CHROMATOGRAPHIC DETERMINATION OF GLYCYRRHIZINIC ACID IN *Glycyrrhiza glabra* PREPARATION

T. P. Arystanova, M. P. Irismetov, and A. O. Sopbekova UDC 547.915.543.422.23

Licorice root preparation, bioslastilin (BS), was prepared in the chemistry of natural compounds branch laboratory of the Chemical Sciences Institute of the Ministry of Science and Higher Education of the Republic of Kazakhstan [1].

BS contains ~80% glycyrrhizinic acid (GA) and ~20% other biologically active substances: flavonoids, monodisaccharides, lipids, etc. It is used as a biological additive in the food, perfumery and cosmetic industry.

At present the production of medicinal preparations with BS, the development of a composition and mass production of tablets, and analytical and standardization methods are under intense scrutiny [2]. The hepatoprotective activity of BS was found in preclinical trials using models of acute toxic hepatitis [3]. The activity is due to a high content of GA.

Previously the possibility of using TLC for determining of glycyrrhizinic acid contents in BC was studied [4]. Chromatography was performed in solvent systems as before [5] and chosen by us experimentally: $CHCl_3$—CH_3OH (8:2), $CHCl_3$—CH_3OH (7:3), $CHCl_3$—CH_3OH—H_2O (30:17:3), *n*-butanol—ethanol—ammonia (7:2:5), *n*-butanol—water—acetic acid (4:5:1), benzene—ethylacetate (1:1), and benzene:acetone (10:1).

Components of BS were detected in the chromatograms using 25% alcoholic phosphotungstic acid, 10% alcoholic phosphomolybdic acid, 1% vanillin in conc. H_2SO_4, iodine vapor, and UV light [6].

Chromatographically pure GA obtained from TsZL Shymkentskii OAO "Khimfarm" was used as a standard sample (SS).

The preparation (0.05 g) was dissolved in CH_3OH (5 mL). The test solution (10 μL, 100 μg) was spotted (2-3 times) at the origin of chromatographic plates using a MSh microsyringe (Russia). The diameter of the spot was < 3 mm. The optimal volume was 4-6 μL. SS solution I (8 μL, 80 μg, 1%) and SS solution II (2 μL, 5 μg, 0.25%) were placed alongside. The plate was dried in air for 10 min and placed in a chamber for ascendant chromatography using $CHCl_3$—CH_3OH—H_2O (30:17:3) or *n*-butanol—ethanol—ammonia (7:2:5).

It was found during the study that Kieselgel 60 plates were better than Silufol UV-254 plates with respect to separation and spot size. They give a good separation, compact spots, and rapid development. The rise of the solvent front sufficient for complete separation is 8 cm. However, developers for which the sensitivity is less than the sensitivity of detection by UV light must be applied if these plates are used because they have no fluorescent indicator.

The detection limit of GA for development by phosphotungstic acid was 5 μg (the spot is colored reddish-purple). The detection limit of GA for spraying with phosphomolybdenic acid was 3 μg (the spot appears blue with a yellow background). The colors of the spots were stable for 3 h.

Treatment with ammonia vapor could lower the detection limit to 0.5 μg.

Vanillin solution colors the GA spot reddish-purple. The detection limit is 1-2 μg. After spraying with vanillin solution, the chromatogram must be kept at 60°C for 1 h.

The results showed that it is inadvisable to use iodine vapor as developer because of poor reproducibility and sensitivity.

The chromatography should be performed on Silufol UV-254 plates despite the fact that the separation of components at high solvent-front rates takes longer and leads to slight broadening of the spots.

The rise of the front necessary for complete separation is 11 cm. Reagents are not needed if these plates are used. The spots of GA and other components can be detected with higher sensitivity at the same time. The spots are visible in UV light as shining violet spots. The sensitivity for GA is 0.5 μg. Two more spots are observed that do not appear after treatment with phosphotungstic and phosphomolybdenic acids.

South Kazakhstan State Medical Academy. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 77-78, January-February, 2001. Original article submitted December 12, 2000.

0009-3130/01/3701-0089$25.00

The quality of the chromatograms improves if the plates are heated to 40°C and chromatography is performed at 20-25°C.

BS components were selectively separated in neutral and basic systems: $CHCl_3$—CH_3OH—H_2O (30:17:3) and *n*-butanol—ethanol—ammonia (7:2:5). The first solvent system is quick. The second contains less toxic components.

Chromatography on Silufol UV-254 plates showed a dominant spot with R_f identical to that of the SS (0.42 ± 0.02) and additional spots with R_f (±0.02) = 0.28, 0.5, 0.58, 0.63, 0.73, and 0.78. The spots of other BS components (additional spots) had fluorescence intensities and dimensions less than those of the spot for 5-10 μg of SS.

Thus, a TLC method is developed for BS on Silufol UV-254 plates that can identify the principal active substance, GA, and other components with high sensitivity (0.5 μg).

The stability of GA in five series of experimental commercial BS samples was studied. It was found that GA is stable on storage during the observation period (2.5 y).

REFERENCES

1. Patent Disclosure RK 65/35-6 (1997).
2. T. P. Arystanova, A. S. Beisenbekov, and A. O. Sopbekova, *Farm. Byull.*, 21 (2000).
3. T. P. Arystanova and K. D. Rakhimov, *Farm. Byull.*, 32 (2000).
4. M. R. Yakubova, G. L. Genkina, and T. T. Shakirov, *Khim. Prir. Soedin.*, 802 (1977).
5. G. V. Khodakova, Yu. A. Akimov, A. S. Shashkova, P. K. Kintya, and V. I. Grishkovets, *Khim. Prir. Soedin.*, 756 (1994).
6. L. A. Baltina, O. B. Fletkher, Zh. M. Putieva, et al., *Khim.-Farm. Zh.*, 47 (1997).