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# STANDARDIZATION OF 8β, 9β-DIHYDROXY-1α, 14α, 16β-TRIMETHOXY-4β-ANTHRONOYLOXY-N-ETHYLACONITAN HYDROBROMIDE SUBSTANCE

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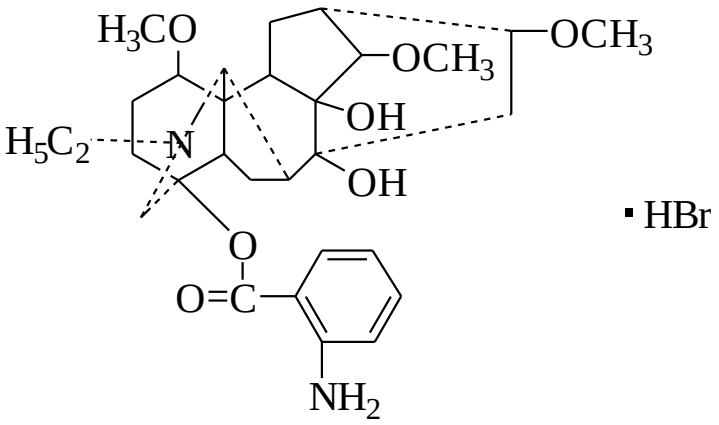
**Annotation**
This article discusses the issue of standardization of the drug substance 8β, 9β-dihydroxy-1α, 14α, 16β-trimethoxy-4β-anthronoyloxy-N-ethylaconitan hydrobromide. As a method for establishing the authenticity of a substance, the use of absorption measurement in the ultraviolet spectrum is recommended. Also, a solution of silicotungstic acid is used to detect alkaloids, which makes it possible to confirm the alkaloid nature of the substance. The characteristic reaction B for bromides was used to detect bromides. As a result of the research, regulatory documentation has been developed that allows standardizing the substance of a real chemical product isolated from medicinal plant materials.

**Keywords:** alkaloid, drug, standardization, identity of the substance, quantitative determination, analytical chemistry.

## Introduction

In recent years, the range of drugs used to treat cardiovascular diseases has expanded around the world. Among these preparations, a special place is occupied by preparations of plant origin. According to experts, drugs developed on the basis of natural compounds have pronounced therapeutic properties, and their toxicity remains low. Our work describes the results obtained during the experimental work on the standardization of the above substance. The substance has the following structure (Fig.1):




$C_{30}H_{42}N_2O_7 \cdot HBr$ M.w. 623

Fig.1. Structural formula of 8β,9β-dihydroxy-1α,14α,16β-trimethoxy-4β-anthronoyloxy-N-ethylaconitan hydrobromide

The substance is a white or off-white crystalline powder. Easily soluble in water, chloroform and alcohol 96%, very slightly soluble in acetone, sparingly soluble in methanol.

## Results of Experiments and their Discussion

***Authenticity*.**

1. The UV spectrum of a 0.01% aqueous solution of the drug in the region from 260 to 360 nm has an absorption maximum at a wavelength of 330 + 2 nm.
2. To 1 ml of a 0.5% aqueous solution of the drug, add 2 drops of a solution of silicotungstic acid, resulting in a yellowish-white precipitate (alkaloids).
3. A 0.5% aqueous solution of the drug gives a characteristic reaction B to bromides.

***Associated alkaloids.*** 0.01 ml (100 μg) of a 1% solution of the drug in the form of a dot (p. mcg) 0.005% solution of the drug in the form of a dot (p.2). The plate is dried in air for 10 min and placed in an unsaturated chamber with a mixture of solvents benzene - chloroform - diethylamine in a ratio of 40:10:3.

When the solvent front passes 15 cm, it is removed from the chamber, dried in a fume hood until the solvent smell disappears, and viewed under UV light at a wavelength of 254 nm. The main violet adsorption zone should appear on the chromatogram from point 1 at the level of the zone from point 2 (8β, 9β-dihydroxy-1α, 14α, 16β-trimethoxy-4β-anthronoyloxy-N-ethylaconitan, Rf = 0.32). In addition to the main zone from point 1, up to 4 more zones of associated alkaloids with Rf from 0.5 to 1.4 can appear, one of which is blue, the others are purple. Only one zone should appear on the chromatogram from point 2. A weak glow at the start is allowed.

The chromatogram is considered reliable if the requirements of the "Chromatographic System Suitability Test" test are met.

***Preparation of 1% and 0.005% solutions of the drug.*** 0.02 g of the drug is dissolved in 2 ml of methyl alcohol (1% solution). To 0.1 ml of a 1% solution of the drug, add 20 ml of methyl alcohol and mix (0.005% solution).

***Checking the suitability of the chromatographic system.*** The chromatographic system is considered suitable if a clear division of the adsorption zones of the accompanying alkaloids is observed; zone 8β, 9β-dihydroxy-1α, 14α, 16β-trimethoxy-4β-anthronoyloxy-N-ethylaconitan on the chromatogram has an Rf of at least 0.3.

0.05 g of the drug is dissolved in 10 ml of purified water; the solution should be clear. The solution obtained during the test for transparency in color should not exceed the standard. pH 5.5 to 6.5 (0.5% aqueous solution, potentiometrically). About 0.5 g of the drug (accurately weighed) is dried at 105°C to constant weight. Loss in weight on drying should be no more than 1.0%. Sulphated ash from 0.5 g of the drug (accurately weighed) should not exceed 0.15%. Sulphated ash from 0.5 g of the preparation must withstand the test for heavy metals - no more than 0.001% in the preparation.

Microbiological purity must meet the standard requirements for drug substances. In 1 g of the drug, the total number of aerobic bacteria and fungi in total is not more than 102. The presence of bacteria of the *Enterobacteriacieae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* families in 1.g of the drug is not allowed.

The drug must be non-toxic. The drug at a concentration of 0.01% aqueous solution when injected into a vein at a dose of 1 mg per 1 kg of animal weight for 5 seconds. should not cause the death of

white outbred mice weighing 18-22 g within 48 hours. The test is carried out in accordance with the requirements of pharmacopoeial documents.

The drug must be non-pyrogenic. The test dose is 0.5 mg of the drug per 1 kg of animal weight. The drug is diluted in a ratio of 1:10 with pyrogen-free saline, preheated to 37°C, and injected into the marginal vein of the rabbit's ear for 30 seconds. the test is carried out in accordance with the requirements of the general pharmacopoeial articles.

***Residual amounts of organic solvents.*** About 0.05 g (accurately weighed) of the drug is placed in a volumetric flask with a capacity of 10 ml, dissolved in methyl alcohol and the volume of the solution is adjusted to the mark and mixed.

1 ml of the resulting solution and a solution of a working standard sample of 96% ethyl alcohol are alternately chromatographed on a gas chromatograph with a flame ionization detector, obtaining at least 5 chromatograms under the following conditions:

- a glass column measuring 0.3x250 cm;
- sorbent N-AW-DMCS with a particle size of 0.16 mm-0.20 mm;
- stationary phase - 15% polymethylpropylsilicol
- the column temperature changes according to the program from 400 to 1100C at a rate of 30 ml/min.
- temperature of the evaporator and detector –2000 C.
- carrier gas velocity (nitrogen) –30 ml/min.

The content of the residual amount of the solvent - ethyl alcohol in the preparation (x) in percent is calculated by the formula:

$$X = \frac{S_1 \cdot m_0 \cdot 100}{S_0 \cdot m_1} \ ,$$

$S_1$ – is the average value of the ratio of the areas of the alcohol peaks to the areas of the peaks of the internal standard and the chromatograms of the test solution;

$S_0$ – is the average value of the ratios of the peak areas of ethanol to the peak areas of the internal standard of their chromatogram of the working standard ethanol sample solution;

$m_1$ – is the mass of the sample of the drug, in g;

$m_0$ – is the mass of a sample of a working standard sample of ethyl alcohol, in g.

The content of ethyl alcohol is not more than 0.5% (5000 ppm).

*Preparation of a solution of a working standard sample of ethyl alcohol*. About 1.16 g (accurately weighed) of ethyl alcohol 96% is placed in a volumetric flask with a capacity of 100 ml, the volume of the solution is adjusted to the mark with methanol. The solution is used freshly prepared.

***Quantitation***. About 0.05 g (accurately weighed) of the powder is placed in a 100 ml volumetric flask, dissolved in 80 ml of water and diluted to the mark with water. Transfer 2 ml of the solution into a 25 ml volumetric flask, dilute to the mark with water and mix.

Measure the optical density of the resulting solution on a spectrophotometer at a wavelength of 330 nm in a cuvette with a layer thickness of 10 mm.

Water is used as a reference solution.

In parallel, the optical density of the standard sample solution 8β, 9β-dihydroxy-1α, 14α, 16β-trimethoxy-4β-anthronoyloxy-N-ethylaconitan is measured.

The content of 8β, 9β-dihydroxy-1α, 14α, 16β-trimethoxy-4β-anthronoyloxy-N-ethylaconitan (X) is calculated by the formula:

$$X = \frac{D_1 \cdot a_0 \cdot в}{D_0 \cdot a_1}$$

$D_1$ – is the optical density of the test solution;

$D_o$ – is the optical density of the standard sample solution of 8β, 9β-dihydroxy-1α, 14α, 16β-trimethoxy-4β-anthronoyloxy-N-ethylaconitan;

ao is the weight of the standard sample of 8β, 9β-dihydroxy-1α, 14α, 16β-trimethoxy-4β-anthronoyloxy-N-ethylaconitan, in grams;

$a_1$ – is the weight of the tested powder, in grams;

$a_0$ – content of standard sample 8β, 9β-dihydroxy-1α, 14α, 16β-trimethoxy-4β-anthronoyloxy-N-ethylaconitan, in percent;

The content of $C_{30}H_{42}N_2O_7$·HBr (8β, 9β-dihydroxy-1α, 14α, 16β-trimethoxy-4β-anthronoyloxy-N-ethylaconitan) must be at least 97.5%.

***Preparation of a standard sample solution of 8β, 9β-dihydroxy-1α, 14α, 16β-trimethoxy-4β-anthronoyloxy-N-ethylaconitan.*** About 0.05 g (accurately weighed) powder of standard solution 8β, 9β-dihydroxy-1α, 14α, 16β-trimethoxy-4β-anthronoyloxy-N-ethylaconitan is placed in a 100 ml volumetric flask, dissolved in 80 ml of water and diluted with water to the mark. Transfer 2 ml of the solution into a 25 ml volumetric flask, dilute to the mark with water and mix.

Quantitative determination is carried out by the spectrophotometric method, by comparing the test preparation with a standard sample. The metrological characteristic of the analysis method from 6 determinations shows that the error of a single determination is ±0.12%.

Metrological characteristics of the analysis method

| n | Xav | $S^2$ | S | P,% | tα | ±Δx | ±ΔE |
|---|---|---|---|---|---|---|---|
| 6 | 98,8 | 0,0025 | 0,05 | 95 | 2,57 | 0,128 | ±0,12% |

## Conclusion

As a method for standardization of the substance of the alkaloid, the method of UV spectrometry is proposed. An optical measurement of the degree of light absorption of a solution of the present substance should be carried out at a wavelength range from 260 to 360 nm, and when measuring a 0.01% solution of the substance, an absorption maximum should be at 330 + 2 nm. The metrological characteristics and the too low error rate in the calculation of the results of repeated analyzes show the correctness of the recommended method.

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