# METHOD FOR THE QUANTITATIVE DETERMINATION OF VINCAMAJINE IN THE HERB Vinca major INTRODUCED INTO GEORGIA

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The alkaloid vincamajine from the herb Vinca major is a pharmacologically active base [1]. For its preparation, raw material collected in the phase of the second autumn flowering is used. We propose a method for the quantitative determination of vincamajine base consisting in obtaining the vincamajine fraction, two-stage TLC (silica gel LS 5/40) in systems 1 (benzene–ethyl acetate–methanol (1:1:1)) and 2 (benzene–ethyl acetate–methanol (2:2:0.5)) and determining the alkaloid in the eluate spectrometrically.

Determination Procedure. The comminuted dry air-dry raw material (100 g) was placed in a flask with a ground-in stopper and was extracted with ethanol. The ratio of raw material to extractant was 1:6 for the first extraction and 1:5 for the three following ones. The ethanolic extracts were combined and concentrated, and the concentrate was treated with a 5% solution of hydrochloric acid. The acid solution was exhaustively extracted with chloroform.

After the conversion of the alkaloids into their bases, the chloroform extract was concentrated and was treated with citrate-phosphate buffers having pH 6.0 and 3.0. Extraction with ethanol of the buffer with pH 3.0 gave the vincamajine fraction, which, after concentration and drying, was dissolved in chloroform, transferred quantitatively to a 25-ml measuring flask, and made up with chloroform to the mark. From this solution, 3 ml was taken and was applied in the form of a continuous line 14 cm long to a plate (24 × 18 cm). As marker was used 0.1 ml of a 0.1% chloroform solution of a chromatographically pure sample of vincamajine (UV spectrum: $\lambda_{max}^{MeOH}$ 249, 293 nm (log ε 3.93, 3.50), $E_{1cm}^{1\%}$ = 85 at λ 293 nm, c = 0.003512 g/100 ml–0.01035 g/ml). Chromatography was performed in system 1. After drying, the band of the marker on the finished chromatogram was revealed with a solution of cerium ammonium sulfate in 85% orthophosphoric acid. The section of sorbent at the level of the vincamajine marker – a crimson band – was eluted with chloroform–methanol (5:1) (3 × 15 ml). The eluate was evaporated to dryness and the residue was dissolved in 2 ml of chloroform and chromatographed by TLC in system 2 with, as marker, 0.1 ml of a 0.1% solution of vincamajine. The alkaloid was extracted similarly. The dry residue was dissolved in 2 ml of methanol. After appropriate dilution, the optical density was measured.

The amount of alkaloid (X, %) calculated on the absolutely dry raw material, was found from the formula

$$X = \frac{K \cdot D \cdot V_1 \cdot V_3 \cdot V_5 \cdot 100 \cdot 100}{E_{1\,cm}^{1\%} \cdot 100 \cdot V_2 \cdot V_4 \cdot a\,(100 - b)}.$$

where D is the optical density of the solution under investigation;

$E_{1cm}^{1\%}$ is the specific absorption index of vincamajine at a wavelength of 293 nm, which is 85;

$V_1$ is the volume of the chloroform solution, ml;

$V_2$ is the volume of the chloroform solution deposited on the chromatogram, ml;

$V_3$ is the volume of the methanolic solution, ml;

$V_4$ is the volume of the methanolic solution taken for dilution, ml;

$V_5$ is the volume of diluted methanolic solution, ml;

a is the weight of raw material, g;

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b is the loss in weight on drying, %; and

K is a correction factor, equal to 1.12.

The metrological characteristics of the method are given below

| $f$ | $\bar{X}$ | $S^2$ | $\pm S$ | $P$ | $t(Pf)$ | $\pm \Delta X$ | $\pm E\%$ | $\frac{Em\%}{m=3}$ |
|---|---|---|---|---|---|---|---|---|
| 9 | 0.0123 | $7{,}85\cdot10^{-8}$ | $2{,}81\cdot10^{-4}$ | 95 | 2,26 | $6{,}34\cdot10^{-4}$ | 5,16 | 2 98 |

It was found that the amount of vincamajine in the herbage of the larger periwinkle in the phase of the second autumn flowering was 0.0123%, calculated on the absolutely dry raw material.

## LITERATURE CITED

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# HAPLAPHINE – A NEW ALKALOID OF Haplophyllum perforatum

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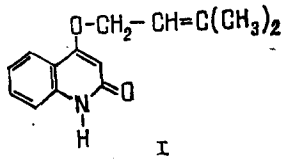
The epigeal part of the plant Haplophyllum perforatum (M.B.) Kar. et Kir. was collected by K. Taizhanov in the Dzhambul province, Kazakh SSR, close to the Kuyuk Pass in the flowering phase on June 17, 1984. The air-dry epigeal part (3100 g) was extracted by steeping with ethanol eight times. The treatment of a small amount of the evaporated ethanolic extract with 10% sulfuric acid showed that the alkaloids were not extracted by an acid solution, and the extract of the raw material, after the ethanol had been distilled off, was therefore boiled with ether. The concentrated ethereal extract was chromatographed on a column of silica gel. The first ethereal eluates yielded the lignan eudesmin, mp 105-106°C (acetone), and the later ones the alkaloid haplamine, mp 201-202°C (decomp., ethanol), with yields of 0.02 and 0.07%, respectively, on the weight of the dry raw material. The mother liquor from the haplamine, after its recrystallization, was chromatographed twice on silica gel. Ethereal eluates yielded 15 mg of a substance with mp 159-160°C (acetone), M+ 229 (mass spectrometry), which we have called haplaphine (I).

The IR spectrum of (I) contained absorption bands at 3160 and 1665 $cm^{-1}$ (–NH–CO–). UV spectrum of (I), $\lambda_{max}$, nm: 215, 225, 229, 238 shoulder, 267, 277, 317, 328, which is typical for 4-alkoxy-2-quinolone alkaloids and, like the latter, did not change on acidification and alkalinization [1].

In the PMR spectrum of (I) ($CDCl_3$, 0 – HMDS, taken on a BS 567A 100 Mz instrument) there were signals at the following δ values (ppm): 1.73 and 1.79 (s, 3 H each, $=C(CH_3)_2$); 4.62 and 5.50 (d, 2 H, J = 6.8 Hz and t, 1 H, J = 6.8 Hz, $O-\underline{CH_2}-CH=$); 5.97 (s, 1H, proton at C-3 of a quinolone nucleus); 7.27 and 7.86 (m, 3 H, and dd, 1 H, aromatic protons at C-6, 7, and 8 and at C-5); 12.11 (br.s, 1 H, NH).

The facts given above indicate that haplaphine has has the structure of 4(γ,γ-dimethylallyloxy)-2-quinolone (I).

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