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Validation of a TLC-densitometric method for quality control of Estradiol valerate in drug combinations

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The aim of the current study was the validation of a TLC-densitometric method for quality control of Estradiol valerate in drug combination dosage forms. The TLC conditions were: glass plates with silicagel $G_{60}F_{254}$; mobile phase: chloroform : water = 90 : 10 v/v. The TLC-densitometric method was validated with respect to the analytical parameters: limearity, LOD, LOQ, accuracy and precision (repeatability). Linear regression analysis was performed. The regression calibration curve was built. 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. LOD = $3.15.10^{-4}$ mg/ml; LOQ = $9.54.10^{-3}$ mg/ml.

For estimating the accuracy the recovery is presented in R [%] ± RSD [%] with the 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 %. Precision is estimated by standard deviation, relative standard deviation and confidence interval. All data for the obtained quantity correspond to the confidence interval: 1.88 mg ÷ 2.17 mg. The proposed validated TLC-densitometric method is appropriate for quality control of Estradiol valerate in commercially available tablets.

Key words: TLC-densitometry, Estradiol valerate, validation, linearity, accuracy.

INTRODUCTION

Osteoporosis is a systemic skeletal disease, characterized by decreased bone mass and altered bone microarchitecture, leading to increased bone fragility and fracture risk in women [1] and men [2]. Estradiol valerate is used for treatment of symptoms of menopause (hot flashes, burning, irritation) and types of prostate cancer (androgen-dependent), for prevention of osteoporosis in postmenopausal women, replacement of estrogen in women with ovarian failure or other conditions that cause a lack of natural estrogen in the body. The risk of osteoporosis is increased in estrogen deficiency [3].

Estradiol valerate is included in combined dosage forms for contraception with Cyproterone acetate (Femilar) [4] and Dienogest [5, 6]. On the pharmaceutical market drug products are available for treatment of climacteric symptoms in postmenopausal women, containing Estradiol valerate 2 mg in combination with: Ciproterone acetate 1 mg (Climen tabl.) [4]; Dienogest 2 mg (Climodien tabl.) [7], Medroxyprogesterone acetate 10 mg (Divina, Farlutes) [8]; Levonogestrel 0.15 mg (Climonorm tabl.) [9].

The following methods for determination of Estradiol valerate in tablets are described: I) UV-spectrophotometry at λ = 280 nm [10]; II) UV-spectrophotometry - first derivative at λ = 270 nm [11] and λ = 292 nm [10]; III) fluorimetry after derivatization reaction with dansyl chloride [12]. For determination of Estradiol valerate in drug combinations containing other components in the tablets the following methods are developed:

I) UV-derivative spectrophotometry:

1) Estradiol valerate and Cyproterone acetate [13]

2) 2nd derivative spectrophotometry: at λ = 297.4 nm for Estradiol valerate and at λ = 273.4 for Medroxyprogesterone acetate [14]

II) chemiluminescence method: inhibition of luminol luminescence by Estradiol valerate [15]

III) 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 [16]

2) Estradiol valerate/Medroxyprogesterone acetate: stationary phase: C_{18}, mobile phase: ammonium nitrate: acetonitrile = 30 : 70 v/v, λ = 280 nm [17]

IV) GC/MS: Estradiol valerate and Medroxyprogesterone acetate [18]

V) electrochemical method: carbon electrode modified with iron tetrapyridinoporphyrazine: 17β-Estradiol valerate in injections [19].

The aim of the current study is to validate the TLC-densitometric method for quality control of Estradiol valerate with respect to the analytical

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parameters: selectivity, linearity, accuracy, precision.

MATERIALS AND METHODS

Materials

I) Reference standard: Estradiol valerate

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

III) TLC glass plates precoated with silicagel $G_{60}F_{254}$, 20 cm × 20 cm (Sigma Aldrich, N: 2364681).

Method: TLC-densitometry.

I) Instrumentation: densitometer VILBER LOURMAT CN-15.LC Serial:16263; sample applicator 10 μl micropipette (Hamilton, Bonaduz, Switzerland, N:18005701); TLC chamber (22 cm × 12 cm × 22 cm); stationary phase: Silicagel $G_{60}F_{254}$; mobile phase: chloroform : distilled water = 90 : 10 v/v, detection at λ = 254 nm.

II) Preparation of solution of RS for linearity check.

Accurately weighed quantities of the reference standard Estradiol valerate: 0.005 g, 0.01 g, 0.015 g, 0.02 g, 0.025 g, 0.03 g were dissolved in separate volumetric flasks of 10.0 ml in 99.98 % ethanol to obtain solutions with concentrations correspondingly: 5.10^{-4} g/ml; 1.10^{-3} g/ml; $1.5.10^{-3}$ g/ml, 2.10^{-3} g/ml, $2.5.10^{-3}$ g/ml, 3.10^{-3} g/ml.

III) Preparation of model mixtures for accuracy check.

Accurately weighed quantities equivalent to 0.015 g, 0.020 g, 0.025 g of the reference standard Estradiol valerate were dissolved in separate volumetric flasks of 10.0 ml in 99.98 % ethanol to obtain 3 samples from 3 different model mixtures with contents equivalent to 80 % (1.5 mg/ml, I), 100 % (2 mg/ml, II) and 120 % (2.5 mg/ml, III) of the theoretical concentration in the tablets (2 mg).

IV) Preparation of model mixtures for precision (repeatability) check.

Accurately weighed quantities equivalent to 0.02 g of the reference standard Estradiol valerate were dissolved in 6 separate volumetric flasks of 10.0 ml in 99.98 % ethanol to obtain 6 model mixtures with Estradiol valerate content equivalent to 100 % (2 mg/ml) of the theoretical concentration in the tablets (2 mg).

RESULTS AND DISCUSSION

The TLC-densitometric method was validated for the analytical parameters: selectivity, linearity, accuracy and precision.

1) Selectivity

A ”placebo” solution without the active substance Estradiol valerate was prepared in the same manner like the solution of the reference standard. The selectivity of the applied TLC-densitometric method was proved by the fact, that on the chromatograms with ”placebo” solutions there were no spots with Rf, corresponding to the Rf of Estradiol valerate (0.92).

2) Linearity

Linearity accordance between the concentration and spot area in the range: 5.10^{-4} g/ml ÷ 3.10^{-3} g/ml was proved by the regression equation: y = 28874286.x + 14290. (Fig. 1.). LOD = $3.15.10^{-4}$ mg/ml; LOQ = $9.54.10^{-3}$ mg/ml.

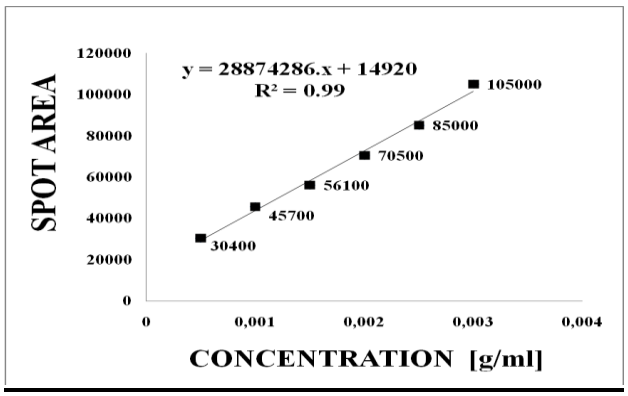

Fig. 1. Linearity for Estradiol valerate

3) Test for system suitability

The suitability of the system was confirmed by the lack of a statistically significant difference between the values of the chromatographic mobility parameter relative to the front (Rf) in the analysis of 6 samples of Estradiol valerate: Rf: 0.43, 0.43, 0.43, 0.43, 0.44, 0.44 (0.43 ÷ 0.005, SD = 1.4).

4) Accuracy

For the estimation of accuracy, in Table 1 are summarized data for the spot area (A), obtained by the method of calibration curve for model mixtures, the quantity [C] and the recovery [RC] of Estradiol valerate, arithmetical mean ($\overline{X}$)**;** standard deviation (SD) and relative standard deviation (RSD) (%); $S\overline{X}$ – mean quadratic error; P – confidence possibility (%); t – Student’s coefficient; $\overline{X} \div t.S\overline{X}$ – confidence interval; E – relative error. Accuracy is presented by the recovery R (%) and RSD [20].

Table 1. Data for spot area (A), quantity [C] and degree of recovery [RC] for Estradiol valerate in model mixtures – estimation of accuracy.

N:	A	[$C_{1.5}$] [mg]	R [$C_{1.5}$] [%]	A	[C_2] [mg]	R [C_2] [%]	A	[$C_{2.5}$] [mg]	R [$C_{2.5}$] [%]
1.	56000	1.42	97.73	68000	1.84	96.84	84000	2.39	97.55
2.	57900	1.49	99.33	71500	1.96	100.51	86200	2.47	98.80
3.	60700	1.59	102.58	76200	2.12	106.0	90300	2.61	102.35
$\overline{X}$ ± SD	58200	1.5 ± 0.09		71900	1.97 ± 0.14		86833	2.49 ± 0.11	
$\bar{R}$ [%] ± RSD[%]			99.95 ± 2.39			101.12 ± 4.56	3197		99.57 ± 2.5
SD	2364	0.09	2.39	4115	0.14	4.61	3.68	0.11	2.49
RSD [%]	4.06	6.0	2.39	5.72	7.11	4.56		4.42	2.5
$S\ \overline{X}$		0.05	1.38		0.08	2.66		0.06	1.44
P [%]		90.0	90.0		90.0	90.0		90.0	90.0
t		2.92	2.92		2.92	2.92		2.92	2.92
$t.S\ \overline{X}$		0.15	4.03		0.23	7.77		0.18	4.2
$\overline{X}$ ± $t.S\ \overline{X}$		1.35 ÷ 1.65	95.92 ÷ 103.98		1.74 ÷ 2.2	93.35 ÷ 108.89		2.31 ÷ 2.67	95.37 ÷ 103.77
E [%]		3.33	1.38		4.06	2.63		2.41	1.45

Table 2. Data for spot area (A), quantity [C] and degree of recovery [RC] for Estradiol valerate in model mixtures – estimation of precision.

N:	C	A	U A	[C]	R [C] [mg/l]	U [C]
1.	1.95	69900	1.14	1.90	97.44	1.22
2.	1.95	70300	0.99	1.92	98.46	1.00
3.	2.00	71900	0.36	1.97	98.50	0.44
4.	2.00	73500	0.27	2.03	101.50	0.22
5.	2.05	75200	0.93	2.09	101.95	0.89
6.	2.05	76100	1.29	2.12	103.41	1.22
$\overline{X}$ ± SD		72817 ± 2584		2.01 ± 0.09		
$\bar{R}$ [%] ± RSD[%]					100.21 ± 2.39	
SD		2584		0.09	2.38	
RSD [%]		3.51		4.48	2.39	
$S\ \overline{X}$				0.04	0.98	
P [%]				98.0	98.0	
t				3.37	3.37	
$t.S\ \overline{X}$				0.13	3.30	
$\overline{X} - t.S\ \overline{X}$ ÷ $\overline{X} + t.S\ \overline{X}$				1.88 ÷ 2.17	96.91 ÷ 103.51	
E [%]				1.99	0.98	

All results are within the 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 %.

5) Precision (repeatability)

Precision is estimated by the uncertainty of the result, determined by standard deviation (SD), relative standard deviation (RSD) and confidence interval ($\overline{X} \div t.S\overline{X}$). Table 2 presents: C – added content of Estradiol valerate in model mixtures, [C] – content obtained by the method of the calibration curve, [RC] – degree of recovery, U[A] – Chauvenet criterion for area, U[C] – Chauvenet criterion for the obtained content. All data for the obtained quantity of Estradiol valerate correspond to the con-fidence interval: 1.88 mg ÷ 2.17 mg (SD = 0.05).

CONCLUSIONS

Estradiol valerate is available on the market in drug combinations with: Ciproterone acetate, Dienogest, Medroxyprogesterone acetate, Levonogestrel. The obtained quantities by the applied method correspond to the relevant confidence interval: 1.88 mg ÷ 2.17 mg. The proposed validated TLC-densitometric method is appropriate for quality control of Estradiol valerate in commercially available tablets.

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ВАЛИДИРАНЕ НА TLC-ДЕНЗИТОМЕТРИЧЕН МЕТОД ЗА КОНТРОЛ НА КАЧЕСТВОТО НА ESTRADIOL VALERATE В ЛЕКАРСТВЕНИ КОМБИНАЦИИ

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(Резюме)

Целта на настоящото изследване е валидирането на TLC-денситометричен метод за контрол на качеството на Estradiol valerate в комбинирани дозирани лекарствени форми. Условията на TLC са: стъклени плаки: Silicagel $G_{60}F_{254}$; подвижна фаза: хлороформ : вода = 90 : 10 v/v. TLC-денситометричният метод е валидиран по отношение на аналитичните параметри: линейност, LOD, LOQ, точност и прецизност (повторяемост). Проведен е линеен регресионен анализ. Построена е калибрационна права. Линейната зависимост между концентрацията и площта на петната в интервала: 5.10^{-4} g/ml ÷ 3.10^{-3} g/ml се доказва от уравнението на регресия: y = 28874286.x + 14290. LOD = $3.15.10^{-4}$ mg/ml; LOQ = $9.54.10^{-3}$ mg/ml.

За оценка на аналитичния параметър точност, е представен аналитичният добив в R [%] ± RSD [%], като резултатите отговарят на съответния доверителен интервал: R[1.5 mg]: 95.92 % ÷ 103.98 %; R[2 mg]: 93.35 % ÷ 108.89 %; R[2.5 mg]: 95.37 % ÷ 103.77 %. Прецизността е оценена чрез изчисляване на стандартно отклонение, относително стандартно отклонение и доверителен интервал. Всички данни за получените количества съответстват на доверителния интервал: 1.88 mg ÷ 2.17 mg.. Предложеният валидиран TLC-денситометричен метод е подходящ за контрол на качеството на Estradiol valerate valetate в таблетки.