RAPID METHOD FOR THE QUANTITATIVE DETERMINATION OF SANGUIRITRINE IN THE HERBS *Macleaya cordata* and *Macleaya microcarpa*

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For analyzing the herb plume poppy a spectrophotometric method has been developed which is distinguished by a high productivity thanks to the absence of a stage of chromatographically separating the combined alkaloids. The error of a single determination is ±5.07%.

In view of the introduction into culture of the pink plume-poppy (*Macleaya cordata* (Wild.) R. Br.) and *Macleaya microcarpa* (Maxim), Fedde — sources of the preparation "sanguiritrine" [1], which consists of the combined bisulfates of the alkaloids sanguinarine and cheleritrine [2, 3] — the question arose of a rapid and sufficiently accurate method of evaluating the quality of the plant raw material.

The known methods of analysis [4-6] are based on the use of TLC to separate the alkaloids in extracts from the raw material, their elution from the sorbent, and photometric determination from the solvent. The careful performance of all these operations makes the analysis laborious and unproductive.

The proposed spectrophotometric method for analyzing the plume poppy is characterized by a greater productivity thanks to the absence of a stage of the chromatographic separation of the combined alkaloids while retaining a satisfactory degree of accuracy and reproducibility.

The main task of the investigation consisted in finding a rapid and reliable method of separating sanguiritrine and cheleritrine from the accompanying ballast substances present in the unpurified extracts from the raw material without using TLC.

The samples of plume poppy upon which the rapid method were developed had already been analyzed by the chromato-spectrophotometric method suggested by us previously [5]. We guided ourselves by the results of these analyses in developing a stage of purifying the extracts. The combined alkaloids were extracted from the plume poppy with chloroform [5]. After the chloroform had been distilled off, the dry extracts were treated with a solution of sulfuric acid, whereupon the sanguinarine and cheleritrine passed into the acid solution in the form of sulfates. Quantitative determination was carried out by a spectrophotometric method at the isobestic point with a wavelength of 447 nm [6]. As the standard solution we used sanguiritrine corresponding to the requirements of VFS 42-948-80.

When the dry extracts were treated with a 2% solution of sulfuric acid, stable results were obtained which were 15-20% higher than those of the chromato-spectrophotometric method. Chromatographing the chloroform extracts both before and after their treatment with the 2% sulfuric acid solution showed that in this case pigments having absorption in the visible region of the spectrum passed partially into the acid solution.

An increase in the concentration of the acid led to the precipitation of the sanguinarine and cheleritrine bisulfates.

The best results were obtained by treating the dry residue after the evaporation of a small volume (0.5-1 ml) of the chloroform extract with a weak (0.015 M) solution of sulfuric acid. Under these conditions, the pigments and other ballast substances do not pass into the solution, and, consequently, do not interfere with the determination.

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TABLE 1. Results of Analyses of the Plume Poppy from the Harvests of Different Years

Year of harvesting and number of the sample	Amt. of sanguinitrine calc. on the abs. dry wt. of the raw material, %		Conversion factor TLC/rapid method
	chromatographic method	rapid method	
1974	0,60	0,62	0,97
1975	0,63	0,58	1,08
1977	—	—	—
1	1,31	1,39	0,94
1/2	1,36	1,34	1,01
2	0,68	0,68	1,00
2/2	0,70	0,72	0,97
3	0,75	0,77	0,97
3/2	0,76	0,76	1,00
4	0,79	0,77	1,03
4/2	0,76	0,75	1,01
5	0,76	0,76	1,00
5/2	0,89	0,90	0,99
6	0,72	0,71	1,01
6/2	0,78	0,78	1,00
		Mean = 0,998	

The tertiary alkaloids present in the raw material (protopine, allocryptopine, and cryptopine) [7] have no absorption bands in the visible region of the spectrum and their presence cannot lead to a distortion of the results. According to the literature [7], the quaternary alkaloids berberine and coptisine have been detected in the plume poppy. In the 14 samples of raw material that we studied they were either absent or present in very small amounts not affecting the results of the determination. Thus, when 0.02-0.04 ml of a chloroform extract, corresponding to about 20-30 μg of sanguiritrine, was deposited on a Silufol plate (Czechoslovakia), they did not show up. Under the conditions of chromatography [5], the two alkaloids remain at the start, and the limit of detection of each of them in the deposit on a plate is 0.1 μg in a spot. Consequently, the amount of their combined material in the extracts from the raw material studied was less than 0.5% of the sanguiritrine content.

The identity of the UV spectra of sulfuric acid solutions of sanguiritrine from the raw material and standard sanguiritrine in the region above 350 nm confirms the possibility of the quantitative determination of the combined sanguinarine and cheleritrine in the plume poppy without the chromatographic purification of the chloroform extract.

The comparative results of the analysis of 14 samples of plume poppy harvested in various years by the chromatographically rapid methods, which are given in Table 1, confirm the reliability of the rapid variant. The conversion factor of the results of the analysis by the TLC method to the rapid method (means of 14 independent determinations in 14 samples of raw material) is close to 1 (0.998), and therefore there is no need to introduce it into the formula for calculation.

The metrological characteristics of the rapid method for the quantitative determination of sanguinitrine in the plume poppy are given below.

f	$\bar{x}$	S	P, %	$t(P_1 f)$	$\Delta\bar{x}$	ε, %
9	0,753	±0,0169	95	2,26	±0,0382	±5,07

EXPERIMENTAL

The raw material ground and passed through a sieve with apertures having a diameter of 1 mm (GOST State Standard 214—70) (5 g) was placed in 250-ml conical flask with a ground-in stopper, 5 ml of 25% ammonia solution was added, the mixture was carefully stirred with a glass rod to obtain a homogeneous moist mass, the flask was closed with the stopper and kept at room temperature for 30 min, and then 100 ml of chloroform was added, the flask was closed with the stopper again, and it was left for 16 h. The contents of the flask (10-20 ml of extract) were stirred for 10-15 min and filtered through filter paper, the first portions

being discarded. Of the filtrate obtained, 2 ml (400-600 μg of sanguiritrine) was transferred by a pipette to a 50-ml beaker, the chloroform was evaporated off to dryness on the water bath, 10 ml of 0.015 M sulfuric acid solution was added to the dry residue with a pipette, the mixture was heated in the boiling water bath for 5 min and cooled to room temperature, the solution was transferred quantitatively to a 25-ml measuring flask, and the beaker was rinsed with two 5-ml portions of 0.015 M sulfuric acid solution. The volume of the solution was made up to the mark with the same acid and it was mixed.

The optical density of the solution so obtained was measured at a wavelength of 447 nm in a cell with a layer thickness of 10 mm. A 0.015 M solution of sulfuric acid was used as the comparison solution. In parallel, and under the same conditions, the optical density of a 0.003% solution of a standard sample of sanguiritrine corresponding to the requirements of VFS 42-948-80 was measured under the same conditions.

SUMMARY

A spectrophotometric method of analyzing the herb plume poppy has been developed which is distinguished by a higher productivity thanks to the absence of a stage of chromatographically separating the combined alkaloids. The error of a single determination is ±5.07%.

LITERATURE CITED

1. A. D. Kodash, O. I. Zakharova, V. T. Sheverdinov, A. M. Zakharov, and E. E. Khlaptsev, Rast. Resur., 11, No. 2, 217 (1975).
2. S. A. Vichkanova and V. V. Adgina, Antibiotiki, No. 10, 902 (1973).
3. S. A. Vichkanova and V. V. Adgina, Antibiotiki, No. 7, 609 (1971).
4. G. A. Maslova, Khim. Prirodn. Soedin., 261 (1974).
5. I. E. Kopylova, G. A. Maslova, and M. E. Perel'son, Khim. Farm. Zh., No. 1, 58 (1980).
6. I. E. Kopylova, G. A. Maslova, and M. E. Perel'son, Khim. Farm. Zh., No. 9, 131 (1978).
7. H. G. Kiryakov, M. S. Kitova, and A. V. Georgieva, C. R. Acad. Bulg. Sci., 20, 189 (1967).