TLC AND HPLC ANALYSIS OF SYRINGIN IN Syringa vulgaris

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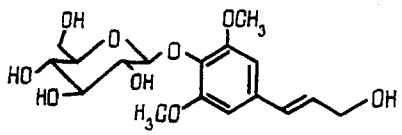
Using HPLC, the component composition of the bark of Syringa vulgaris has been studied and a procedure has been developed for the quantitative determination of syringin in raw material from this plant. The seasonal dynamics of the accumulation of syringin in the bark of common lilac has been studied with the aid of a chromatospectrophotometric method and HPLC analysis.

It has been established previously that the chemical composition of the bark of the common lilac Syringa vulgaris L., family Oleaceae, is represented by substances of different natures: phenylethanol derivative (tyrosol, hydroxytyrosyl, and their glycosides salidroside and hydroxysalidroside), phenylpropanoids (the acylglycosides acteoside and forsythiaside; the cinnamylglycosides syringin and coniferin; and the lignan glucosides lariciresinol and olivil), and also iridoids in the form of conjugates with tyrosol and hydroxytyrosol (oleuropein, noroleuropein, ligustroside, nüzhenide) [1-3].

It has been found that syringin (I), like other glycosides of cinnamyl alcohol, possesses neurotropic properties [4]. It is also known that syringin (eleutheroside B) is one of the biologically active substances of prickly eleutherococcus and is responsible for the tonic, adaptogenic, and immunostimulating properties of extracts of it [5, 6].



I

In the standardization of the raw material and preparations of eleutherococcus, the quantitative determination of syringin and of the lignan syringaresinol diglucoside (eleutheroside E) by the HPLC method is used [7].

The aim of the present investigations was to study the component composition of the bark of the common lilac by the HPLC method and to develop a procedure for the quantitative determination of syringin, and also to study the dynamics of the accumulation of syringin in the bark of this plant during the vegetation period.

Syringin can be used as a standard in the analysis of lilac, eleutherococcus, mistletoe [8], and other plant sources containing syringin and other related phenylpropanoids.

A chromato-spectrophotometric method of determining syringin in lilac bark has been proposed previously [9] in which use is made of the extraction of the raw material with 70% ethanol, chromatography of the extract and of a standard sample of syringin on a Silufol UV 254 plate, elution of the syringin from cut-out bands of the plate containing this substance (detection in UV light at a wavelength of 254 nm), and the spectrophotometry of the eluates at a wavelength of 266 nm. The UV spectrum of an aqueous solution of syringin has an intense absorption maximum at a wavelength of 266 nm (Fig. 1). In this region of the spectrum, the optical densities of solutions of syringin are directly proportional to the concentrations of the substance, which permits the use of spectrophotometry for the quantitative determination of syringin in the raw material after its separation from other components. We confirmed the individuality of the syringin zone by the HPLC of an eluate obtained after the chromatography of the extract.

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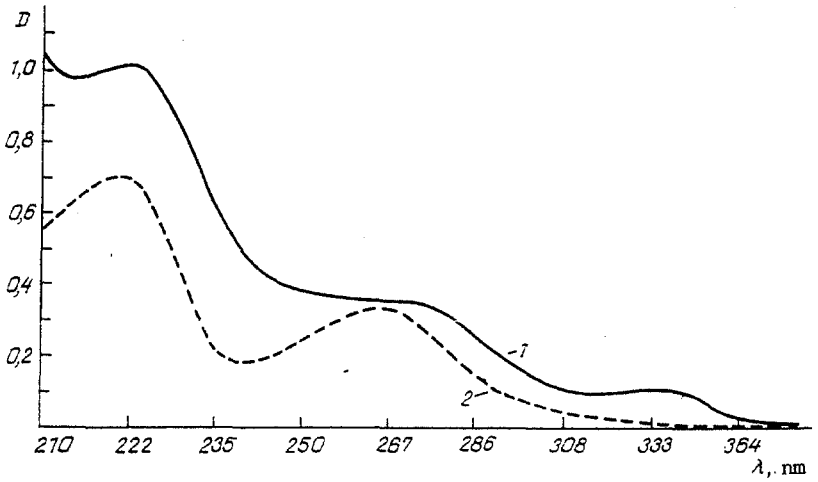

Fig. 1. UV spectra in 70% ethanol: 1) extract of lilac bark; 2) solution of syringin.

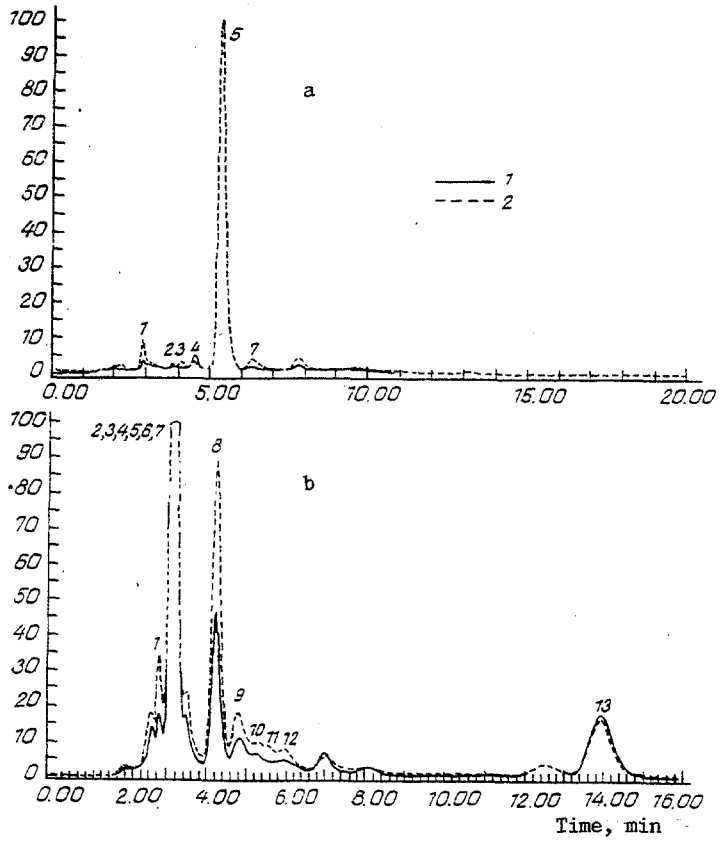

Fig. 2. HPLC of common lilac bark (the numbers of the peaks correspond to those given in Table 1): a) eluent mixture 1: 1) at 266 nm (0.28 O.D.); 2) at 280 nm (0.21 O.D.); b) eluent mixture 2: 1) at 266 nm (0.18 O.D.); 2) at 280 nm (0.14 O.D.).

The results of the investigation showed the possibility of performing the rapid analysis of lilac bark with the aid of direct spectrophotometry of 70% ethanol extracts of the raw material which likewise have an absorption maximum at a wavelength of 266 nm (see Fig. 1) with the subsequent recalculation of the syringin content using a coefficient of 0.7 enabling an overestimation of the results of analysis to be avoided.

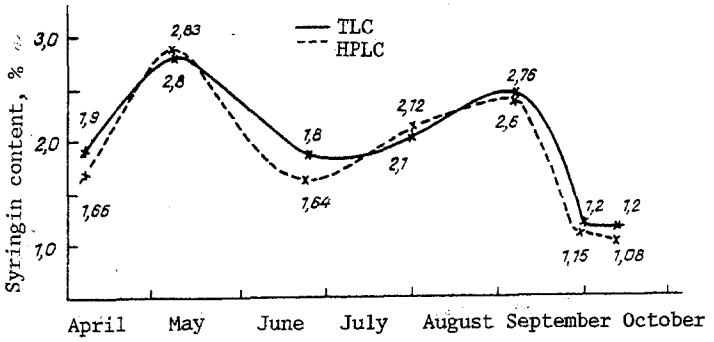

Fig. 3. Seasonal dynamics of the accumulation of syringin in lilac bark.

TABLE 1. Retention Times of Individual Substances

Name of the substance	Number of the peak on HPLC	Retention time, min	
		eluent mixture 1	eluent mixture 2
Hydroxysalidroside	1	2,9	2,8
Hydroxytyrosol	2	3,8	3,1
Salidroside	3	4,1	3,1
Coniferin	4	4,5	3,1
Syringin	5	5,4	3,1
Tyrosol	6	6,0	3,1
Olivil 4-glucoside	7	6,3	3,1
Forsythiaside	8		4,2
Lariciresinol 4-glucoside	9		4,8
Acteoside	10		5,3
Noroleuropein	11		5,6
Nuzhenide	12		6,0
Oleuropein	13		14,0
Ligustroside	14		26,5

The component composition of common lilac bark was determined by the HPLC method using mixtures 1 and 2 (Fig. 2). The substances were detected at two wavelengths (266 and 280 nm). On the basis of the retention times of the individual substances and of model mixtures, 14 components were identified in extract (Table 1).

On elution with mixture 1 (12% ethanol), the peaks of compounds (I-VII) were identified on the chromatogram (Fig. 2a), and among these syringin considerably predominated. Elution with the more polar mixture 2 (25% ethanol) permitted the identification of the remaining components (peaks 8-14), among which forsythiaside (peak 8) and oleuropein (peak 13) predominated (Fig. 2b).

A comparative investigation of the component compositions of the bark of common lilac and the Hungarian lilac Syringa josikeae Jacq. fil showed a closeness of their chemical composition, but, at the same time, certain differences were detected. Thus, the bark of Hungarian lilac contained only half as much syringin (1.4%) and the dominating component was oleuropein (peak 13). This plant was also found to contain a high level of noroleuropein (peak 11) but a considerably smaller amount of forsythiaside (peak 8).

The results of investigations by the HPLC method of the leaves of common lilac are also interesting, their dominating components being oleuropein and other iridoids, syringin being present in only small amounts (0.1%).

For the quantitative determination of syringin extracts, as the eluent we used mixture 1, with the aid of which the optimum time of chromatography and the best separation of syringin from accompanying substances are achieved (Fig. 2a). Detection was effected with a UV detector at a wavelength of 266 nm. The peak intensity–concentration and peak area–concentration relationships were linear within the range of concentrations of syringin of 6-400 μg/ml. The concentrations of syringin determined in the extract from the peak intensities of the peak areas coincided almost completely.

The reproducibility was checked by three determinations for each concentration (δ = ±3%).

The amounts of syringin in various samples of common lilac bark gathered during the vegetation period were determined by HPLC analysis (Fig. 3). In parallel, these samples of raw material were analyzed by the chromato-spectrophotometric method [9]. As can be seen from Fig. 3, the results of the quantitative determination of syringin in extracts of common lilac by the two methods practically coincided. During the vegetation period two maxima of the accumulation of syringin in lilac bark were found: in May (2.8%) and September (2.6%).

EXPERIMENTAL

The plant raw material of common lilac and Hungarian lilac was collected in Moscow province during the vegetation period (from April to October in 1988, 1989, and 1990).

HPLC analysis of the extracts, of the individual substances, and of model mixtures of them was carried out with the aid of a Gilson chromatograph (France) on a column (0.39 × 30 cm) filled with the reversed-phase sorbent Silasorb C_{18} (10 μm, Chemapol, Czechoslovakia). The recording of the substances being eluted was carried out from their absorption at two wavelengths (266 and 280 nm) simultaneously. The eluents used were: mixture 1 – ethanol–0.2% CH_3COOH (12:88 by volume) (rate of elution 1.4 ml/min); and mixture 2 – ethanol–0.2% CH_3COOH (25:75 by volume) (rate of elution 1.0 ml/min).

Preparation of the Extract. A weighed sample of the air-dry raw material was extracted with 70% ethanol in a ratio of 1:30 under the conditions described in [9]. The extract was diluted with water in a ratio of 1:5, and 20 μl was injected into the HPLC chromatograph.

Where the chromato-spectrophotometric method was used, the extract, without dilution, was deposited on a Silufol UV 254 plate and was chromatographed under the conditions described in [9] in the presence of a standard sample of syringin. After the syringin zones had been cut out and eluted, the amounts of this substance in the eluates were determined by spectrophotometry.

To confirm the individuality of the syringin zone, the eluate was evaporated to dryness, the residue was dissolved in 0.1 ml of eluent, and 20 μl of this solution was injected into the HPLC chromatograph.

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