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INVESTIGATION OF NATURAL COMPOUNDS BY THE HPLC METHOD

II. HPLC FINGERPRINT METHOD FOR THE EPIGEAL ORGANS OF *Ferula kuhistanica* AND *F. tenuisecta*

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A microcolumn HPLC fingerprint method has been developed for the epigeal organs of *Ferula kuhistanica* and *F. tenuisecta*. The possibility has been shown of control by the microcolumn HPLC method of the qualitative and quantitative yields of ferutinin in the initial plant raw material and in preparations extracted from it, and also at each stage of the isolation of the desired product. The method is universal and does not depend on species and chemical composition of the plant.

Ferula tenuisecta Korov. and *F. kuhistanica* Korov. (fam. *Apiaceae* contain esters of terpene alcohols [1]. Extracts from these plants have not hitherto been studied in the quantitative respect for their contents of various components. Furthermore, great interest is presented by changes in the component spectra of substances isolated from these plants resulting from a number of factors.

The samples of extracts studied consisted of the total substances from the epigeal parts of *F. kuhistanica* of the 1979 and 1989 harvests. Chromatograms of them are given in Fig. 1a and b, respectively. HPLC analyses of the corresponding samples partially purified by adsorption chromatography on packed columns of silica gel are shown by dashed lines. An extract from the epigeal part of *F. tenuisecta* freed from chlorophyll and ballast substances was also studied (Fig. 1c).

The aim of the work performed was to determine the amounts of ferutinin and the number of substances accompanying it in extracts and, on this basis, to optimize the process of isolating ferutinin from the epigeal parts of *F. kuhistanica* and *F. tenuisecta*. The amount of the main component was determined by the method of area standardization.

The HPLC method of separation that we have developed permits the most complete determination of the chemical composition of a biological material, which is a characteristic feature of the fingerprint method. In plant material that had been stored for more than 10 years (1979 harvest) we detected about 25 components, and in a plant of the 1989 harvest about 15 substances. But in both cases ferutinin was preserved in large amounts. In all the specimens, ferutinin was the dominating component. The smaller number of components in *F. kuhistanica* of the 1989 harvest is probably connected with a difference in the vegetation periods of the plants.

The fingerprints of the epigeal parts of the species *F. tenuisecta* are characterized by nine components (Fig. 1c), and ferutin and teferin were identified among them, in addition to the main substance ferutinin.

The investigations performed showed that the HPLC fingerprint method permits the qualitative and quantitative control of the whole technological process for the isolation of ferutinin. This method, realized on a Milikhrom, is being supplemented by a new dimension – the simultaneous use of the UV spectrometry of the components being chromatographically separated. The method is universal even for other plant species and other classes of compounds.

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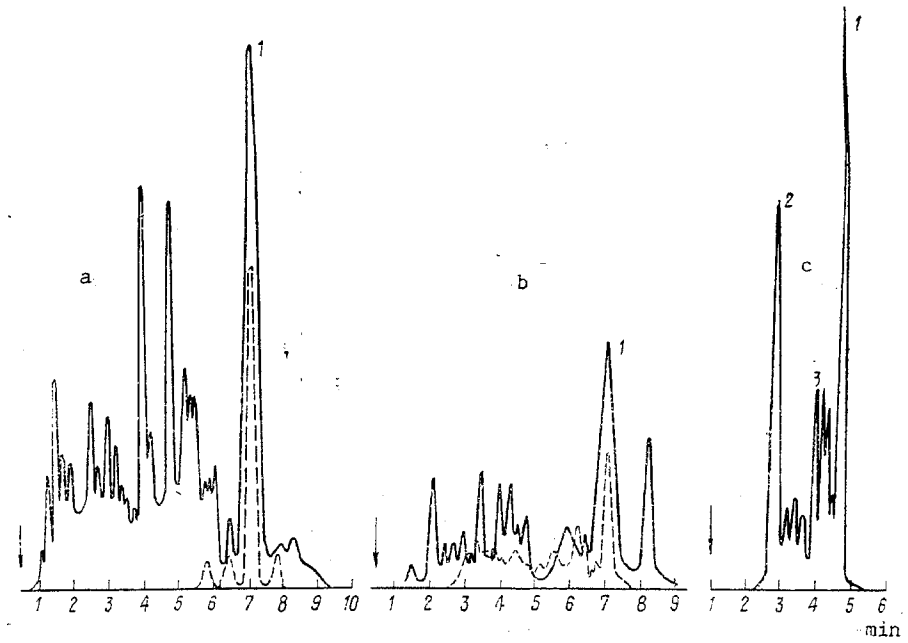

Fig. 1. HPLC of extracts from the epigeal parts of Ferula kuhistanica, (a, 1979 harvest, and b, 1989 harvest) and F. tenuisecta (c). 1) Ferutinin; 2) teferin; 3) ferutin.

EXPERIMENTAL

The preparation of the extracts from the plants and also the isolation of ferutinin, ferutin, and teferin were performed by methdos described previously [1].

Chromatography was conducted on a Milikhrom microcolumn liquid chromatograph (Nauchpribor Scientific Production Combine, Orel). A steel microcolumn with dimensions of 2 × 62 mm containing the reversed-phase sorbent Silasorb C18 was used. UV detection was carried out at 260 nm. The same mobile phase was used as in [2].

To identify the peaks on the chromatograms we used the individual substances ferutinin, ferutin, and teferin, and also a model mixture of them. Where necessary in an experiment the substances to be identified and the model mixture of them were added to the extracts.

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