DETERMINING TOCOPHEROLS BY THIN-LAYER CHROMATOGRAPHY

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A method for the identification and quantitative determination of α-tocopherol by means of thin-layer chromatography (TLC) is developed. The proposed technique has been tested on vegetable oils and oil extracts. Using this method the contribution of plant raw materials to the total content of tocopherols in oil extracts has been estimated.

The Russian pharmaceutical market is currently flooded with oils and oil extracts that differ in chemical composition, properties, and preparation methods. The State Pharmacopoeia (SP), XIth Ed., does not have a general article for vegetable oils. These preparations are standardized according to a general pharmacopoeic article (Art. 472) from the SP, Xth Ed. This edition included only one medicinal oil, henbane oil (Oleum Hyoscyami) [1]. The following parameters are determined in assessing the quality of vegetable oils: color, odor, taste, solubility, density, index of refraction, acidity, and iodine and peroxide numbers. The purity of the oils is monitored by the inclusion of impurities of paraffin, wax, mineral oils, and resin acids and the presence of peroxides, aldehydes, and soap. However, the content of biologically active compounds (BAC) that are responsible for the pharmacotherapeutic effectiveness of the oil and oil extracts is not addressed in the Russian regulations [2].

Practically all vegetable oils and oil extracts contain tocopherols, phospholipids, carotinoids, and other BAC. Considering the useful physiological and biological properties of tocopherols, the isolation and analysis of this groups of compounds is very critical. The solution of this problem is very difficult because of the complicated structure and properties of tocopherols and their low content in the studied samples. Furthermore, tocopherols are very reactive and unstable. During the isolation they can undergo various changes. Therefore, isolation and determination methods must be selected according to the ability to ensure conditions that avoid changing the native properties of the tocopherols.

Our goal was to develop a TLC method for determining tocopherols in vegetable oils and oil extracts.

Titration methods are based mainly on the ability of tocopherols to be easily oxidized to form quinoid structures. The SP, Xth Ed., recommends for quantitative determination of tocopherols the use of cerimetry in the presence of diphenylamine indicator [1]. Titration methods typically are highly accurate but nonselective and are applied mainly to analyze drug ingredients and are unstuiable to analyze finished drugs that contain compounds with similar properties (tocopherol isomers).

Literature from the last five years showed that physicochemical methods are preferred for quality control of tocopherols because they are the fastest, most sensitive, and have the greatest information value. Electrochemical methods are currently used sparingly in pharmaceutical analysis, apparently because of the scarcity of the equipment and the toxicity of the mercury used in polargraphs [3]. Optical methods can identify and determine quantitatively vitamins in biological samples, as a rule, after their preliminary separation from other components. Polarimetry gives important information for the analysis of optically active ingredients (α-tocopherol) but is unsuitable for analyzing total isomers of optically active compounds without their preliminary separation [3].

Chromatographic methods, of which the most used are TLC and HPLC [4], are more objective for qualitative and quantitative analysis of tocopherols. GC was used successfully to determine vitamin E and is often recommended by researchers for this purpose [3, 5, 6]. This is certainly a fast and accurate analytical method but lengthy sample preparation including preliminary conversion of vitamin E into the methyl ester is required because the tocopherols are not determined in the native state. This increases the uncertainty of the analysis. HPLC has recently been recognized more

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1.5

Fig. 1. Chromatogram of standard α-tocopherol solution °C, % = 1.5) using eluent system No. 4.

widely in pharmaceutical analysis for the quantitative determination of vitamin E [5, 7]. However, the high cost of the equipment and the shortage of qualified operators limits significantly the practical use of HPLC [8].

TLC is traditionally used in pharmaceutical analysis for assessing the authenticity and purity of ingredients and drugs. High-performance thin-layer chromatography (HPTLC) has recently been becoming more significant for quality control of tocopherols. The advantages of this method include the availability, sensitivity, rapidity, and low cost.

The optimum conditions for separating complicated biochemical samples, which includes tocopherols, by TLC are selected experimentally taking into account the effect of several factors such as the solution concentration, the ratio of solvents in the eluent, the type of TLC plates, etc. The solvent has the greatest effect on the separation of compounds by TLC. Therefore, we studied the selection of eluent for determining tocopherols. The polarity was calculated and the relative rate of displacement of α-tocopherol (R_f value) was determined for each of the eluents. Table 1 lists the results. The separation of tocopherols and the quality of the chromatographic bands were best using the single-component eluent No. 4. For this system, the relative rate of displacement of the compound was optimal and the spots were round. This is consistent with a linear sorption isotherm.

TABLE 1. Properties of Certain Eluent Systems for TLC Separation of Tocopherols

System	Eluent	Solvent ratio	Polarity	R_f
1.	$CHCl_3$:C_2H_5OH	3:1	4.1	0.97 ± 0.01
2.	$CHCl_3$:C_2H_5OH	2:1	4.2	0.95 ± 0.01
3.	$CHCl_3$:C_2H_5OH	1:1	4.2	0.94 ± 0.01
4.	$CHCl_3$	–	4.1	0.59 ± 0.02

Furthermore, $CHCl_3$ is a cheap eluent that is readily available and relative nontoxic (class III toxicity). Figure 1 shows the chromatogram obtained using system No. 4.

Method. Eluents were prepared by mixing components in the specified ratios (Table 1) immediately before use. Attention must be paid to accurate measurement of the components during the preparation of mixed eluents because even small composition changes can degrade the separation. The angle at which the plate is placed in the chamber affects the shape of the spots and the rate of separation. If the plate is inclined with the silica gel layer on top, then the rate of development increases. However, the spots become more diffuse. If the plate is inclined with the sorbent layer underneath, the rate of separation also increases but the spots in this instance are clearer. The experiments established that the optimum angle of the plate was from 60° to 45°.

The chromatographic plate must be treated with a developer to assess visually the quality of the separation after the elution. The developer is a compound that reacts with the components of the separated mixture to form a colored compound. The following developers have been proposed in the literature: alcoholic PMA (5%), potassium hexacyanoferrate(III), Emmer-Engel reagent [9], etc. It is well known that PMA is a nonspecific developer and detects many other components and mixtures besides tocopherols as dark blue spots on a yellowish-green background. We also found that potassium hexacyanoferrate(III) solution was not sensitive enough and cannot be used for these purposes. We first chose concentrated nitric acid as the developer because it forms with α-tocopherol the reddish-orange compound *o*-tocopherylquinone. This reaction has been listed in the SP [1]. This developer is highly sensitive, specific, cheap, and available. Before treatment, the plates are removed from the chromatography chamber and dried at room temperature to remove solvent. After drying, the plate is fixed horizontally and treated dropwise with developer. Then, the plate is placed in a thermostatted oven for 5 – 7 min. Chromatographic bands are colored reddish-orange on a white background. The detection limit using this developer was 3×10^{-6} g.

Thus, the results were used to select the optimal chromatographic conditions as Sorbil (type A, 10 × 10 cm) silica gel plates on a polymer substrate; eluent, $CHCl_3$; developer, conc. nitric acid; sample volume, 10 μL; saturation time of the chromatographic chamber with eluent vapor, 20 min; elution time, 25 min; and time of storage of the plate in the thermostatted oven at 80°C, 5 – 7 min.

Figure 2 shows chromatograms from a series of standard α-tocopherol solutions (α-tocopherol of 95% purity from ICN Biomedical was used as the standard) that were obtained under the aforementioned conditions. α-Tocopherol has the highest biological activity. Therefore, it was selected

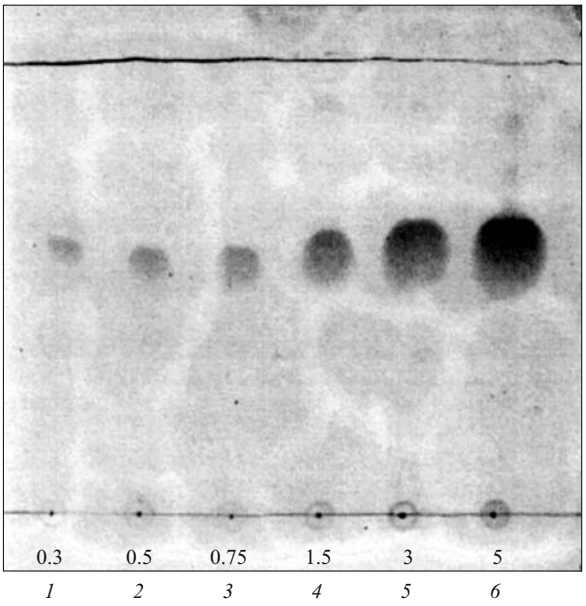

Fig. 2. Chromatogram with a series of standard α-tocopherol solutions °C, % = 0.3 – 5.0): 0.3% (*1*), 0.5 (*2*), 0.75 (*3*), 1.5 (*4*), 3.0 (*5*), 5.0 (*6*).

as the standard for the vitamin E group [9]. Plates were scanned immediately after developing the chromatographic bands using an Epson Perfection 2480 Photo (China) planchette scanner. The resulting images (Fig. 2) were processed using the Sorbfil Videodensitometer (RF) computer program.

The operating principle of the program consists of constructing an analog curve of the chromatogram according to the deviation of the spot brightness from that of the background and subsequent identification of peaks on this curve and calculation of their area. This produced tracks in coordinates of R_f vs. intensity. A maximum on the curve corresponded to the center of a band; the area under the curve, the spot area; the peak height, the intensity of the band color [10]. The R_f values of the compound and the areas of the

TABLE 2. Content of α-Tocopherol in Oil Extracts and Medicinal Plant Raw Material

Medicinal plant raw matl.	Content of α-tocopherol, %	
	in oil extracts	in medicinal plant raw matl
Grape seeds	0.383	–
Castor beans	–	–
Calendula flowers	< 0.03	< 0.03
Nettle leaves	< 0.03	–
Seabuckthorn berries	0.086	–
Chamomile flowers	0.223	< 0.03
Yarrow herb	0.047	–
Marigold herb	0.255	0.165
Rose hips	0.450	0.21

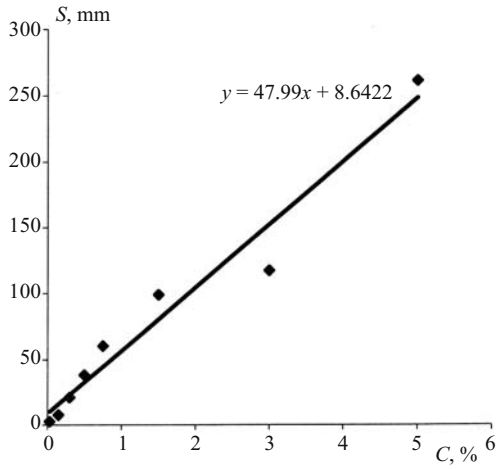

Fig. 3. Calibration curve for determining α-tocopherol content °C, % = 0.03 – 5.0).

chromatographic bands were calculated. It should be noted that calculation of R_f values using a special program gave more accurate results because in this instance the spot center was considered the area with the maximum coloration. Such determination of the center for irregular diffuse bands was more accurate than a visual assessment because the true band center did not coincide with the geometric one. Furthermore, the visible size of the spot is almost half its actual area [10].

A linear dependence between α-tocopherol concentration (C, %) and the coloration of the chromatographic band (S, mm^2) in the studied concentration range (0.03 – 5.0%) was obtained (Fig. 3). The equation of the calibration curve was (Fig. 3):

$$S = 47.99C + 8.6422$$

The developed method was tested on oils from seabuckthorn (*Hippophae rhamnoides*), castor beans (*Ricinus communis*), and grape seeds (*Vitis vinifera*) in addition to oil extracts of chamomile (*Matricaria Chamomilla*), calendula (*Calendula officinalis*), nettle leaves (*Urtica dioica*), rose hips pulp (*Rosa cinnamonea*), marigold herb (*Bidens triportita*), and yarrow (*Achillia Millifolium*). TLC analyses were performed under the aforementioned conditions. The results were processed using the Sorbfil Videodensitometer program.

Considering that sunflower and olive oils, which themselves contain native tocopherols, are used most often as extractants to produce industrial oil extracts [11], it became necessary to assess the tocopherol content of the medicinal plant raw material used as the source to produce them. Therefore, alcohol extracts of the studied species of medicinal plant raw material were analyzed. Table 2 gives the results.

It was found that the α-tocopherol content in the studied samples was the total tocopherols of the oil and the medicinal plant raw material. Tocopherols of the oils themselves

contributed most to the total content. It should be noted that the medicinal plants "transfer" to the oil extracts highly active tocopherol forms, tocotrienols [9, 12].

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