INVESTIGATION OF NATURAL COMPOUNDS BY THE HPLC METHOD

I. MICROCOLUMN HPLC SEPARATION OF ESTERS OF CAROTANE ALCOHOLS FROM THE ROOTS OF Ferula tenuisecta

M. R. Nuriddinova, A. I. Saidkhodzhaev, and V. M. Malikov

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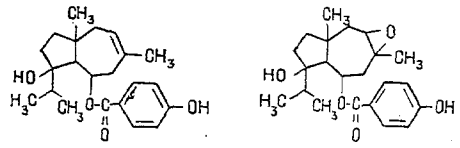
Reversed-phase and normal-phase variants of microcolumn high-performance liquid chromatograph (HPLC) for the separation of the preparation Teféstrol, isolated from the roots of Ferula tenuisecta have been developed. Minor components have been isolated. The amounts of the major and minor components in Tefestrol have been determined.

The preparation Tefestrol, which has been approved for use in medical practice and is based on esters isolated from the roots of Ferula tenuisecta Korov (fam. Apiaceae), consists mainly of two components – esters of the carotane alcohols ferutinol and tenuferol with p-hydroxybenzoic acid [1].

In order to study the component composition of the preparation, as before, we have used the method of HPLC analysis.



I

II

Chromatography was conducted on a Milikhrom microcolumn liquid chromatograph. Ferutinin (I) and tenuferidin (II) have their UV absorption maxima at 261 nm (log ε 4.16) and 263 nm (log ε 4.15), respectively, and therefore UV detection was conducted at 260 nm. The UV spectra of substances were taken on the same instrument by the method of halting the elution peak. The UV spectra of ferutinin and tenuferidin, which were identified by the method of additives, agreed completely with the UV spectra of the individual substances.

Quantitative analyses were carried out by the method of external calibration.

The reversed-phase variant of HPLC showed the presence of eight substances in different quantitative proportions in different batches of the substance. More than 96% of it consisted of ferutinin and tenuferidin, in ratios of 70:30-85:15.

Figure 1 gives a chromatogram of the reversed-phase HPLC separation of Tefestrol. The main component of Tefestrol – ferutinin, an ester of the carotane series – undergoes oxidation, and even during its isolation the amount of oxidized product in the preparation increases. The normal-phase variant of HPLC separation of the compounds under study proved to be more convenient. Figure 2 gives a chromatogram of the normal-phase HPLC analysis of the samples of Tefestrol that were studied, which had been isolated by various methods. In the normal-phase variant of HPLC, up to eight components were determined.

The results of calculation showed that it is possible to achieve a considerable purification of Tefestrol (sample E): (See following page, below figures.) The minor components, which have been isolated only by the method of HPLC analysis are being studied by chromato-mass spectrometric methods: they are substances of the same carotane series.

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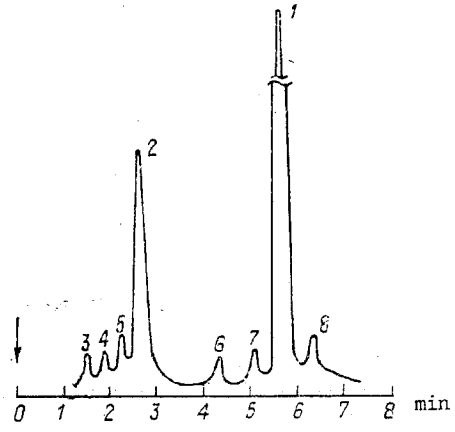

Fig. 1

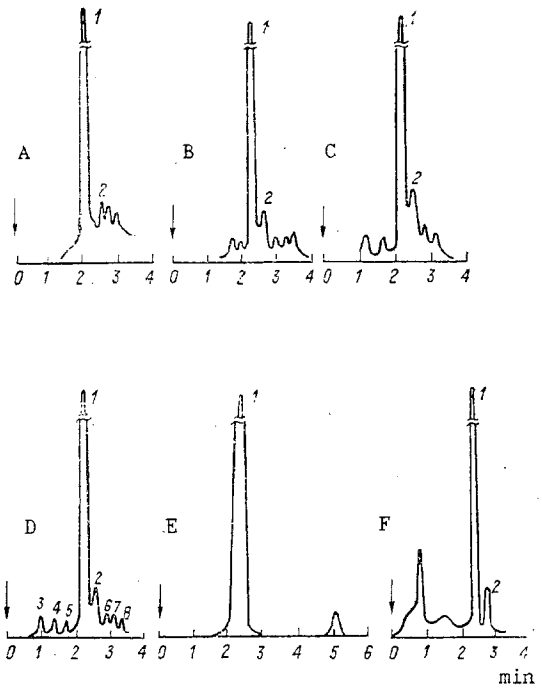

Fig. 2

Fig. 1. Reversed-phase HPLC analysis of Tefestrol: 1) ferutinin; 2) tenuferidin; 3-8) minor components.

Fig. 2. Normal-phase HPLC analysis of samples of Tefestrol: 1) ferutinin; 2) tenuferidin; 3-8) minor components.

Sample of the preparation	Ferutinin, %	Tenuferidin, %	Total amount of minor components, %
A	82,90	7,04	10,06
B	79,50	3,21	17,29
C	78,41	2,56	19,04
D	51,91	10,89	37,20
E	94,73	—	5,27
F	72,03	3,25	24,73

Thus, the proposed methods of reversed-phase and normal-phase microcolumn HPLC analysis permit the rapid quantitative determination of the composition of the preparation Tefestrol from the roots of F. tenuisecta, and control of the technological process of obtaining the substance is being carried out by these methods.

EXPERIMENTAL

Samples of the preparation Tefestrol were isolated by a method described previously with some modifications [3].

Chromatography was conducted on a Milikhrom microcolumn liquid chromatograph of the Nauchpribor Scientific–Production Association, Orel. A steel microcolumn with dimensions of 2 × 62 mm containing the sorbent Silasorb C18 was used for reversed-phase chromatography and a microcolumn of the same dimensions with the sorbent Silasorb 600 for normal-phase chromatography. UV detection was carried out at 260 nm. For one analysis a $2 \cdot 10^{-6}$-g sample of Tefestrol was used. The rate of elution was 100 μl/min. For normal-phase chromatogrpahy our mobile phase was a mixture of hexane, chloroform, methanol, and acetic acid. For reversed-phase chromatography we used the same mobile phase as in [2].

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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*

M. R. Nuriddinova, V. M. Malikov, A. Yu. Kushmuradov, and A. I. Saidkhodzhaev

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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.

Institute of the Chemistry of Plant Substances, Uzbekistan Academy of Sciences, Tashkent.
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