of 50 μg mL^{-1} and kept stored at -20 ^{0}C. Working standard solutions were prepared from the stock standard solutions. A calibration graph was constructed in the range of 1.25, 2.5, 5, 7.5, 10 and 12.5 μg mL^{-1} for estradiol valerate (n=6). For quality control samples containing 2, 7, 11 μg mL^{-1} of estradiol valerate, the stock solution was diluted with methanol.

Procedure for pharmaceutical preparation

Climen dragee drug was weighed and finely powdered. The average weight of drug was determined with the help of weight of 10 dragees. A portion of powder equivalent to the weight of one dragee was accurately weighed into 100 mL volumetric flask and 70 mL methanol was added. The volumetric flask was sonicated for 15 min to effect

complete dissolution of the estradiol valerate, the solution was then made up to volume with methanol. The solution was filtered through a Whatman No 42 paper. Approximate dilutions were made at concentrations of 2.5 and 10 µg mL^{-1} with methanol. Zero- and first-order derivative spectra were recorded against methanol.

Data analysis

All statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 10.0. Correlations were considered statistically significant if calculated P values were 0.05 or less.

RESULTS AND DISCUSSION

Method development and optimization

The derivative wavelength difference (Δλ) depends on the measuring wavelength range and n values (smoothing factor). Generally, the noise decreases by increasing Δλ. Optimal wavelength range should be chosen since the broad peaks become sharper, the ratio of signal/noise elevates and the sensitivity of the method increases by controlling the degree of low pass filtering or smoothing. Therefore, a series of *n* values (*n*=1-9) were tested in the first-order derivative spectra of estradiol valerate in methanol. Optimum results were obtained in the measuring wavelength range 250-310 nm, *n*=5 (Δλ=17.5 nm) for first- order derivative spectrophotometric method.

Figure 2 presents the overlay of UV spectra of estradiol valerate in methanol gives two characteristic maxima at 280 and 289 nm. These two shouldered peaks were separated by using derivative spectrophotometer. Figure 3 presents the overlay of first-order ultraviolet spectra of estradiol valerate standard samples in methanol, respectively. As demonstrated in the Figure 3, the spectra present characteristic a maximum and two minima. Maximum is represented at 270 nm and minima are shown at 282 and 292 nm.

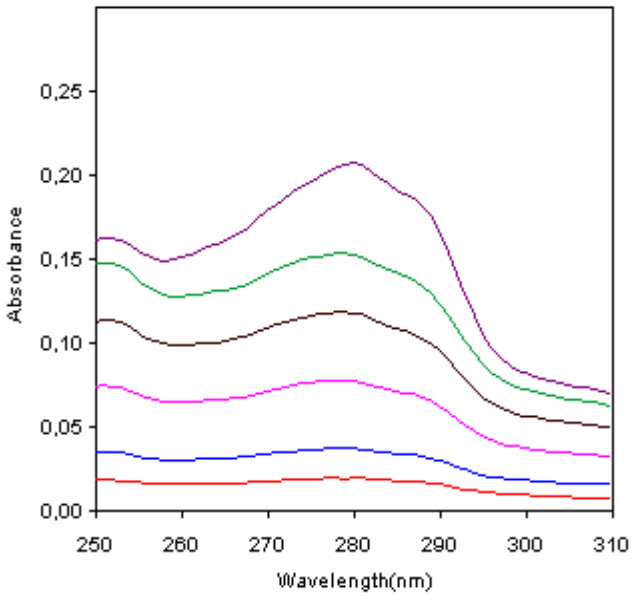

Figure 2: Zero-order derivative spectrum of standard solution of estradiol valerate

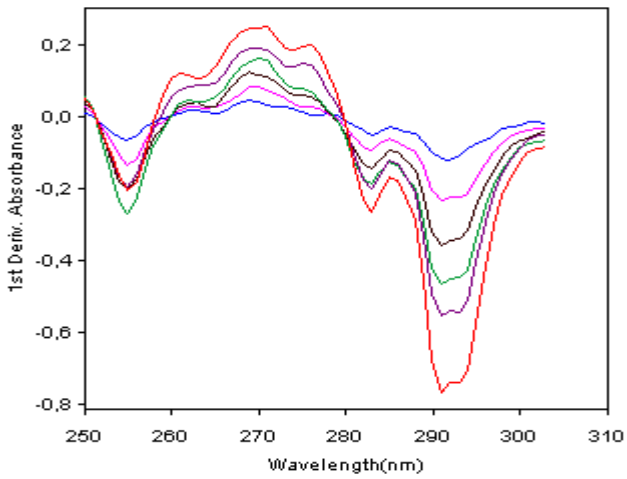

Figure 3: First-order derivative spectrum of standard solution of estradiol valerate

As no difference was observed between spectra of estradiol valerate standard and dragee solutions and in the maximum wavelengths of all spectra, it was suggested that the developed methods allowed complete elimination of the background absorption due to the capsule excipients at the chosen wavelengths both in zero- and first-order derivative spectra of estradiol valerate (Figures 4, 5).

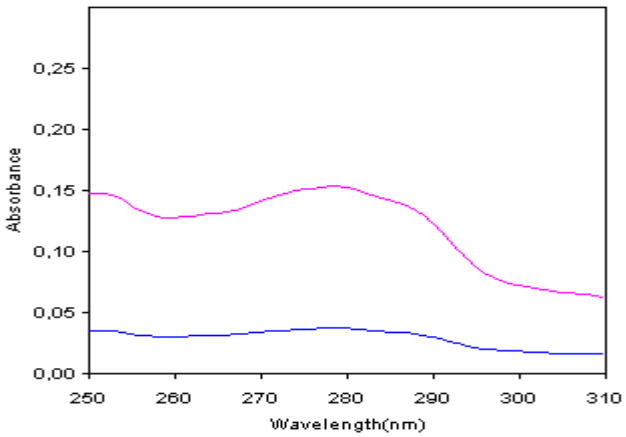

Figure 4: Zero-order derivative spectrum of solutions of Climen dragee containing estradiol valerate (2.5 and 10 µg mL^{-1})

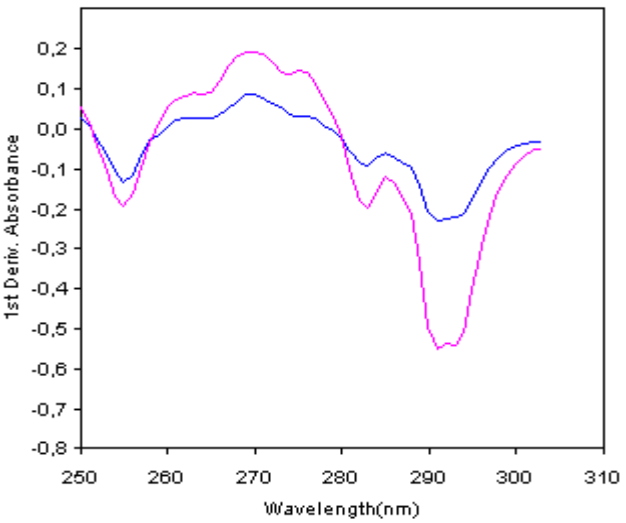

Figure 5: First-order derivative spectrum of solutions of Climen dragee containing estradiol valerate (2.5 and 10 µg mL^{-1})

Method validation

Linearity

For quantitative analysis of estradiol valerate, the calibration curves were plotted for each spectrophotometric method over the concentration ranges cited. The peak to zero method for calibration curve in the first-order derivative spectrophotometric method was used. The linearity ranges of all spectrophotometric methods were found to be 1.25-12.5 µg mL^{-1}. The statistical parameters and regression equations which were calculated from the calibration curves along with the standard error of the slope and the intercept are given in Table 1.

Limits of detection (LOD) and quantitation (LOQ)

The LOD and LOQ of estradiol valerate by the proposed methods were determined using calibration standards. LOD and LOQ values were calculated as 3.3 σ/S and 10 σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation (n=6) [10] (Table 1).

Table 1. Results of regression analysis of estradiol valerate by the proposed methods

Methods	Range (µg mL^{-1})	LR[a]	Sa	Sb	R	LOD	LOQ
Zero-order Spectrophotometric Method	1.25-12.5	$A_{280\ nm}$=0.132x+0.071	0.016	0.003	0.9995	0.399	1.212
First-order Spectrophotometric Method	1.25-12.5	$^1D_{292}$ nm =0.956x-0.449	0.115	0.011	0.9994	0.397	1.203

λ: Wavelength, [a]Based on six calibration curves, LR: Linear regression Sa: Standard deviation of intercept of regression line, Sb: Standard deviation of slope of regression line, R: Coefficient of correlation, x: estradiol valerate concentration (µg mL^{-1}), LOD: Limit of detection, LOQ: Limit of quantitation, A: Absorbance, 1D: First-order absorbance

Table 2. Precision and accuracy of estradiol valerate by the proposed methods

Method	λ (nm)	Added (µg mL^{-1})	Within-day			Between-day		
			Found±S.D (µg mL^{-1})	Accuracy	Precision R.S.D%[a]	Found±SD (µg mL^{-1})	Accuracy	Precision R.S.D%[a]
Zero-order Spectrophotometric Method	$A_{280\ nm}$	2	2.01±0.021	0.50	1.04	2.04±0.026	2.00	1.27
		7	7.11±0.209	1.57	2.94	7.12±0.221	1.71	3.10
		11	10.90±0.186	-0.91	1.71	10.80±0.226	-1.82	2.09
First-order Spectrophotometric Method	$^1D_{292}$ nm	2	2.03±0.029	1.50	1.43	2.06±0.032	3.00	1.55
		7	7.09±0.246	1.29	3.47	7.13±0.265	1.85	3.72
		11	11.16±0.345	1.45	3.09	11.22±0.427	2.00	3.81

S.D: Standard deviation of six replicate determinations, R.S.D: Relative standard derivation, [a]Average of six replicate determinations, Accuracy: (%relative error) (found-added)/addedx100

Specificity

Comparison of the zero- and first-order derivative spectrum of estradiol valerate in standard and drug formulation (Climen dragee) solutions show that the wavelength of maximum and minimum absorbance did not changed (Figures 4, 5). According to the results obtained, the zero- and first-order derivative spectrophotometric methods are able to access estradiol valerate in presence of excipients and hence, methods can be considered specific.

Accuracy and precision

The precision of the analytic methods were determined by repeatability (within-day) and intermediate precision (between-day). Three different concentrations which were quality control samples (2, 7, 11 µg mL^{-1}) were analyzed six time in one day for within-day precision and once daily for three days for between-day precision. Repeatability was ≤2.94% and ≤3.09% (n=6) and intermediate precision was ≤3.10% and ≤3.81% (n=6) for zero- and first-order derivative spectrophotometric methods, respectively (Table 2). Accuracy of zero- and first-order derivative spectrophotometric methods showed acceptable relative error values were ≤2.00% and ≤3.00% (n=6), respectively (Table 2).

Recovery

To determine the accuracy of the zero- and first-order derivative spectrophotometric methods and to study the interference of formulation additives, the recovery was checked as three different concentration levels (2, 6, 10 µg mL^{-1}) and analytical recovery experiments were performed by adding known amount of pure drugs to pre-analyzed samples of commercial dosage form (Climen). The percent analytical recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. The recoveries of zero- and first-order derivative spectrophotometric methods were between 101.2%-101.5% and 98.5%-99.8% (Table 3).

Stability

To evaluate the stability of estradiol valerate, standard solutions were prepared separately at concentrations covering the low, medium and higher ranges of calibration curve for different temperature and times. These solutions were stored at room temperature, refrigeratory (4^0C) and frozen (-20^0C) temperature for 24 h and 72h. Stability measurements were carried out with zero- and first-order derivative spectrophotometric methods. The results were evaluated comparing these measurements with those of standards and expressed as percentage deviation and estradiol valerate was found as stable at room temperature, 4 and -20^0C for at least 72h (Table 4).

Table 3. Recovery values of estradiol valerate in pharmaceutical preparation

Commercial preparation			Climen dragee (2.5 μg mL^{-1})		
Method	**λ (nm)**	**Added (μg mL^{-1})**	**Found±S.D (μg mL^{-1})**	**Recovery (%)**	**R.S.D [a] (%)**
Zero-order Spectrophotometric Method	$A_{280\ nm}$	2	2.03±0.038	101.5	1.87
		6	6.08±0.142	101.3	2.34
		10	10.18±0.147	101.2	1.44
First-order Spectrophotometric Method	$^1D_{292}$ nm	2	1.97±0.058	98.5	2.94
		6	5.98±0.149	99.7	2.49
		10	9.98±0.366	99.8	3.67

S.D : Standard deviation of six replicate determinations, R.S.D: Relative standard derivation,
[a]Average of six replicate determinations

Table 4. Stability of estradiol valerate in solution

Stability (%)		Room temperature stability (Recovery % ± S.D)		Refrigeratory stability, +4^{0}C (Recovery % ± S.D)		Frozen stability, - 20°C (Recovery % ± S.D)	
λ (nm)	**Added (μg mL^{-1})**	**24 h**	**72 h**	**24 h**	**72 h**	**24 h**	**72 h**
A_{280} nm	3	101.2±2.82	99.4±0.09	98.7±0.19	101.4±2.62	107.8±0.06	98.8±0.64
	8	102.1±2.17	101.2±0.08	101.5±0.07	98.4±0.65	103.7±0.08	97.3±0.01
	12.5	101.3±1.84	99.6±1.54	98.0±4.52	101.6±1.59	101.2±2.87	101.2±0.06
$^1D_{292}$ nm	3	99.4±0.72	102.1±0.06	103.0±1.24	101.5±0.09	101.1±1.92	101.2±0.64
	8	99.1±1.21	99.3±0.04	98.5±0.21	102.3±1.97	93.8±0.14	100.4±0.22
	12.5	99.7±2.52	102.5±0.07	98.5±0.45	98.5±0.15	102.0±0.14	102.4±0.55

S.D : Standard deviation of six replicate determinations

Table 5. Determination of estradiol valerate in pharmaceutical preparation

Commercial preparation	Method	λ (nm)	n	Found ± S.D (mg)	Recovery (%)	R.S.D[a] (%)	Confidence interval
Climen dragee 2 mg	Zero-order Spectrophotometric Method	$A_{280\ nm}$	18	2.01±0.452	100.5	2.15	1.99-2.02
	First-order Spectrophotometric Method	$^1D_{292}$ nm	18	1.99±0.386	99.5	2.08	1.97-2.01

S.D: Standard deviation of six replicate determinations, R.S.D: Relative standard derivation, [a]Average of six replicate determinations

Table 6. Statistical comparison (t-test) of the results obtained by proposed methods

Commercial preparation	Statistical Values	Zero-order derivative Spectrophotometric Method	First-order derivative Spectrophotometric Method	t values
Climen dragee 2 mg	n	18	18	t_c=1.13 t_t=1.69
	X	2.01	1.99	
	S.D	0.452	0.386	
	Std.Error	1.68	1.35	
	CI	1.99-2.02	1.97-2.01	

n: Number of determination, X: mean, S.D: Standard deviation, CI: Confidence interval, t_c: Calculated F values, t_t: Tabulated t values, H_o: Hypothesis: no statitically significant difference exists between two methods, $t_t > t_c$: H_o hypothesis in accepted (α=0.05)

Comparison of two spectrophotometric methods

Zero and first-order derivative spectrophotometric methods were applied for determination of the commercial dragee (Table 5). The results show the high reliability and reproducibility of two methods. The best results obtained at 280 nm and 292 nm for zero- and first-order derivative spectrophotometric methods were statistically compared using the t-test. At 95 % confidence level, the calculated t-values do not exceed the theoretical values (Table 6). Therefore, there is no significant difference between zero- and first-order derivative spectrophometric methods. This is suggested that the two methods are equally applicable.

The proposed methods are very effective for the assay of estradiol valerate in dragees. The validity of the proposed methods was presented by recovery studies using the standard addition method. For this purpose, a known amount of reference drug was spiked to formulated dragees and the nominal value of drug was estimated by the proposed methods. Each level was repeated six times. The results were reproducible with low S.D and R.S.D. No interference from the common excipients was observed.

CONCLUSION

In conclusion, zero- and first-order derivative spectrophometric methods were developed for determination of estradiol valerate in dragee dosage form. Estradiol valerate can be directly determined in dragees in presence of excipients without sample pre-treatment procedures by using spectrophotometric methods. The apparatus and reagents used seem to be accessible even for the simple laboratories. Also, no significant difference was found between the proposed spectrophotometric methods (t_t=1.69>t_c=1.13). Therefore, developed methods can be recommended for routine and quality control analysis of estradiol valerate.

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