Diazoline – 3-methyl-9-benzyl-1,2,3,4-tetrahydro-γ-carboline naphthalene-1,5-disulfonate (I) – is widely used in medical practice as an H₁ histamine receptor blocker. In contrast to other antihistamine drugs, I does not show any sedative effect. This feature makes Diazoline even more attractive for practical medicine [1].

Diazoline is prepared by reacting 3-methyl-9-benzyl-1,2,3,4-tetrahydrocarboline sulfate (II) with naphthalene-1,5-disulfonic acid [2]. From the experience in synthesizing I, we believe that diazoline may contain the following compounds as impurities: II, 3-methyl-9-benzyl-1,2,3,4-tetrahydro-γ-carboline (III), and naphthalene-1,5-disulfonic acid.

The aim of this study was to examine I for purity and elaborate procedures for its analysis in the presence of impurities. We used HPLC as an analytical tool.

EXPERIMENTAL PART

In this study, we analyzed samples of I, II, III, and naphthalene-1,5-sulfonic acid, and pelletized diazoline produced by the Shchelkovo vitamin plant. The studies were carried out with a Milikhrom-2 chromatograph equipped with a KAKh-4-64-3 column packed with Silasorb C₁₈. The detection wavelength – 286 nm corresponds to the UV absorption maximum of I and is near the absorption maxima of other
Fig. 3. Chromatographic pattern for diazoline. Mobile phase is 80% methanol. Peak 1 is associated with compound I, peak 2, with compound III, and peak 3, with compound II.

analyzed compounds. The registration scale was 1.6, and the measurement time was 0.2 sec.

Dimethyl sulfoxide (chemical-purity grade) was used as solvent.

A precisely weighed sample (about 0.3 g or 0.1 g) of the powder prepared by grinding 25 pellets of diazoline was placed in a 50-ml volumetric flask, 30–40 ml of dimethyl sulfoxide was added, and the mixture shaken until the powder dissolved. The same solvent was then added to the capacity mark. Solutions of I and II with a concentration of 2 mg/ml were also prepared and were used as reference solutions. The samples for chromatographic analyses were 2 μl in volume.

The weight fraction \( X (%) \) of II in compound I was calculated according to

\[
X = \frac{S_2 C \times 50 \times 100}{S_2 a},
\]

where \( S_2 \) is the area of the peak associated with compound II in the chromatogram of the solution of compound I; \( S_2 \) is the area of the peak of compound II in the chromatogram of the standard solution of compound II; \( C \) is the concentration of the standard solution, mg/ml; and \( a \) is the average weight of a single pellet, g.

The content \( X (%) \) of I in a pellet was calculated by the formula

\[
X_I = \frac{S_1 C \times 50 P}{S_1 a},
\]

where \( S_1 \) is the area of the peak associated with compound I in the chromatogram of the solution prepared with powdered Diazoline pellets; \( S_2 \) is the area of the peak of compound I in the chromatogram of the standard solution of compound I; \( C \) is the concentration of the standard solution, g/ml; \( P \) is the average weight of a single pellet, g; and \( a \) is the weight of the analyzed sample of powdered pellets, g.

**RESULTS AND DISCUSSION**

The chromatographic behavior of I and the impurities was studied using 1 mg/ml solutions. Methanol – water and ethanol – water mixtures and 0.01 M solution of sodium hydroxide were used as eluents, because it is known that the solubility of I in alkaline media is higher [3]. The elution rate was varied in the range from 40 to 100 μl/min with a step of 5 μl/min. The content of organic solvent in the eluent varied from 40% to 100% with a step of 5%. It was found that all samples of I contain an admixture of II; in some samples, trace amounts of compound III were also detected. Therefore, the task was to determine the conditions that would enable optimal chromatographic separation of compounds I and II.

As the parameters to be optimized, we took the retention time for compound III (peak 2 in Fig. 3) and the separation coefficient for the peaks \( R_\alpha \), which was calculated according to a conventional method [4]. The results obtained are presented in Figs. 1 and 2. As can be seen from the figures, using 80% methanol or 90% ethanol solutions as the mobile phase provides satisfactory separation of the peaks. The addition of 0.01 M sodium hydroxide increases the separation efficiency; however, strongly alkaline eluents reduce the lifetime of the column, and this variant may be appropriate only with very low-efficient columns.

Under the conditions selected, the separation coefficient did not exceed unity, and the last component was eluted from the column not later then in 15 min. Figure 3 illustrates the chromatographic pattern for sample I under the conditions selected.

To make the determination even more rapid, we studied the effect of the elution rate on the parameters being optimized (for 80% methanol and 90% ethanol solutions as examples). The results are summarized in Table I.

Increasing the elution rate to 90 μl/min reduces the separation coefficient, whereas the higher viscosity of ethanol (than that of methanol) requires that the pump operate at the maximum admissible pressure, which reduces the lifetime of both the column and the pump. Decreasing the elution rate below 50 μl/min results in a significantly longer retention...