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Application of Validated TLC-Densitometric Method for Determination of Estradiol Valerate in Tablets

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Abstract **Original Research Article**

The aim of current study was the application of validated TLC-densitometric method for quality control of pharmaceutical dosage products, containing steroid component Estradiol valerate. The used materials were: Climonorm tabl. CN1 (Estradiol valerate 2 mg); Climonorm tabl. CN2 (Estradiol valerate 2 mg/Levonogestrel 0.15 mg); Climen tabl. CM1 (Estradiol valerate 2 mg); Climen tabl. CM2 tabl. (Estradiol valerate 2 mg/Ciproterone acetate 1 mg). Thin layer chromatographic-densitometric method was applied. The used instrumentation was densitometer VILBER LOURMAT CN-15 LC. Chromatographic system used was: stationary phase: Silicagel $G_{60}F_{254}$ glass plates; mobile phase: chloroform: acetone = 90: 10 v/v, migration distance of mobile phase: 120 mm, detection at λ = 254 nm. The amount of Estradiol valerate in Climonorm tabl. and Climen tabl. was determined by method of calibration curve by using of regression equation: y = 28874286.x + 14290 in concentration range: 5.10^{-4} g/ml ÷ 3.10^{-3} g/ml. LOD = $3.15.10^{-4}$ g/ml; LOQ = $9.54.10^{-3}$ g/ml. Analytical parameter precision (repeatability) is presented by standard deviation (SD) and related standard deviation (RSD) and is proved by the fact that all of the experimental results for the content of Estradiol valerate correspond to the respective confidence interval: $\overline{\overline{X}} \pm t.S_{\overline{\overline{X}}}$: Climonorm CN1 tabl.: 1.78 mg ÷ 2.1 mg; SD = 0.09; Climonorm CN2 tabl.: 2 mg ÷ 2.16 mg; SD = 0.08; Climen CM1 tabl.: 1.81 mg ÷ 2.13 mg; SD = 0.09; Climen CM2 tabl.: 1.77 mg ÷ 2.09 mg; SD = 0.09. The proposed TLC-densitometric method is appropriate for identification and determination of Estradiol valerate in commercially available tablets.

Keywords: Estradiol valetate, TLC, densitometry, tablets, determination.

INTRODUCTION

Osteoporosis is designated as the third socially significant disease in the world and is caused by the reduced levels of estrogen [1], which lead to increasing of the expression of Interleukin 1, Interleukin 6 and tumor necrosis factor α (TNF-α) [2]. Interleukin 6 enhances the viability of the osteoclasts, inhibits apoptosis and increases the duration of their life cycle, as a result of which occurs osteoporosis [3]. Receptor activator of nuclear factor (RANK) kB and reactive oxygen species stimulate the expression of TNF-α in osteoclast precursor cells, which leads to increased bone resorption [4]. Phytoestrogens are polyphenolic plant metabolites [5], have estrogene activity and show a higher affinity for β-estrogen receptors due to the structural similarity with 17β-Estradiol [6].

In therapy of osteoporosis the combined drugs from different classes show additive effects in fracture treatment [7, 8]. The use of bisphosphonates with hormones has additive effects on decreasing of bone resorption markers [9]. Estrogens (0.625 mg/day) in combination with Bazedoxifene (20 mg/day) enhance bone density [10]. Combination Estradiol/Progestin reduces the risk of fractures [11].

Estradiol valerate is available on market in drug combinations: Estradiol valerate/Cyproterone acetate [12]; Estradiol valerate/Dienogest for treatment of primary dysmenorrhea [13] and as oral contraceptive [14-17].

For analysis of drugs have been reported different analytical instrumental methods:

1. RP-HPLC with UV-detection: for drugs in dosage preparations: Asenapine at λ = 220 nm [18]; Zolpidem tartrate at λ = 300 nm [19]; simultaneous determination of Metformin and Benfotiamine at λ = 230 nm [20].

2. GC-MS: for analysis of herbal extracts [21].
3. HPTLC: for Bosentan in tablets [22].
4. UV-spectrophotometry: for determination of drugs in pharmaceutical dosage formulations: Stavudine at λ = 270 nm [23]; simultaneous estimation of Artesunate λ = 242 nm and Mefloquine hydrochloride λ = 256 nm [24].
5. visible apectrophotometry: Lamivudine after derivatization reaction with methyl red reagent at λ = 570 nm [25].

For quantification of Estradiol valerate in tablets is described first order derivative UV-spectrophotometry at λ = 270 nm [26] and HPLC - RP with UV-detection at λ = 220 nm using μBondapak Phenyl column (3.9 mm x 30 mm x 5 μm), mobile phase: acetonitrile: water = 80: 20 v/v and flow rate: 0.8 ml/min [27].

For determination of Estradiol valerate in drug combinations with other components in tablets are developed the following methods:

- First derivative UV-spectrophotometry: 1) at 283.1 nm for Estradiol valerate and 269.9 nm and 297.7 nm for Cyproterone acetate [28].
- Gas chromatography-mass spectrometry: Estradiol valerate and Medroxyprogesterone acetate [29].
- HPLC with UV-detection: 1) Estradiol valerate/Dienogest: stationary phase: ACE C_8, mobile phase: ammonium nitrate: acetonitrile = 30: 70 v/v, flow rate: 2 ml/min., λ = 280 nm, internal standard: Cyproterone acetate [30].

In our previous works have been applied TLC-densitometry [31] and HPLC-HILIC method for determination of Estradiol hemhydrate [32]. The aim of current study was the application of the validated TLC-densitometric method for identification and determination of Estradiol valerate in dosage forms.

MATERIALS AND METHODS

Drug products – tablets

- Climonorm CN1 tabl. (WEKSBH, Bayer, Germany): Estradiol valerate 2 mg
- Climonorm CN2 tabl. (WEKSBH, Bayer, Germany): Estradiol valerate 2 mg/Levonogestrel 0.15 mg
- Climen CM1 tabl. (344418, Bayer, Germany): Estradiol valerate 2 mg
- Climen CM2 tabl. (344418, Bayer, Germany): Estradiol valerate 2 mg/Ciproterone acetate 1 mg

Reference standard: Estradiol valerate with chemical purity.

Reagents with analytical grade quality: chloroform (Sigma Aldrich, N: SZBD 074SV UN 1888); acetone (Sigma Aldrich, N: SZBC 1861 SV); 99.98 % ethanol (Sigma Aldrich, N: SZBD 0500V UN 1170), distilled water.

METHODS: **TLC-Densitometry**

Instrumentation

Densitometer VILBER LOURMAT CN-15 LC Serial: 16263; sample applicator 10 μl micropipette (Hamilton, Bonaduz, Switzerland, N:18005701); TLC glass chamber (22 cm x 12 cm x 22 cm); stationary phase: TLC glass plates Silicagel $G_{60}F_{254}$, 20 cm x 20 cm (Sigma Aldrich, N: 2364681) were used.

Chromatographic Conditions

Stationary phase: TLC glass plates precoated with Silicagel $G_{60}F_{254}$, mobile phase: chloroform: acetone = 90: 10 v/v, migration distance of mobile phase: 120 mm, detection at λ = 254 nm.

Preparation of test solutions from dosage formulations (tablets), containing Estradiol valerate for investigation of analytical parameter precision (repeatability)

Separately from every drug formulation 20 tablets were weighed accurately. From homogenous powdered tablets from every drug preparation accuratelly were weighed 6 samples, containing an amount equivalent respectively to 20 mg Estradiol valerate. Every sample was transferred separately to a 10.0 ml volumetric flask and was diluted with 99.98 % ethanol. The solutions were filtered through a blue band filter and were analyzed by densitometric method described.

Chromatographic Procedure

Chromatographic analysis was achieved by using glass TLC plates**.** From every solution separatelly were spotted aliquot parts of 10 μl onto glass plates Silicagel $G_{60}F_{254}$, keeping 10 mm distance between bands. The plate was developed about at 25 ^{o}C ± 1 ^{o}C in ascending vertical manner in glass chromatographic chamber, previously presaturated for 1 h with mobile phase: chloroform: acetone = 90 : 10 v/v. The migration distance of the mobile phase in all experiments was 12 mm. The developed plates were dried on air. Densitometric scanning was performed on scanner VILBER LOURMAT CN-15 LC, operated in the absorbance mode at λ = 254 nm.

RESULTS AND DISCUSSION

In recent work the validated TLC-densitometric method was appliyed for identification and determination of Estradiol valerate in dosage pharmaceutical formulations – tablets. The obtained by TLC-densitometric method chromatograms of tablets are illustrated on Figure-1. for Climonorm CN1 tabl. and Climonorm CN2 tabl. and on Figure-2. for Climen CM1 tabl. and Climen CM2 tabl.

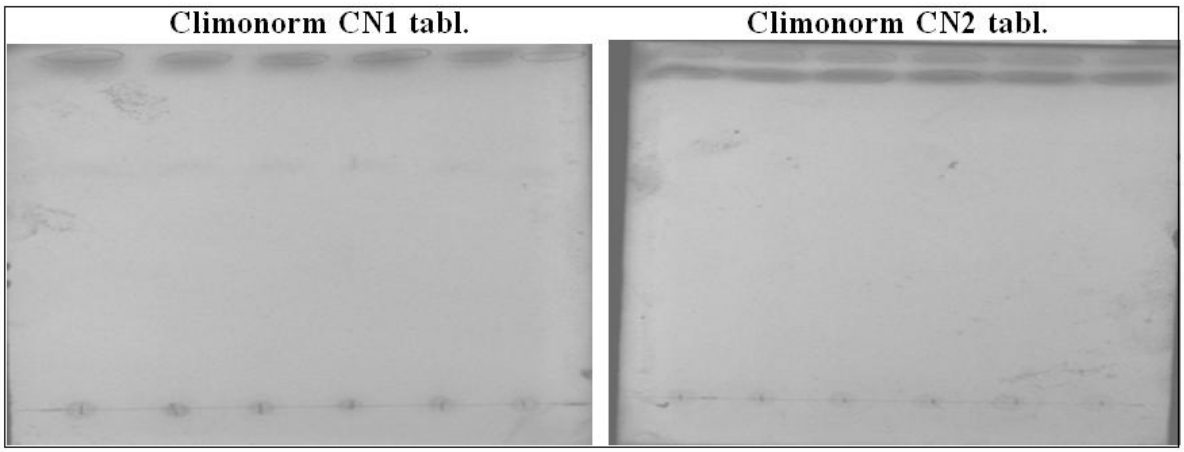

Fig-1: Chromatograms of Estradol valerate in Climonorm CN1 tabl. and Climonorm CN2 tabl.

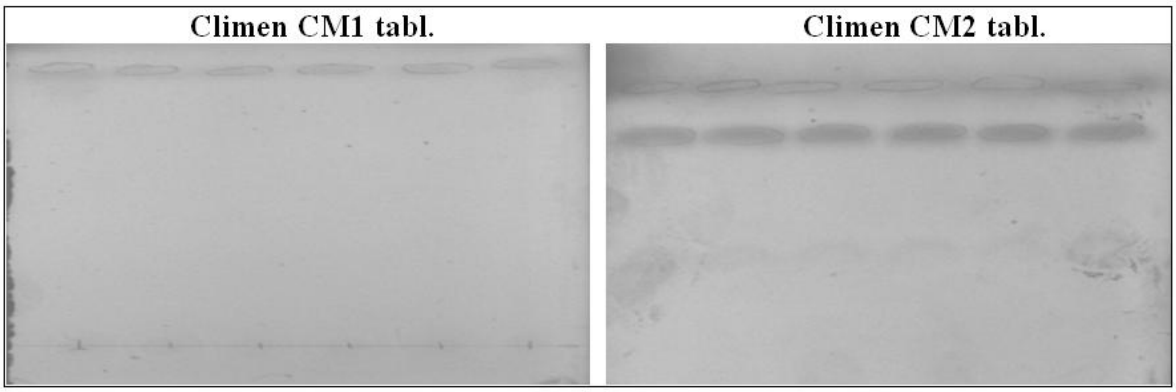

Fig-2: Chromatograms of Estradol valerate in Climen CM1 tabl. and Climen CM2 tabl.

On Table-1 are presented the data for the values of Rf, radius r [cm] and area of the spots S [cm^2] for Estradiol valerate in Climonorm tabl. and Climen tabl.

Table-1: Results for Rf and r [см] and S [$см^2$] for Estradol valerate in Climonorm tabl. and Climen tabl

N:	Climonorm CN1 tabl.			Climonorm CN2 tabl.		
	Rf	r [см]	S [$см^2$]	Rf	r [см]	S [$см^2$]
1.	1.08	0.14	0.0615	1.09	0.15	0.0707
2.	1.09	0.15	0.0707	1.1	0.16	0.0804
3.	1.09	0.15	0.0707	1.08	0.14	0.0615
4.	1.08	0.14	0.0615	1.09	0.15	0.0707
5.	1.08	0.14	0.0615	1.1	0.16	0.0804
6.	1.1	0.16	0.0804	1.09	0.15	0.0707
N:	Climen CM1 tabl.			Climen CM2 tabl.		
	Rf	r [см]	S [$см^2$]	Rf	r [см]	S [$см^2$]
1.	1.1	0.16	0.0804	1.09	0.15	0.0707
2.	1.09	0.15	0.0707	1.09	0.15	0.0707
3.	1.08	0.14	0.0615	1.08	0.14	0.0615
4.	1.09	0.15	0.0707	1.09	0.15	0.0707
5.	1.1	0.16	0.0804	1.08	0.14	0.0615
6.	1.09	0.15	0.0707	1.1	0.16	0.0804

On Table-2 are summarized the results for spot area (A) and Chauvenet's criterion for spot area (UA) and quantity (UC) of Estradiol valerate in Climonorm tabl. and Climen tabl.

Table-2: Spot area and Chauvenet's criterion for the spot area (UA) and quantity (UC) of Estradiol valerate in Climonorm tabl. and Climen tabl

N:	Climonorm CN1 tabl.			Climonorm CN2 tabl.		
	A	UA	UC	A	UA	UC
1.	70200	0.35	0.33	72500	0.62	0.63
2.	71800	0.27	0.33	77000	1.28	1.38
3.	72400	0.51	0.56	70700	1.38	1.38
4.	68100	1.18	1.11	73100	0.37	0.38
5.	69000	0.82	0.78	76200	0.95	1.0
6.	75100	1.57	1.56	74300	0.14	0.25
$\overline{\overline{\overline{X}}}$	71100			73967		
SD	2546			2361		
RSD	3.58			3.19		
N:	**Climen CM1 tabl.**			**Climen CM2 tabl.**		
	A	**UA**	**UC**	**A**	**UA**	**UC**
1.	72500	0.25	0.22	69100	0.61	0.56
2.	71400	0.19	0.11	71200	0.17	0.22
3.	70000	0.74	0.67	68800	0.72	0.67
4.	72600	0.29	0.33	72400	0.62	0.67
5.	76000	1.64	1.66	67900	1.06	1.11
6.	68700	1.26	1.22	75000	1.6	1.66
$\overline{\overline{\overline{X}}}$	71867			70733		
SD	2520			2670		
RSD	3.51			3.77		

The amount of Estradiol valerate in analysed tablets was determined by method of a calibration curve and results are included in Table-3.

Table-3: Content of Estradiol valerate in Climonorm tabl. and Climen tabl

N:	Climonorm tabl.		Climen tabl.	
	[CN1]	[CN2]	[CM1]	[CM2]
1.	1.91	1.99	1.99	1.88
2.	1.97	2.15	1.96	1.95
3.	1.99	1.93	1.91	1.87
4.	1.84	2.01	2.00	1.99
5.	1.87	2.12	2.12	1.83
6.	2.08	2.06	1.86	2.08
$\overline{\overline{\overline{X}}} \pm$ SD	1.94 ± 0.09	2.04 ± 0.08	1.97 ± 0.09	1.93 ± 0.09
SD	0.09	0.08	0.09	0.09
RSD [%]	4.64	3.92	4.57	4.66
S $\overline{\overline{\overline{X}}}$	0.04	0.03	0.04	0.04
P [%]	99.0	99.0	99.0	99.0
t	4.03	4.03	4.03	4.03
t.S $\overline{\overline{\overline{X}}}$	0.16	0.12	0.16	0.16
$\overline{\overline{\overline{X}}} - t.S\overline{\overline{\overline{X}}} \div \overline{\overline{\overline{X}}} + t.S\overline{\overline{\overline{X}}}$	1.78 ÷ 2.1	1.92 ÷ 2.16	1.81 ÷ 2.13	1.77 ÷ 2.09
E [%]	2.06	1.47	2.03	2.07

DISCUSSION

In our previous work [33] a TLC-densitometric method for Estradiol valerate was validated in accordance with International Conference on Harmonization guidelines for validation of analytical procedures for analytical parameters: limearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy and precission (repeatability) [34, 35].

Placebo solution, containing as supplement starch, without the active substance Estradiol valerate was prepared. The selectivity of the applied method was confirmed by the fact that on chromatogram with placebo preparation did not exist spot with Rf, corresponding to Rf of Estradiol valerate in reference standard solution. This fact confirms that there was no interference from the commonly present in tablets excipient starch [33].

The calibration curve was obtained by using the data for 6 different concentrations of standard solutions of Estradiol valerate and was generated by plotting the sample concentration versus the mean peak area. Linear regression analysis was performed. Linearity accordance between the concentration and spot area in range: 5.10^{-4} g/ml ÷ 3.10^{-3} g/ml was proved by the regression equation: y = 28874286.x + 14290 (y – peak area, x – concentration of analyte). The least squares regression yielded a correlation coefficient R = 0.99. Limit of detection and limit of quantitation were determined by using of the standard deviation of the response and the slope of the regression equation for the calibration curve. The limit of detection, defined the concentration giving a signal with signal to noise ratio of 3, is LOD = $3.15.10^{-4}$ g/ml. The limit of quantitation, defined the concentration giving a signal with signal to noise ratio of 10, is LOQ = $9.54.10^{-3}$ g/ml [33].

The content of drug in model mixtures was determined by method of calibration curve using the regression equation. For the estimation of analytical parameter accuracy the degree of recovery is presented in R [%] ± RSD [%] and suit respective confidence interval: R [1.5 mg]: 95.92 % ÷ 103.98 %; R [2 mg]: 93.35 % ÷ 108.89 %; R [2.5 mg]: 95.37 % ÷ 103.77 % [33].

For the estimation of an analytical parameter precision (repeatability) was used the uncertainty of the result, which is determined by: standard deviation (SD), relative standard deviation (RSD) and confidence interval. SD was calculated sample standard deviation (SD), by the applying of the Bessel's correction, in which the denominator N − 1 (degrees of freedom) is used instead of N and in this case $(S)^2$ is an unbiased estimator for $(SD)^2$. All data for the obtained quantity correspond to the confidence interval: 1.88 mg ÷ 2.17 mg [33].

In recent study the validated TLC-densitometric method was applied for identification and determination of Estradiol valerte in dosage forms – tablets: Climonorm CN1 tabl. and Climen CM2 tabl.: Estradiol valerate 2 mg; Climonorm CN2 tabl.: Estradiol valerate 2 mg/Levonogestrel 0.15 mg; Climen CM2 tabl. Estradiol valerate 2 mg/Ciproterone acetate 1 mg.

Chemical structures of Estradiol valerate, Levonogestrel and Ciproterone acetate are illustrated on Figure-3.

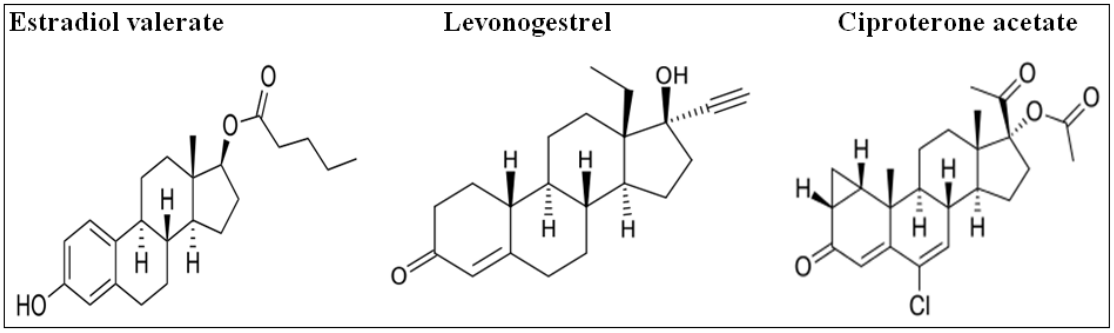

Fig-3: Chemical structures of Estradiol valerate, Levonogestrel and Ciproterone acetate

The amount of Estradiol valerate in Climonorm tabl. and Climen tabl (Table-3) was determined by a calibration curve using the regression equation: y = 28874286.x + 14290.

From Table-2 it is obvious that for all of the analysed tablets the calculated Chauvet's criterion for the area of the spots (UA) and for the quantities (UC) of Estradiol valerate are lower than the maximum value of the criterion (Umax = 1.73; N = 6), which proves that the results suit to the requirements of the criterion for the analysis of 6 separete samples from Climonorm tabl. and Climen tabl. All of the experimental results for the content of Estradiol valerate correspond to the respective confidence interval: Climonorm CN1 tabl. (1.78 mg ÷ 2.1 mg, SD = 0.09); Climonorm CN2 tabl. (2 mg ÷ 2.16 mg, SD = 0.08); Climen CM1 tabl. (1.81 mg ÷ 2.13 mg, SD = 0.09); Climen CM2 tabl. (1.77 mg ÷ 2.09 mg, SD = 0.09).

CONCLUSIONS

Estradiol valerate is available on market in drug combinations with: Ciproterone acetate, Dienogest, Medroxyprogesterone acetate, Levonogestrel. The validated TLC-densitometric method was appliyed for the identification and determination of Estradiol valerate in tablets. The analytical parameter repeatability for the content of

drug in tablets is characterized with SD and RSD. The results showed that all of the experimental results for the content correspond to the respective confidence interval. The proposed validated TLC-densitometric method is appropriate for quality control of Estradiol valerate in commercially available tablets.

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