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QUANTITATIVE DETERMINATION OF TRITERPENE GLYCOSIDES IN THE FRUIT OF *Sophora japonica*

V. I. Grishkovets and L. A. Gorbacheva

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An extract of the fruit of the Japanese pagoda tree *Sophora japonica* L. possesses an immunostimulating action [1]; phenolic compounds have been found in the fruit of this plant [2]; and we have isolated polysaccharides [3] and triterpene glycosides [4].

Preparations of the fruit of the Japanese pagoda tree are widely used in pharmaceutical and phytotherapeutic practice [5]. In view of this, the quantitative determination of the triterpene glycosides in the preparations obtained is of great importance since it determines the nature of the dose-dependent effect.

The quantitative determination of triterpene glycosides in plant raw material is difficult because of their low level in the raw material (usually less than 1%) and the noneffectiveness of the usual physicochemical methods of analysis (UV, IR, and NMR spectroscopy) owing to the absence of specific signals (absorption bands), while the preparation of colored derivatives for photometry in the visible region of the spectrum requires the use of concentrated strong acids that cause resinification of the accompanying substances. Methods for the HPLC of triterpene glycosides have been developed [6], but the quantitative variants of these methods require the use of standard glycosides, which, together with the poor availability of the equipment, substantially limits the possibilities of their use in bulk analyses

We propose a quantitative method for the TLC analysis of triterpene glycosides in extracts of the Japanese pagoda tree which is based on determining the diameters of the glycoside spots or a comparison of the intensities of coloration of the spots with standards. We have used Silufol plates and the chromatographic solvent system chloroform—methanol—25% aqueous ammonia (10:45:15), the spots of the glycosides being detected with a 20% solution of tungstophosphoric acid in alcohol, followed by heating the chromatogram at 110°C for 5-10 min until the coloration was stable.

To determine the nature of the relationship between the weight of the sample (weight of the glycoside in the solution) and the diameter of the spot, we investigated samples of the two main triterpene glycosides of the Japanese pagoda tree — adzukisaponin-V (**1**) and soyasaponin (**2**) [4] in a wide range of weights of applied samples — from 0.5 to 20 μg (diameter of the initial spot not more than 2-3 mm) with a single elution of the chromatograms. The results obtained are shown in Table 1 and Fig. 1. For (**1**) and (**2**), these showed the same linear dependence of the diameter of the spot on the logarithm of he weight of the sample in the range from 0.5 to 16 μg:

$$D=3.18\cdot\lg m+2.00,$$

where D is the diameter of the spot, mm; and m is the weight of the sample, μg; or, conversely,

$$\lg m=0.314(D=2.00),$$

where D and m are expressed in the same units.

The limit of detection for glycosides is about 0.5 μg, and the working range of weights of the samples is 1-16 μg (spot diameters 2-6 mm). The error found in the determination of the weight of a sample within the working range is usually 40-50% and is due mainly to the inaccuracy of determining the diameter of the spot (±0.5 mm) in view of the subjectivity of the operator in deciding on the boundaries of the spots. However, the error can be brought down to 30-40% by depositing 3-4

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TABLE 1. Results of a Determination of the Dependence of the Diameter of the Spot on the Weight of the Sample

Weight of the sample m, μg	lg m	Diameter of the spot, D, mm	Weight of the sample m, μg	lg m	Diameter of the spot, D, mm
0.5	–0.30	1.0	6.0	0.78	4.5
1.0	0.00	2.0	8.0	0.90	5.0
2.0	0.30	3.0	10.0	1.00	5.2
3.0	0.48	3.5	12.0	1.08	5.4
4.0	0.60	4.0	16.0	1.20	5.8
5.0	0.70	4.3	20.0	1.30	6.0

*Mean of 10 determinations.

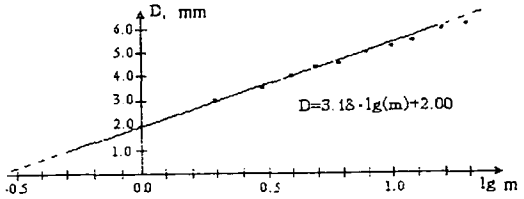

Fig. 1. Dependence of the diameter of the spot D (in mm) on the logarithm of the weight of the sample m (in μg).

volumes (weights) of the sample covering the range of 1-16 μg, or to 20-30% by chromatographing a comparison scale simultaneously (samples of pure glycosides weighing 1, 2, 4, 8, and 16 μg).

To determine the levels of (**1**) and (**2**) in the fruit of the Japanese pagoda tree, we subjected seeds to analysis, since it is just these that contain the bulk of the triterpene glycosides, as we have shown previously [4]. The seeds, making up about 50% of weight the air-dry fruit, were ground to a particle size of 0.25 mm and extracted with 70% ethanol (10 ml per 1 g of seeds) at 40-60°C for 10 h with periodic stirring. The residue was separated off by centrifugation, and the extract was analyzed by TLC under the conditions described above. After revelation, the chromatograms showed a main violet spot with R_f 0.3, consisting of the sum of (**1**) and (**2**) (they can be separated on repeated elution of the chromatogram) and a less intense spot with R_f 0.4 consisting of the sum of adzukisaponin-II (**3**) and soyasaponin-III (**4**) (also separable on repeated elution) [4]. A yellow-brown spot with $R_f < 0.25$ represented phenol glycosides and a pink-violet spot with $R_f > 0.8$ substances of lipid nature.

For the quantitative determination of the sum of (**1**) and (**2**), 2-30 μl samples of the extract obtained were deposited on plates and chromatographed by the method described above. The weight of glycosides in each sample and their concentration (c) in the extract in μg/μl, which is equivalent to mg/ml, were determined from the diameter of the corresponding spot. When the volume of extractant is 10 ml, the weight of glycosides in 1 g (1000 mg) of seeds is 10c (mg), which, expressed as a percentage, is $10c/1000 \cdot 100\% = c$ (%), or $c/2$ (%) calculated to the fruit. The sum of (**3**) and (**4**) can be determined analogously. The combined amount of (**1**) and (**2**) in the fruit of the Japanese pagoda tree gathered in accordance with the requirements of FS [Pharmaceutical Specification] 42-452-72 is from 0.3 to 0.6%.

REFERENCES

1. G. A. Drozd and L. A. Gorbacheva, Farmatsiya, No. 1, 34 (1994).
2. W. Karrer, Konstitution und Vorkommen der organischen Pflanzenstoffe, Birkhäuser Verlag, Basel and Stuttgart (1958).
3. L. A. Gorbacheva, V. I. Grishkovets, G. A. Drozd, and B. Ya. Chirva, Khim. Prir. Soedin., 748 (1995).
4. V. I. Grishkovets and I. A. Gorbacheva, Khim. Prir. Soedin., 709 (1995).
5. D. A. Murav'eva, Pharmacognosy [in Russian], Moscow (1978), p. 587.
6. V. V. Makhan'kov, N. F. Samoshina, G. V. Malinovskaya, L. N. Atopkina, V. A. Denisenko, V. V. Isakov, A. I. Kalinovskii, and N. I. Uvarova, Khim. Prir. Soedin., 57 (1990).