METHOD FOR THE QUANTITATIVE DETERMINATION OF SCHIZANDRIN AND SCHIZANDROL IN *Schisandra chinensis*

L. I. Samoilenko and N. I. Suprunov

UDC 547.562.1:543.062

The active substances of *Schisandra chinensis* (Trucz.) Baill. (Chinese magnoliavine) are lignans of the dibenzocyclooctadiene series [1-3]. They were first found in the seeds [4], and then in the stems and rhizomes [5]. The lignans possess valuable pharmacological properties and are therefore used in medicine. A polar fraction of the lignans containing the main component – schizandrin – has a relatively high biological activity [6].

As shown previously [5], because of their close physicochemical properties schizandrin and schizandrol are extremely difficult to separate by chromatography. In view of this, their amount can be determined only as a total.

For quantitative control in the production of lignans and for the determination of the quality of the Chinese magnoliavine raw material we have developed a chromatophotocolormetric method. It is based on the color reaction of schizandrin and schizandrol with concentrated sulfuric acid. The method enables the optical densities of the yellow solutions of mixtures of schizandrin and schizandrol to be measured at a wavelength of 350 nm. The absorption of the solution of the lignans obeys the Lambert–Beer law in the range of working concentrations from 0.1 to 1 mg/ml.

In addition to schizandrin and schizandrol, and magnoliavine raw material contains another eight, less polar, lignans. To separate the mixture of lignans preparatively, we used thin-layer chromatography. They were separated on a plate with a fixed layer of silica gel of type KSK in the ethyl acetate–petroleum ether (1 : 1) system. Under the action of concentrated sulfuric acid, the spots of the schizandrin and schizandrol assumed a bright yellow color which changed when the chromatograms were heated.

In the extraction of the comminuted seeds, the lipids of the seed core pass into the extract, which distorts the results of the analysis. In view of this, it has been proposed to use the skin of the seeds for analysis since the seed core contains no lignans. The best solvent for extracting the lignans from the skin of the seeds is chloroform. The extraction of the lignans takes place satisfactorily at ordinary temperatures. The lignans are obtained from the stems and rhizomes with the minimum amount of impurities by the simultaneous extraction of the raw material with chloroform and filtration of the extract through a layer

TABLE 1. Statistical Analysis of the Results of the Determination of the Amount of Schizandrin and Schizandrol in the Skin of the Seeds of the Chinese Magnoliavine

Extract taken, ml	Optical density	Lignans found		$x - \bar{x}$	$(x - \bar{x})^2$	Metrological characteristics
		mg	%			
0,2	0,210	16,0	3,2	+0,05	0,0025	$S = \pm 0{,}06$
0,2	0,200	15,5	3,1	−0,05	0,0025	$S_{\bar{x}} = \pm 0{,}03$
0,2	0,200	15,5	3,1	−0,05	0,0025	$\varepsilon_\alpha = \pm 0{,}09$
0,2	0,210	16,0	3,2	+0,05	0,0025	$t_\alpha = \pm 3{,}182$
			$\Sigma = 12{,}6$		0,0100	$\varepsilon_{rel} = \pm 2{,}8\%$
			$\bar{X} = 3.15$			

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Scientific Center, Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 148-152, March-April, 1974. Original article submitted March 19, 1973.

TABLE 2. Statistical Analysis of the Results of a Determination of the Amounts of Schizandrin and Schizandrol in the Bark of the Stems of the Chinese Magnoliavine

Extract taken, ml	Optical density	Lignans found		$x - \bar{x}$	$(x-\bar{x})^2$	Metrological characteristics
		mg	%			
0,2	0,390	55	5,5	+0,1	0,01	$S = \pm 0,11$
0,2	0,385	54	5,4	—	—	$S_{\bar{X}} = \pm 0,05$
0,2	0,390	55	5,5	+0,1	0,01	$\varepsilon_\alpha = 3,182$
0,2	0,380	53	5,3	—0,1	0,01	$t_\alpha = \pm 0,16$
0,2	0,380	53	5,3	—0,1	0,01	$\varepsilon_{rel} = \pm 2,9\%$
			$\sum = 27,0$		0,04	
			$\bar{X} = 5,4$			

TABLE 3. Statistical Analysis of the Results of a Determination of the Amounts of Schizandrin and Schizandrol in the Bark of the Rhizomes of the Chinese Magnoliavine

Extract taken, ml	Optical density	Lignans found		$x - \bar{x}$	$(x-\bar{x})^2$	Metrological characteristics
		mg	%			
0,2	0,540	83	8,3	+0,05	0,0025	$S = \pm 0,10$
0,2	0,530	81	8,1	—0,15	0,0225	$S_{\bar{X}} = \pm 0,05$
0,2	0,540	83	8,3	+0,05	0,0025	$\varepsilon_\alpha = 3,182$
0,2	0,540	83	8,3	+0,05	0,0025	$t_\alpha = \pm 0,16$
			$\Sigma = 33,0$		0,0300	$\varepsilon_{rel} = \pm 1,9\%$
			$\bar{X} = 8,25$			

TABLE 4. Dynamics of the Accumulation of Lignins according to the Phases of Development of the Chinese Magnoliavine

Phases of development of the liana	Date of collecting the samples (1971)	Content of lignans, %			
		in the bark of the stems		in the bark of the rhizomes	
		combined fraction	schizandrin and schizandrol	combined fraction	schizandrin and schizandrol
Dormancy	6.I	6,00	2,90	—	—
	2.II	6,50	5,00	—	—
	4.III	6,00	5,00	—	—
Movement of the sap	10.IV	4,90	1,42	6,00	3,00
	8.V	7,50	3,75	7,80	3,26
Budding	14.VI	9,12	7,26	8,90	3,75
Flowering	12.VII	10,44	9,14	11,50	8,25
Beginning of fruit-bearing	4.VIII	8,10	6,75	9,3	3,25
Ripening of the fruit	5.IX	7,16	2,90	11,00	3,00
Fall of the leaves	3.X	8,10	3,05	10,30	2,50
Dormancy	2.XI	7,20	2,75	9,23	2,02

of alumina, which retains the sterols and the green and yellow pigments. Because of the low content of lignans in the wood of the stems and the rhizomes (about 0.5%) they were determined only in the bark.

The amounts of schizandrin and schizandrol were determined in the seeds of the ripe fruit in the 1971 harvest and in the stems and rhizomes as a function of the phase of development of the liana.

As a statistical treatment of the results of analysis has shown (Tables 1-3), the method developed is distinguished by accuracy, reproducibility, and a relative error of the results of ± 2.8% for the seed skin, 2.9% for the stems, and 1.9% for the rhizomes. The seeds contain an average amount of schizandrin and schizandrol of 3%, the stems 5%, and the rhizomes 4%. It can be seen from Table 4 that the greatest amount of lignans accumulates in the vegetative organs in July in the flowering phase. In the autumn and winter months, the amount of schizandrin and schizandrol falls to one half. A marked fall in the amounts of these lignans in the stems is found in the phase of movement of the sap. Thus, the best type of raw material for obtaining schizandrin and schizandrol is the bark of the stems and the rhizomes which must be collected in July.

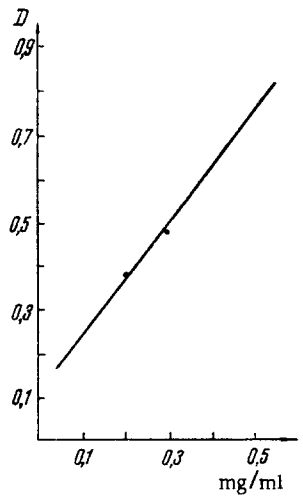

Fig. 1. Calibration graph of a pure mixture of schizandrin and schizandrol.

EXPERIMENTAL

Construction of a Calibration Graph. To construct the calibration graph (Fig. 1), 0.1 g (accurately weighed) of a pure mixture of schizandrin and schizandrol (1 : 1) was dissolved in chloroform in a 25-ml measuring flask. By means of a micropipette, 0.05, 0.1, 0.15, and 0.20 ml of the resulting 0.4% solution of the mixture of schizandrin and schizandrol were transferred to a number of test tubes and in each the volume was made up to 1 ml with chloroform, after which 0.2 ml of concentrated sulfuric acid was added with a micropipette, the tubes were shaken, 1 ml of acetone was added to each, they were shaken again, and their colorations were measured on a FÉK-56 photoelectric colorimeter with a No. 2 filter. The test tubes theoretically contained 100, 200, 300, and 400 μg of the mixture of schizandrin and schizandrol. The dependence of the optical density on the concentration is linear in the range of concentrations used.

Determination of the Mixture of Schizandrin and Schizandrol in the Seeds. The skin was separated from the seed core. A 0.5-g sample of it (accurately weighed) was ground in a mortar with 2 g of Al_2O_3 for chromatography, and the mixture was placed in a glass tube (d = 0.7 cm, h = 30 cm). It was extracted with 15 ml of chloroform into a 25-ml measuring flask and the extract was made up to the mark with pure chloroform. By means of a micropipette 0.2 ml of the extract was deposited on a layer of silica gel fixed with gypsum at two points on a plate (9 × 12) at a distance of 3 cm, and the chromatogram was run in the ethyl acetate–petroleum ether (1 : 1) system. After drying, one half of the plate was covered with a strip of polyethylene, and the other was sprayed with concentrated sulfuric acid. When the schizandrin and schizandrol had been shown up on the control part of the plate (yellow color), the positions of their spots on the untreated part were determined. These sections of the silica gel with the schizandrin and schizandrol (R_f 0.33 and 0.40, respectively) were scraped off, transferred to a glass tube of the same size as that used for extraction, and eluted with 5 ml of chloroform into 50-ml beakers. For a control, the same area of pure sorbent as that of the sorbent with the lignans was taken and was eluted. The beakers were placed under a hood and the chloroform was evaporated off. The dry residue in each beaker was treated with 1 ml of chloroform, 0.2 ml of concentrated sulfuric acid was added with a microburette, mixing was brought about by rotation, 1 ml of acetone was added, mixing was again performed, and the colored solution was placed in a cell with a thickness of 3.040 mm. The optical density was determined at 350 nm on a FÉK-56 photoelectric colorimeter with a No. 2 filter.

Determination of a Mixture of Schizandrin and Schizandrol in the Bark of the Stems and of the Rhizomes. An accurately weighed sample of about 1 g of air-dry raw material finely ground in a coffee mill was placed in a glass tube (d = 0.7 cm, h = 30 cm) containing 2 g of Al_2O_3 for chromatography. The mixture was extracted with 20 ml of chloroform, the extract was collected in a 20-ml measuring flask, and the solution was made up to the mark. The chromatography and determination of the optical density of the solutions under test was performed as described above.

The percentage of the mixture of schizandrin and schizandrol in the seeds, stems, and rhizomes was calculated from the formula

$$X = \frac{A\ V\ V_1\ 100}{V_2\ P\ 1000},$$

where A is the amount of schizandrin and schizandrol (mg) in 1 mg of extract, found from the calibration graph; V is the amount of extract, ml; V_1 is the volume in which the mixture of schizandrin and schizandrol was dissolved after their separation on the chromatogram and elution from the sorbent, ml; V_2 is the volume of the extract deposited on the chromatogram, ml; and P is the weight of the raw material, g; the factor 100 is to convert the result into a percentage and the factor 1000 is for converting g into mg.

CONCLUSIONS

A chromatophotocolorimetric method for the quantitative determination of the mixture of schizandrin and schizandrol in various organs of Schisandra chinensis (Turcz.) Baill. has been proposed. It has been established that the approximate amount of schizandrin and schizandrol in the seeds is 3%, in the stems 5%,

and in the rhizomes 4%. The best type of raw material is the bark of the stems and of the rhizomes collected in July.

LITERATURE CITED

1. N. K. Kochetkov, A. Ya. Khorlin, and O. S. Chizhov, Zh. Obshch. Khim., 10, 31, 3454 (1961).
2. N. K. Kochetkov, A. Ya. Khorlin, O. S. Chizhov, and V. I. Shevchenko, Izv. Akad. Nauk SSSR, Otd. Khim. Nauk, No. 5, 850 (1962).
3. N. K. Kochetkov, A. Ya. Khorlin, and O. S. Chizhov, Izv. Akad. Nauk SSSR, Otd. Khim. Nauk, No. 6, 1036 (1964).
4. D. A. Balandin, in: Materials for the Study of the Stimulants and Tonics Ginseng Root and Magnoliavine Root [in Russian], No. 1 (1951), p. 45.
5. N. I. Suprunov and I. V. Vetlugina, Farmatsiya, 3, 34 (1972).
6. A. A. Lebedev, in: Materials for the Study of the Stimulants and Tonics Ginseng Root and Magnoliavine Root [in Russian], No. 1 (1951), p. 103.