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QUANTITATIVE DETERMINATION OF TEFESTROL IN *Ferula tenuisecta* ROOTS

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A method for determining tefestrol in Ferula tenuisecta *roots, based on its TLC separation from accompanying substances and spectrophotometric determination, was proposed.*

Key words: esters of terpene alcohols and phenolcarboxylic acids, determination method.

Tefestrol preparation, which has been created and included in medical practice at the Institute of the Chemistry of Plant Substances of the Academy of Sciences of the Republic of Uzbekistan, is a mixture of esters of terpene alcohols and phenolcarboxylic acids, ferutinine [4-hydroxy-6(4′-hydroxybenzoyloxy)-dauk-8,9-ene], tenuferidin [4-hydroxy-6(4′-hydroxybenzoyloxy)-8,9-epoxydaukane], fertidine (4-hydroxy-6-α-4′-hydroxybenzoyloxy,10-α-angeloyloxy-dauk-8,9-ene), and two minor components of unknown structure [1-3].

Tefestrol tablets (0.005 g) are widely used as an estrogen preparation for ovary malfunction, infertility, primary and secondary amenorrhea, disfunctional maternal hemorrhage, etc. [4].

The preparation is a white powder with creamy shades. It is soluble in $CHCl_3$, slightly soluble in 95% ethanol, and practically insoluble in water.

Methods for quantitative determination of ferutin and ferutinine in *Ferula* roots have been published [5].

We performed a quantitative determination of the content of the total tefestrol preparation in *Ferula tenuisecta* roots.

The UV spectrum of tefestrol in 95% ethanol in the range 220-350 nm has an absorption maximum at 260 nm, which is characteristic of the *p*-hydroxybenzoyl group. UV spectroscopy in combination with TLC forms the basis of the developed method for determining tefestrol in *Ferula tenuisecta* roots.

We used tefestrol that satisfied requirements of the pharmacopeic article as a standard in developing the method. We recommend the maximum at 260 nm, which is specific for ferutinine and tenuferidin, as the analytical band. At working concentrations of 0.005-0.013 μg/mL (100% tefestrol), the absorption (D = 0.2-0.6) of alcoholic solutions of tefestrol obeys Beer's law.

During development of the method, we studied the following steps: 1) extraction of tefestrol from plant material; 2) chromatographic separation of it from accompanying substances; 3) elution of the desired substances from sorbent and their quantitative spectrophotometric determination.

Tefestrol was separated from accompanying substances by TLC. Stationary phases on glass plates using sorbents such as KSK, silica gel L 5/40 μ (Czech Rep.), and Silufol UV-254 (Czech Rep.) and mobile phases of various solvent systems were tested.

The sensitivity of tefestrol detection increases on Silufol UV-254 plates (0.1 μg instead of 1 μg on KSK and L 5/40 μ sorbents) with good resolution of the total extracted substances on all three sorbents. Furthermore, the preparation composition is visible in UV light, which provides an advantage for more accurate determination of the tefestrol content.

We chose $CHCl_3$—C_2H_5OH (95%) (9.5:0.5) as the mobile phase after testing several solvent systems. Under these conditions, the tefestrol band is sufficiently well separated from accompanying substances: R_f ferutinine 0.50; R_f tenuferidin 0.36. Chromatograms are developed in 40 min. The chromatography was performed in ascending mode without preliminary saturation.

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TABLE 1. Tefestrol Addition to Extract of *Ferula* Roots

Tefestrol content in extract, mg	Added tefestrol, mg	Calc., mg	Average from three determinations, mg	Relative uncertainty, %
168.53	51.32	219.85	226.70	+3.11
152.60	102.11	254.71	260.45	+2.20
172.12	154.36	326.48	327.60	+4.50
170.05	95.35	265.40	262.55	-1.44
172.12	154.36	326.46	324.79	-0.51
152.60	75.05	227.85	221.85	-1.54

The purity of the bands containing the tefestrol components was determined by comparing UV spectra of the eluted bands of chemically pure ferutinine and tenuferidin with bands obtained by chromatography of the plant extract.

The raw material was extracted with 95% ethanol in a Soxhlet extractor. It was determined experimentally that tefestrol is completely extracted from the raw material after 2.5 h (six washes). Data for the extraction dynamics are presented below.

Extraction time, min	Raw matl. wt., g	Tefestrol content, %
30	2.6005	1.83
60	2.6489	3.64
90	2.5211	5.29
120	2.5032	6.41
180	2.5148	6.41

We also studied the completeness of tefestrol extraction as a function of raw material particle size. The results showed that grinding roots to a particle size that passes through a 2-mm sieve is optimal.

Raw matl. size, mm	Grinding time, h	Tefestrol found, %
1	7.0	6.42
1.5	7.5	6.42
2.0	2.5	6.43
2.5	2.5	6.40

The optimal elution conditions were found by studying the desorption of chemically pure ferutinine and tenuferidin. It has been found that shaking for 2 h on a vibrational shaker produces 96-98% desorption of tefestrol from the sorbent.

Samples of *Ferula tenuisecta* roots were analyzed six times (n = 6) in order to determine the uncertainty of the analysis: f = 5, X = 6.43%, S^2 = 0.0141, S = 0.1187, P = 95%, t(p,f) = 2.57, ±X = 0.305, ±E = 4.74%.

The uncertainty of a single determination is ±4.74% with 95% probability.

A check of the method using added tefestrol in the root extract (Table 1) showed that the relative uncertainty of the determination is within the random uncertainty and, therefore, the desired components are not lost during the analysis.

Thus, we developed a method for quantitative determination of tefestrol in roots of *Ferula tenuisecta* that consists of extraction of raw material with 95% ethanol, separation of the desired product by chromatography, and determination of the content of active substance by spectrophotometry.

EXPERIMENTAL

Quantitative Determination of Tefestrol. An analytical sample of raw material was ground to a particle size that passed through a 2-mm sieve (GOST 214-77). Sieved material (~2.5 g, accurate weight) was placed in a cup of filter paper,

dropped into a Soxhlet extractor (~200-mL working volume), treated with ethanol (180 mL, 95%), and extracted for 2.5 h (six rinses) on a boiling-water bath. The alcoholic extract was concentrated on a rotary evaporator to a volume of 15 mL, quantitatively transfered to a 25-mL volumetric flask, made up to the mark with the same solvent, and thoroughly mixed. Three tracks were developed on Silufol UV-254 plates (15×15 cm). The first track was a control. A band 3-cm in length of an alcoholic solution (0.01 mL, 50 μg) of the working standard sample (WSS) of tefestrol was placed at the origin of the second track. The third track was a band of the same length from prepared alcoholic extract (0.01 mL). The plate with the samples was dried in air until the odor of the solvent disappeared, placed in a chamber with $CHCl_3$—C_2H_5OH (95%) (9.5:0.5), and chromatographed in ascending mode (without saturation).

When the solvent front reached ~12 cm, the plate was removed from the chamber and dried in air until the odor of the solvent disappeared. The bands located at the level of tefestrol (WSS) spots were observed in UV light (254 nm). Bands were quantitatively transfered into 50-mL flasks with stopcocks, treated with ethanol (10 mL, 95%), shaken for 2 min, left to stand for 30 min, and filtered through a glass filter (16 pore size).

The optical density of the resulting eluates was measured on a spectrophotometer at 260 nm in 10-mm cuvettes. The reference solution was an eluate from the band of the control track.

The tefestrol content was calculated based on absolutely dry raw material in percent (X) according to the formula:

$$X = (D \cdot m_0 \cdot 100 \cdot b)/[D_0 \cdot m \cdot (100 - w)],$$

where D is the optical density of the eluate of the tested solution, D_0 is the optical density of the tefestrol WSS eluate, m_0 is the mass of tefestrol WSS in g, m is the mass of raw material in g, w is the mass loss upon drying in %, and b is the tefestrol content in the tefestrol WSS in %.

Preparation of Tefestrol WSS. Tefestrol (~0.125 g, accurate weight) was dissolved in ethanol (95%) in a 25-mL volumetric flask. The volume was adjusted to the mark with ethanol. The contents were mixed.

The developed method for quantitative determination of tefestrol provides the basis for a temporary pharmacopeic article on *Ferula tenuisecta* roots.

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