Chemistry of Natural Compounds, Vol. 29, No. 5, 1993

SPECTROPHOTOMETRIC DETERMINATION OF FLAVONES AND ISOFLAVONES IN *Thermopsis alterniflora*

L. D. Kotenko, A. U. Mamatkhanov, and M. T. Turakhozhaev

UDC 615.322:547.814:543.42

A spectrophotometric method has been developed for the quantitative separate determination of the total flavonones and isoflavones when they are present together in an extract of Thermopsis alterniflora.

Thermopsis alterniflora Regl. et. Schmalh. (family Fabaceae) is a medicinal plant and is used for obtaining cytisine [1]. The epigeal part of the plant contains the total flavonoids [2-7], the main representatives of which are flavones — luteolin and chrysoeriol — and an isoflavone — formononetin.

For quantitative estimation of the flavonoids in the raw material, a spectrophotometric method is proposed for the separate determination of total flavones and isoflavones when they are present simultaneously, using Vierordt's method [8, 9].

On the basis of the properties of the groups of flavonoids to be analyzed, we selected two analytical wavelengths — 262 and 352 nm [9]. The absorption at 353 nm corresponds to the flavone content and that at 262 nm to the flavones and isoflavones combined. The UV spectrum of an alcoholic extract of thermopsis has an absorption maximum at 260 ± 2 nm and a shoulder at 350-360 nm. The flavone content was calculated as luteolin and the isoflavone content as formononetin. As the standard samples we used luteolin satisfying the requirements of VFS [All-Union Pharmaceutical Standard] 42-1709-87 and formononetin obtained by synthesis (mp 255-257°C) [10].

EXPERIMENTAL

Analysis of the Raw Material. Approximately 1.0 g (accurately weighed) of the comminuted (particle size 3 mm) air-dry herb was charged into a 100-ml flask and, after the addition of 30 ml of 80% ethanol, the flavonoids were extracted with continuous shaking for 1 h. Then the mixture was allowed to settle for 10-15 min and the liquid was poured off through a glass filter (160 pore) without allowing the particles of raw material to reach the filter. Extraction was repeated three times, the filtrates being collected in a 100-ml measuring flask (solution A). After the transfer of 1.0 ml of solution A to a 50-ml measuring flask it was made up to the mark with 80% ethanol. The optical density of the resulting solution was measured on a spectrophotometer in a cell with a layer thickness of 1 cm relative to 80% ethanol at a wavelength of 353 nm, corresponding to the most pronounced absorption maximum of flavones, and at 262 nm, corresponding to the most pronounced absorption maximum of isoflavones. The concentrations of flavones (C_1) and of isoflavones (C_2) were calculated from the formulas

$$C_1 = \frac{D \cdot V}{E_1 \cdot l \cdot a},$$

$$C_2 = \frac{(E_1 D_2 - E_2 D_1) \cdot V}{E_3 \cdot E_1 \cdot l \cdot a},$$

where D_1 and D_2 are the optical densities of the extract under investigation at 262 and 353 nm, respectively; E_1 is the specific absorption index of luteolin in 95% ethanol at a wavelength of 353 nm, which is 762; E_2 is the specific absorption index of luteolin in 95% ethanol at a wavelength of 262 nm, which is 600; E_3 is the specific absorption index of formononetin at a wavelength of 262 nm, which is 858; l is the layer thickness of the cell, 1 cm; a is the weight of raw material, g; and V is the dilution.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent.
Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 667-669, September-October, 1993. Original article submitted March 29, 1993.

0009-3130/93/2905-0590$12.50

TABLE 1. Metrological Characteristics of the Method

Found, %	Metrological characteristics	Found, %	Metrological characteristics
Formononetin	S^2=97.80·10^{-4}	Luteolin	S^2=42.80·10^{-4}
2.65	S=9.89·10^{-2}	1.72	S=6.54·10^{-2}
2.58	$S_{\bar{X}}$=4.04·10^{-2}	1.68	$S_{\bar{X}}$=2.58·10^{-2}
2.63	X=0.10	1.60	X=0.07
2.46	$t_{\alpha,k}$=2.57	1.74	$t_{\alpha,k}$=2.57
2.41	E=±4.09%	1.58	E=±3,97%
2.49	X=2.54%±0.10	1.70	X=1.67%C0.07

TABLE 2. Results of the Quantitative Determination of Flavonoids in an Extract of *Thermopsis alterniflora* (experiments with additions)

Amount in 1 ml of extract, mg		Added, mg		Found, mg		Relative error, %	
formononetin	luteolin	formo-nonetin	luteolin	formo-nonetin	luteolin	formo-nonetin	luteolin
0.369	0.218	0.360	0.332	0.753	0.538	+3.18	-2.23
0.369	0.218	0.176	0.145	0.522	0.370	-4.40	+1.89

Table 1 gives the metrological characteristics of the method. When the analysis was performed in six replicates the relative error of the method with 95% probability was ±4.09% for luteolin and ±3.97% for formononetin. The absence of systematic errors was confirmed by experiments with additions, the results of which are shown in Table 2.

REFERENCES

1. Kh. Kh. Khalmatov, I. A. Kharlamov, P. K. Alimbaeva, M. O. Karryev, and I. Kh. Khaitov, The Main Medicinal Plants of Central Asia [in Russian], Meditsina, Tashkent (1984), p. 139.
2. N. Sh. Kattaev and G. K. Nikonov, Khim. Prir. Soedin., 115 (1973).
3. N. Sh. Kattaev and G. K. Nikonov, Khim. Prir. Soedin., 648 (1972).
4. N. Sh. Kattaev and G. K. Nikonov, Khim. Prir. Soedin., 140 (1975).
5. S. S. Yusupova, É. Kh. Batirov, F. Kiyamitdinova, and V. M. Malikov, Khim. Prir. Soedin., 639 (1979).
6. É. Kh. Batirov, M. P. Yuldashev, G. A. Nezhinskaya, and V. M. Malikov, Khim. Prir. Soedin., 727 (1979).
7. M. P. Yuldashev, É. Kh. Batirov, A. D. Vdovin, V. M. Malikov, and M. R. Yagudaev, Khim. Prir. Soedin., 352 (1989).
8. I. Ya. Bershtein and Yu. A. Kaminskii, Spectrophotometric Analysis in Organic Chemistry [in Russian], Leningrad (1975), pp. 65-97.
9. A. B. Dmitriev and A. L. Kazakov, Khim.-farm. Zh., 90 (1981).
10. G. N. Dorofeenko, A. L. Shinkarenko, A. L. Kazakov, A. I. Pyshchev, and V. V. Mezheritskii, Khim. Prir. Soedin., 160 (1974).