Ergot alkaloids are widely used in medicine for the treatment of diseases involving either increased levels of prolactin or an irregular rhythm of prolactin secretion, such as female infertility, menstrual cycle irregularities, pituitary tumors, prolactinomas, acromegalia, galactorrhea, and Itsenko - Cushing's disease, and for the prophylaxis or suppression of lactation [1].

Abergin (I) is a new drug developed at the VILAR Co. [1, 2] and used for the treatment of such diseases. The drug consists of an approximately equimolar mixture of two modified alkaloids: 2-bromo-α-ergocryptine mesylate (A) and 2-bromo-β-ergocryptine mesylate (B) [2]. Compound I is obtained by bromination of a natural mixture of α- and β-ergocryptines extracted from Claviceps purpurea (Fries) Tul., an ergocryptine strain parasite ergot BKM F-2642D belonging to the Clavicepiaceae ergot family [3].

Pharmacological investigations showed that I offers more prolonged manifestations of prolactin-inhibiting and neurotropic activity as compared to other preparations of this type (bromocryptine mesylate, parlodel). Abergin had been successfully tested under clinical conditions and was adopted as a dopaminergic drug since 1993 [4, 5].

One of the most difficult problems involved in the analysis of the quality of ergot alkaloid based preparations is the determination and standardization of the content of impurities. Indeed, ergoalkaloids are highly labile compounds: ambient factors such as oxygen from air, light, temperature, acids, bases, and others may cause their chemical modification and destruction [6 - 8]. The resulting products may lead to undesired correction of pharmacological action of the drug. For this reason, technical specifications on the ergoalkaloid drugs restrict both the concentrations of individual impurities and their total content [9, 10]. The analysis of drug preparations is performed by TLC under thoroughly controlled conditions, which is necessary to prevent decomposition of ergoalkaloids during the chromatographic procedure. The content of impurities is visually assessed against a reference scale; sometimes, the procedure stipulates the use of standard samples [10].

The purpose of this work was to develop a TLC procedure for the determination of impurities in a two-component abergin substance in the absence of reference samples, which increased the requirements with respect to reliability and reproducibility of the results.

EXPERIMENTAL PART

The samples of 2-bromo-α- and 2-bromo-β-ergocryptine mesylate and abergin tablets were provided by the Department of Chemistry and Technology of Natural Substances (VILAR Co.) and by the VILAR Experimental Production Plant. Analytical procedures were carried out in a dark room under illumination of a red lamp.

Reagents: methylene chloride (chromatographic reagent grade), dioxane (analytical grade), petroleum ether (b.p., 70 - 100°C), and reagents according to the Russian State Pharmacopeia (Eds. X and XI). The solvent solutions and mixtures were freshly prepared. Equipment: monochromators KF-4M and Khromatoskop (Russia), TLC plates Sorbil of the PTSKh-P-A and PTSPh-P-A-UF grades (Plastmash Production-Design Bureau, Institute of Macromolecular Compounds, Russian Academy of Sciences).

Solutions for the reference scale were obtained by diluting the working solution A (see below) with a calculated amount of chloroform - methanol mixture (4 : 1) to obtain solution 1 (3 μg of abergin per 0.01 ml sample volume), solution 2 (1.5 μg of abergin per 0.01 ml), and solution 3 (0.75 μg of abergin per 0.01 ml).

Method for the Analysis of Impurities in Medicinal Substances

An exactly weighed amount (about 0.03 g) of the preparation is dissolved in 2 ml of a chloroform - methanol (4 : 1) mixture (solution A). A 0.01 ml volume of sample A and the same amounts of reference solutions 1 - 3 are applied onto
TLC Determination of Alkaloid Impurities in Abergin

Fig. 1. TLC chromatogram of a medicinal substance of abergin on a Sorbfil plate: I) test sample, 150 μg of abergin; II – IV) reference solution scale representing 3, 1.5, and 0.75 μg abergin samples, respectively; spot А, 2-bromo-α-ergocryptine mesylate; spot B, 2-bromo-β-ergocryptine mesylate; spots I, 2, 4, and 5, impurities.

Fig. 2. TLC chromatogram of abergin tablets on a Sorbfil plate: I) test sample, 75 μg of abergin from dissolved tablets; II – IV) reference solution scale representing 3, 1.5, and 0.75 μg abergin samples, respectively; spot А, 2-bromo-α-ergocryptine mesylate; spot B, 2-bromo-β-ergocryptine mesylate; spots I – 5, impurities.

The start line of a Sorbfil TLC plate (PTSKh-P-A, 10 x 15 cm, preactivated for 5 min at 100°C) (Fig. 1):
Path I, solution A (150 μg, 100%);
Path II, solution 1 (2 spots x 1.5 μg, 1%);
Path III, solution 2 (2 spots x 0.75 μg, 1%; 0.5%);
Path IV, solution 3 (2 spots x 0.375 μg, 0.25%).

The plate with applied samples is dried in a flow of cold air, placed into a cell with a solvent mixture (dichloromethane – dioxane – petroleum ether – concentrated ammonia solution, 30 : 6 : 4 : 0.06, preequilibrated for 1 h), and chromatographed in the ascending mode. Then the plate is extracted from the cell, dried in a flow of cold air, treated with a 4% chloramine-B solution, heated for 15 min at 120°C, and analyzed under UV illumination at 365 nm. The content of impurities in the sample preparation is assessed by comparing the size and coloration intensity of foreign spots in the chromatogram of solution A to one of the two abergin spots in the reference chromatograms (Fig. 1).

The reference chromatograms II – IV must contain only a couple of closely spaced abergin spots of light-brown color with equal intensity. The chromatogram of solution A is allowed to display overlapped abergin spots and some additional spots both above and below the main ones. The size and coloration intensity of the spot with maximum mobility must not exceed those for either of the two spots in the reference path II (≤ 1%). The size and coloration intensity of the other foreign spots must not exceed those for either of the two spots in the reference path III (≤ 0.5%). The total content of the impurities must not exceed 2%.

Method for the Analysis of Impurities in Abergin Tablets

An exactly weighed amount (about 1.3 g) of powdered tablets is placed in a flask with ground-glass stopper. Then 5 ml of a chloroform – methanol (4 : 1) mixture is added and the mixture is stirred for 20 min. The suspension is centri-

fugated for 10 min at 3500 rpm. A 0.01 ml volume of the supernatant liquid (test solution) and the same amounts of reference solutions 1 – 3 are applied onto the start line of TLC plate (Fig. 2):
Path I, test solution (75 μg, 100%);
Path II, solution 1 (2 spots x 1.5 μg, 2%);
Path III, solution 2 (2 spots x 0.75 μg, 1%);
Path IV, solution 3 (2 spots x 0.375 μg, 1%; 0.5%).

The chromatography conditions and the method of chromatogram description are the same as above; the requirements to the impurity contents are as follows. The size and coloration intensity of the spot with maximum mobility must not exceed those for either of the two spots in the reference path II (≤ 2%). The dimensions and coloration intensities of the other foreign spots must not exceed those for either of the two spots in the reference path III (≤ 1%). The total content of the impurities must not exceed 4%.

RESULTS AND DISCUSSION

Our method for the determination of impurities in 1 was developed taking into account the requirements of other established procedures [9, 10] used for assessment of the quality of preparations containing ergoalkaloids.

The conditions of TLC chromatography and detection of ergoalkaloids, known from the literature, did not allow us to solve the problems of analysis on the Sorbfil plates. These TLC plates differ by many parameters (layer thickness, filler composition, fluorescent indicator, etc.) from the previously employed [9] plates of the Silufol (Czechia) and Silicagel 60-F (Merck, Germany) types, which results in higher TLC detection thresholds for ergoalkaloids. For example, Sorbfil plates of the PTSKh-P-A-UF grade with a fluorescent indicator visualized at a wavelength of 254 nm allowed us to detect about 2 μg of an ergoalkaloid, while the Silufol-254 plates were capable of detecting 0.5 μg, and the Silicagel 60-F plates — even 0.2 μg. The detection threshold for ergoal-