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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN THE ANALYSIS OF PLANT TANNIN SUBSTANCES

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Inadequate attention is being devoted to the separation and analysis of condensed tannin substances and also of tannins of the mixed type. In the present communication we give the results of a choice of conditions for separating the total preparations from some plants of the Kazakhstan flora. Literature sources on the use of the HPLC method in the analysis of tannin substances are few in number and are devoted mainly to their separation [1-6].

Chromatographic analysis was carried out mainly on a DuPont (USA) model 8800 liquid chromatograph with a UV detector and a flow-through cell at the wavelengths 254 and 287 nm, with a rate of flow of 0.5-1.0 ml/min. A Zorbax ODS column (4.6 × 250 mm) was used, with 5-μm particles at a column temperature of 25°C. The mobile phases employed were: 1) 0.01 M H_3PO_4-0.01 KH_2PO_4–ethanol–ethyl acetate (42.5:42.5:10:5), and 2) water–acetonitrile (3:7) to (1:1).

We investigated preparations based on condensed tannin substances and also on tannins of the mixed type, which we obtained from *Ephedra equisetina, Alnus cinera, Sedum hybridum*, and *Agrimonia asiatica*. To identify the signals in the chromatograms and also for the quantitative analysis of the components we used the individual compounds ephedrine hydrochloride, (+)-catechin, dimer B-3, amino acids, polyflavans from the above-mentioned plants, herbacetin 7-O-β-*D*-glucopyranoside, 7-methoxygossypetin 3-O-β-*D*-glucopyranoside, 2,3:4,6-di-O-(S)-4,4′,5,5′,6,6′-hexahydroxydiphenoyl-*D*-glucopyranose, and 2,6-digalloylglucose.

As a result of the investigation performed, we selected the optimum conditions for the separation of the components of preparations containing components of the condensed type: mobile phase 1, rate of flow of eluent 0.5 ml/min, wavelength 254 nm; and for preparations containing tannin substances of the mixed type: mobile phase 2 (3:7), rate of flow of eluent 1 ml/min, wavelength 278 nm.

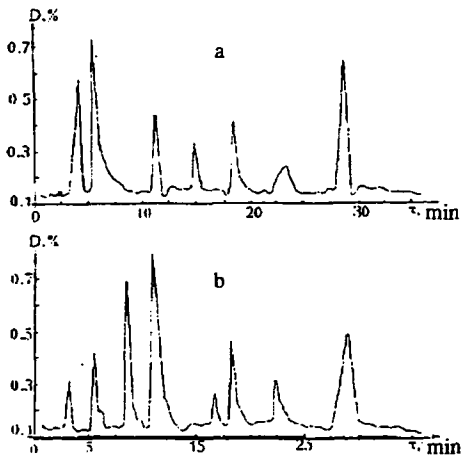

Fig. 1. Chromatograms of total preparations: *a*) based on tannin substances of the condensed type; *b*) based on tannin substances of the mixed type from *Sedum hibridum*.

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It is important to note that, under the conditions found, a high similarity of the results of total multicomponent phytopreparations based on the tannin substances of all the above-mentioned plants was observed, regardless of the times of storage of the preparations (we analyzed freshly gathered samples and samples stored for various times up to two years). Of practical importance is the fact that both the relative percentage levels of the components and their retention times were unchanged. Figure 1 gives some results of the analyses.

Thus, the possibility and the reliability of the use of the HPLC method for proving the authenticity and the quality of phytopreparations in dependence of the times of storage for tannin substances of different types has been shown, and a dependence of chromatographic behavior on the type of tannin substances under the conditions of a reversed-phase process has been revealed.

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