ISSN 1061-9348, Journal of Analytical Chemistry, 2017, Vol. 72, No. 7, pp. 810–814. © Pleiades Publishing, Ltd., 2017.
Original Russian Text © A.S. Khaliullina, R.Sh. Khaziev, A.A. Salamatin, 2017, published in Zhurnal Analiticheskoi Khimii, 2017, Vol. 72, No. 7, pp. 681–685.

ARTICLES

Quantitative Determination of Diterpene Acids in Garden Sage Leaves

A. S. Khaliullina[a], *, R. Sh. Khaziev[a], and A. A. Salamatin[b]

[a] *Pharmaceutical Faculty, Kazan State Medical University, Kazan, 420012 Russia*

[b] *Lobachevsky Institute of Mathematics and Mechanics, Kazan (Volga Region) Federal University, Kazan, 420008 Russia*

**e-mail: anela_90@mail.ru*

Received June 27, 2016; in final form, January 23, 2017

Abstract—A procedure is developed for the quantitative determination of diterpene acids in garden sage leaves by UV spectrophotometry at the wavelength 285 nm. The target group of compounds was selectively extracted by petroleum ether 40/70. It was shown that the completeness of extraction is determined mainly by the number of portions of the pure solvent: at the optimum ratio of the mass of the weighed portion to the volume of solvent 1 g/200 mL, double extraction is sufficient. The duration of each extraction is 20 min. The procedure was used in the analysis of samples of garden sage leaves from various producers. It was found that the concentration of diterpene acids in samples varied from 2.1 to 3.6 wt % (in terms of carnosic acid). The error of a single determination of the sum of diterpene acids in garden sage leaves is ±2.38% ($P = 0.95$).

Keywords: garden sage, diterpene acids, carnosic acid, spectrophotometry, standardization, petroleum ether

DOI: 10.1134/S1061934817070073

Garden sage (*Salvia officinalis*) is known in medicine, first of all, for the anti-inflammatory and antimicrobial properties, determining the efficiency of preparations on its basis in the treatment of infectious and inflammatory diseases of throat and mouth cavity [1–3]. Leaves of *S. officinalis* are a constituents of teas "Grudnoi sbor no. 3," "Sal'varom" (mixture for inhalations no. 1), "Elakosept," etc., and their extracts enter into the composition of complex preparations ("Shalfei" pastils, "Parodontotsid," "Stomatofit," etc.) [4].

Salvia officinalis is a source of a great number of chemical compounds, possessing unique and diverse structures and a wide range of biological activity. Substances of terpenic and phenolic nature, such as diterpenes, tannins, hydroxycinnamic acids, flavonoids, etc. [5, 6] play the key role in the formation of anti-inflammatory, antioxidant, and antimicrobial activity of garden sage leaves. The most famous and widely studied representatives of these groups of compounds are carnosic acid and its derivatives, and also caffeic acid oligomers (rosmarinic acid, etc.). At the same time, the medicinal plants are a source of essential oils, exemplified mainly by mono- and sesquiterpenoids [7].

In domestic standard documents, the analyzed raw materials are traditionally standardized just by the quantitative concentration of essential oils, which is determined according to the requirements of the general pharmacopoeia article "Determination of the Concentration of Essential Oil in Medicinal Vegetable Raw Materials and Medicinal Vegetable Preparations" using the method of wet distillation [8]. It was also proposed to standardize garden sage according to the concentration of the sum of diterpene acids [9] by the procedure based on the extraction of biologically active substances (**BAS**) from garden sage with acetone followed by the extraction and purification of the diterpene fraction and determination of concentrations by spectrophotometry at 285 nm. Calculations were performed using specific absorbance $E_{1\text{cm}}^{1\%}$, found for a standard sample of carnosic acid dissolved in ethyl alcohol [9].

In the standardization of garden sage leaves by the concentration of BAS responsible for the main pharmacological properties of the raw materials (in particular, diterpene acids), there is a probability of obtaining unreliable results. This is, on one hand, due to multistep purification, leading to the loss and decomposition of the substances to be determined and underestimation of the results of analysis, and, on the other hand, with a possible presence of polyphenolic BAS in the solution, which absorb light at the same wavelength as the analytes.

The aim of this work is to develop an advanced procedure for the quantitative determination of diterpene acids in garden sage leaves.

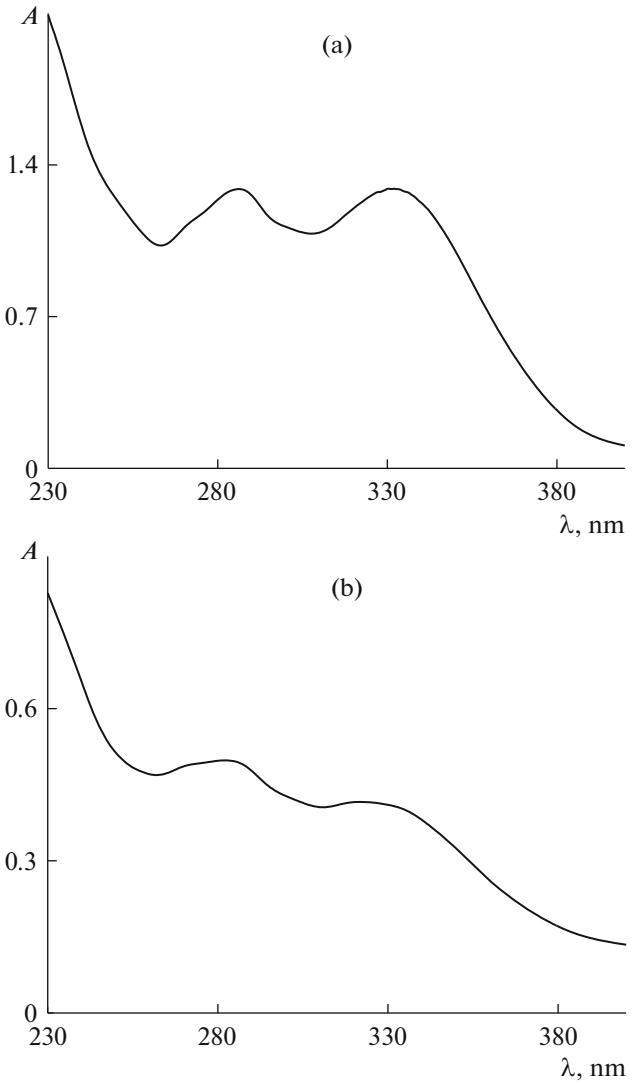

Fig. 1. Absorption spectra of (a) acetone and (b) alcohol extracts from garden sage leaves obtained by the procedure [9].

EXPERIMENTAL

The main test samples were garden sage leaves, packed in drip bags (JSC St-Medifarm, series 030210 and 050410). According to the instruction of the producer, the raw material is crushed leaves passed through a sieve with 2 mm openings. We also analyzed sage leaves grown and prepared in the Botanical Garden of the Kazan State Medical University in 2012 and 2013 and packed crushed sage leaves from various producers: OAO Krasnogorskleksredstva (series 141108), OOO Fitobot (11209), JSC Zdorov'e (060708), and OOO Fitofarm (061109).

The concentration of diterpene acids was determined on a LAMDA 25 spectrophotometer (Perkin Elmer, United States) using 10-mm cells.

In the study of the extraction kinetics, the concentration of active ingredients was determined at the initial instant of solvent boiling (0 min) and in 5, 10, 20, 30, 60, 90, and 120 min after it. Calculations were performed using the specific absorbance of carnosic acid $E_{1\,\mathrm{cm}}^{1\%} = 40.92$ determined in [9]. We considered the following ratios (L) of weighed portion (g) to solvent volume (mL): 1 : 50, 1 : 100, 1 : 150, and 1 : 200.

The data obtained were statistically processed using the Student test at a confidential probability of $P = 0.95$.

RESULTS AND DISCUSSION

The reliability of the procedure [9] was checked using UV spectra of a crude (primary) acetone extract (Fig. 1a) and an alcoholic solution (Fig. 1b) after its purification. In both cases, characteristic absorption maxima were observed at the same wavelengths, 283–285 nm, and ~330 nm. The intensity of the first absorption maximum in the alcohol solution was determined by the contributions of hydroxycinnamic acids and diterpene acids to be determined, present in the extract of garden sage. The second maximum was characteristic only for hydroxycinnamic acids [10]. After the purification of the solution, the second maximum remained still clear (Fig. 1b); therefore, part of hydroxycinnamic acids remained in the solution and affected the intensity of the first absorption maximum. Therefore, the concentration of diterpene acids found by the procedure [9] is unreliable. The necessity of performing a procedure of the primary extract purification also affects the correctness of the experimental results interpretation.

Choice of selective solvent. The replacement of an extractant with a selective one, extracting mainly the target group of compounds, can provide an approach to the development of a procedure yielding reliable results. Garden sage diterpenes are lipophilic compounds and, according to a number of authors, well dissolved in nonpolar solvents, like petroleum ether [11]. Figure 2 presents an UV spectrum of an extract of sage leaves with petroleum ether 40/70. The spectrum has a clear maximum at 285 ± 3 nm and corresponds to the UV spectrum of carnosic acid [12].

In comparing absorption spectra presented in Figs. 1 and 2, one can see that the last spectrum contains no absorption maximum at 330 nm. First of all, this is due to the low dissolving ability of petroleum ether for hydrophilic compounds, hydroxycinnamic acids among them. This fact was also confirmed by the impossibility of the dissolution of a standard sample of caffeic acid in petroleum ether. Therefore, taking into account the selectivity of petroleum ether 40/70 for the target group of compounds, we recommended it for extraction from garden sage raw materials.

The values of specific absorbance $E_{1cm}^{1\%}$ and analytical wavelength, which generally depend on the solvent, are necessary for the quantitative determination of the target compounds concentration in the extracts. These parameters were determined on the basis of the following experiment: after extraction with petroleum ether and recording an UV spectrum of the solution, the solution was evaporated to dryness under vacuum, the dry residue was dissolved in ethyl alcohol, and an UV spectrum of the new solution was recorded. The spectra obtained were identical in the wavelength region 270–300 nm; this suggests that the values of specific absorbance for different solvents are equal. The position of the maximum at 285 nm also did not change with the solvent. Therefore, the concentration of diterpene acids was determined by spectrophotometry at the wavelength 285 nm and specific absorbance $E_{1cm}^{1\%} = 40.92$, found in [9].

The other extracted substances can affect the characteristics of UV spectra of the extracts. The concentration of the other compounds absorbing radiation at 285 nm must be rather high for their effect become significant. Compounds bearing a benzene ring in the structure absorb light at the specified wavelength; among vegetable BAS, these are various phenolic compounds. In the garden sage, in addition to carnosic acid derivatives, these are hydroxycinnamic acids (rosmarinic, salvianolic, etc., and caffeic acid derivatives) and flavonoids [13]. The last two groups of substances because of their relatively high hydrophilic ability cannot be extracted by lipophilic solvents like petroleum ether and, therefore, affect the spectrum of the extract in the vicinity of the analytical wavelength. In addition, there is no data that, among the lipophilic compounds of garden sage dissolving in petroleum ether (essential oil mono- and sesquiterpenes, vegetable wax components, triglycerides, carotinoids, etc.), some substances absorb light in the specified region.

Study of the kinetics of extraction of diterpene acids from raw materials with petroleum ether 40/70. Studies [14] conducted earlier confirmed the well-known fact that the rate of extraction significantly depends both on the average size of sample particles and on the range of their characteristic sizes. To reduce the time of the diffusion transfer of substances in particles of the raw material and ensure the uniform dispersity of the weighed portion, raw materials were additionally crushed in an electric mill to characteristic size (diameter) smaller than 200 μm. The results of the study of the kinetics of extraction are shown in Fig. 3. Here amount x of the sum of diterpene acids in garden sage leaves in terms of carnosic acid in absolutely dry raw materials calculated by Eq. (1) at $V = 100$ (mL) is plotted along the ordinate axis, and extraction time is plotted along the abscissa axis. A significant (more than 85% of the maximum value for the specified conditions) transfer of the target substances to the solution was observed just by the time of solvent boiling, which

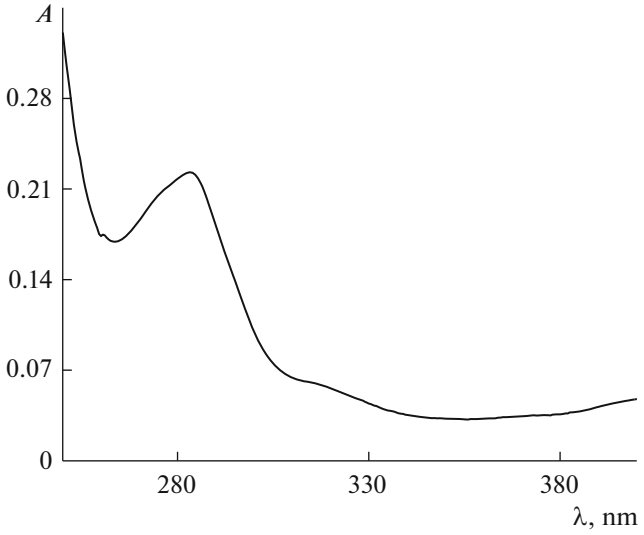

Fig. 2. Absorption spectrum of an extract from *S. officinalis* leaves in petroleum ether 40/70.

coincides with the results of works [14, 15]. Within the next 10–20 min, the yield of the extract increased, reached a maximum value, and remained constant to the end of the experiment ($t \leq 120$ min.). Therefore, considerable part of diterpene acids (~2.1% of the weighed portion) was extracted to the boiling solvent almost instantaneously, and we could not significantly increase the yield of target substances within reasonable time.

The results obtained can be explained both by the sorption of the target compounds with time leading to the equalization of the chemical potentials of the extracted substances in raw materials and in the solution and to the saturation of the solution with these compounds. However, the data in Fig. 4a show that the saturation of the solution in the studied range of values L was not attained. Therefore, the main mechanism is sorption, the effect of which was also noted in the works [14, 15].

The effect of sorption is that an equilibrium between the solution and the crushed raw materials is gradually attained in terms of concentrations of the extracted substances. The extracted substances are, on one hand, bound with the developed inner surface of the raw materials and, on the other hand, tend to pass to the solvent. The intensity of this transition is determined by the chemical affinity of the solvent to the target group of compounds and also by the concentration of the solution. At the initial step of the experiment, the force of the interaction of substances with the raw materials is much weaker than the dissolving ability of the solution of a low concentration. As a result, substances are extracted to the solution, its concentration increases, and the dissolving ability of the solvent drops. At the same time, the difference in the chemical potentials of the target compounds in the

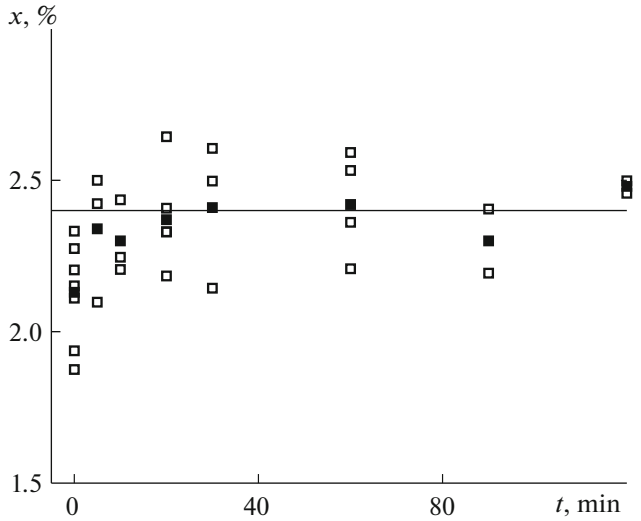

Fig. 3. Dependence of the recovery of diterpene acids on extraction time at the value $L = 1 : 100$; (□) experimental data, (■) average values for the specified time; solid line designated the value $x = 2.4\%$, the average of all experiments ($20 \le t \le 120$ min).

solution and raw materials approaches zero. The moment of the equalization of chemical potentials corresponds to the attainment of an equilibrium, and the process of dissolution is terminated. For the more complete extraction of substances, one should use a new portion of a pure solvent.

Multiple extraction. One and the same weighed portion of raw materials was triply extracted at the fixed ratio L within 20 min. The results are presented in Fig. 4. The fourth column in Fig. 4b corresponds to the sum of values x obtained as a result of triple extraction (total recovery). An analysis of the measured values of absorbance A showed that, under the studied conditions, starting from the ratio 1 : 150, the error of instrument readings may be high, particularly after the second extraction, which can considerably distort the total result. The results of the third extraction, and also their contribution to the total yield, are shown in Fig. 4 in white color. From the viewpoint of the completeness of extraction and the reliability of the results, double extraction at the ratio of the mass of the weighed portion to the solvent volume 1 : 200 is optimum; in this case, total recovery was $x = 2.94\%$.

Procedure of the quantitative determination of the sum of diterpene acids in garden sage leaves. A sample of raw materials is crushed to particles passing through a sieve with 0.25 mm openings. About 1 g (precisely weighed portion) of the sample is placed in a 500.0-mL flask, 200.0 mL of petroleum ether 40/70 is added, and the mixture is subjected to extraction in a boiling water bath within 20 min. After cooling, the solution is filtered through a paper filter to a 500.0-mL volumetric flask. The extraction procedure is repeated as

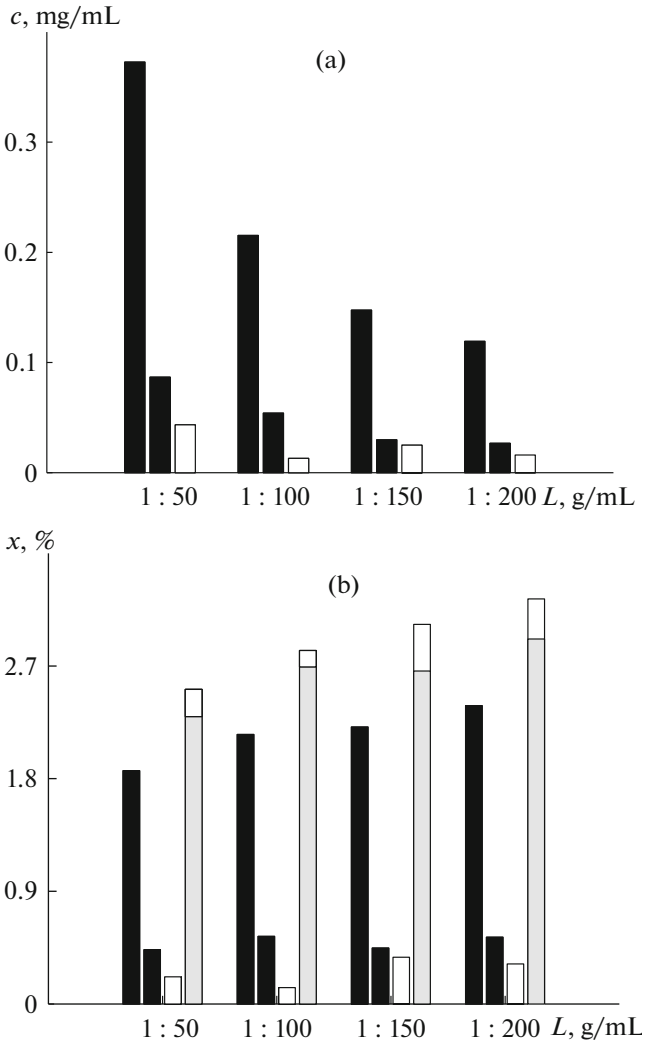

Fig. 4. (a) Concentration (mg/mL) and (b) content (%) of diterpene acids in the extract obtained by triple extraction with petroleum ether 40/70 as functions of the ratio of the weighed portion mass to the solvent volume. Black columns show results of the first two consecutive extractions; white columns, of the third extraction; and gray columns, total of the first two extractions (duration of each extraction 20 min).

described above. The extract is filtered to the same 500.0-mL volumetric flask, and the volume of the solution is brought to the mark with petroleum ether 40/70. The absorbance of the solution is measured at 285 nm using a 10-mm cell against a reference solution of petroleum ether 40/70.

Concentration (x, %) of the sum of diterpene acids in garden sage leaves in terms of carnosic acid in absolutely dry raw materials is calculated by the equation

$$x = \frac{AV}{E^{1\%}_{1\,\mathrm{cm}} m} \times \frac{100}{100 - W}, \qquad (1)$$

where A is the absorbance of the analyzed solution; V is total volume of the extract, mL (500 mL in the

Determination of diterpene acids in various samples of garden sage leaves ($n = 10$, $P = 0.95$)

Sample	Found, %
Botanical garden (2013)	3.6 ± 0.1
Botanical garden (2012)	3.02 ± 0.09
OAO Krasnogorskleksredstva (series 141108)	3.00 ± 0.07
JSC St-Medifarm (series 050410)	2.94 ± 0.07
OOO Fitobot (series 11209)	2.81± 0.07
JSC Zdorov'e (series 060708)	2.20 ± 0.06
OOO Fitofarm (series 061109)	2.10 ± 0.06

proposed procedure); $E^{1\%}_{1cm}$ is specific absorbance of carnosic acid at 285 nm in petroleum ether 40/70, equal to 40.92; m weighed portion, g; and W is loss of weight in drying raw materials, %.

The performance characteristics of the developed procedure of the quantitative determination of diterpene acids in garden sage leaves are presented below:

f	x_{av}	s^2	s	P	$t(0.95, 9)$	Δx	ε, %
9	2.94	0.0096	0.0979	0.95	2.26	0.07	± 2.38

The results obtained satisfy the norm of acceptable errors for spectrophotometry.

Using the developed procedure, we analyzed a number of samples of garden sage leaves (see table). The concentration of diterpene acids varied from 2.10 to 3.6% in terms of carnosic acid in absolutely dry raw materials.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, project no. 16-31-00007 mol_a. The authors are grateful to Victoria Rodionova for her help in the preparation of experiments.

REFERENCES

1. Bulfon, C., Volpatti, D., and Galeotti, M., *J. World Aquacult. Soc.*, 2014, vol. 45, no. 5, p. 545.
2. Mattazi, N., Farah, A., Fadil, M., Chraibi, M., and Benbrahim, K.F., *Int. J. Pharm. Pharm. Sci.*, 2015, vol. 7, no. 9, p. 73.
3. Kryshen', K.L., Guseva, S.I., Tesakova, S.V., and Atsapkina, A.A., *Tsitokin. Vospalenie*, 2009, vol. 8, no. 4, p. 67.
4. VIDAL: Reference Book of Medicines. http://www.vidal.ru. Cited January 21, 2017.
5. Miura, K., Kikuzaki, H., and Nakatani, N., *J. Agric. Food Chem.*, 2002, vol. 50, no. 7, p. 1845.
6. Bors, W., Michel, C., Stettmaier, K., Lu, Y., and Foo, L.Y., *Biol. Res.*, 2004, vol. 37, no. 2, p. 1099.
7. Alimkhozhdaeva, N.Z., *Extended Abstract of Cand. Sci. (Pharm.) Dissertation*, L'vov, 1974.
8. *Gosudarstvennaya Farmakopeya Rossiiskoi Federatsii* (State Pharmacopoeia of Russian Federation), Moscow: Ministry of Health of Russian Federation, 2015, 13th ed., vol. 3. http://www.femb.ru/feml. Cited January 21, 2017.
9. Zilfikarov, I.N. and Zhilin, A.V., *Farmatsiya*, 2007, no. 2, p. 7.
10. Moreno, S., Scheyer, T., Romano, C.S., and Vojnov, A.A., *Free Radical Res.*, 2006, vol. 40, no. 2, p. 223.
11. Al-Bayati, F.A., *Tikrit J. Pure Sci.*, 2011, vol. 16, no. 4, p. 6.
12. Thorsen, M.A. and Hildebrandt, K.S., *J. Chromatogr. A*, 2003, vol. 995, nos. 1–2, p. 119.
13. Lu, Y. and Foo, L.Y., *Phytochemistry*, 2002, vol. 59, no. 2, p. 117.
14. Salamatin, A.A., Khaziev, R.Sh., Makarova, A.S., and Ivanova, S.A., *Theor. Found. Chem. Eng.*, 2015, vol. 49, no. 2, p. 200.
15. Bucic-Kojic, A., Sovova, H., Planinic, M., and Tomas, S., *Food Chem.*, 2013, vol. 136, nos. 3–4, p. 1136.

Translated by E. Rykova