MEDICINAL PLANTS

DETERMINING THYMOL AND CARVACROL BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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A reversed-phase high-performance liquid chromatography (RP-HPLC) technique for the qualitative and quantitative determination of carvacrol and thymol in plants and related medicinal preparations has been developed. A chromatographic system consisting of a reversed-phase Diasorb 130-C18T column eluted with a MeOH:H_2O:THF (50:50:22, v/v) mixture separates effectively the carvacrol and thymol isomers and ensures their quantitative determination.

Key words: thymol and carvacrol, high-performance liquid chromatography, qualitative and quantitative determination.

Two species of the genus *Thymus* (Lamiaceae), *T. serpyllum* (wild thyme) and *T. vulgaris* (common thyme), are recognized and widely used in Russian official medicine [1]. The liquid extract of thyme is included in the preparation Pertussin, which is used as a cholegogic agent for bronchitis and other diseases of the upper respiratory tract [2]. The antimicrobial [3], antinematode [4], and antioxidant activity [5] of plants from the genus *Thymus* is due to the thymol and carvacrol content in them [4 – 6]. It is known that *T. vulgaris* of the phenolic chemotype, where thymol and carvacrol dominate, has high antioxidant activity compared with the nonphenolic chemotype, where linalool dominates [7]. The habitat, moisture, temperature variation, and elevation above sea level can affect the content of essential oil components in *Thymus* species [8 – 10]. Thus, the quality of wild and common thyme herb is determined not only by the total content of essential oil [11] but also its components, i.e., thymol and carvacrol (Fig. 1).

Our goal was to determine quantitatively the content of thymol and carvacrol in medicinal raw material using an HPLC technique.

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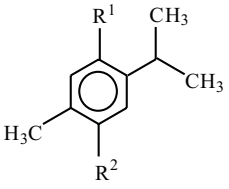
EXPERIMENTAL PART

The studies were carried out on an HPLC analytical system consisting of an HPP 4001 pump (Czech Rep.), an LCD 2536 UV-Vis detector (λ = 277 nm) (Czech Rep.), a Knauer injector with a 20-μL loop (Germany), a Diasorb 130-C18T reversed-phase column (250 × 4.6 mm) packed with 7-μm particles (ZAO Biokhimmak, Russia). The flow rate was 1 mL/min. A Multikhrom system (ZAO Ampersend, Russia) was used to process and record chromatograms.

Doubly distilled water and MeOH and THF (chromatographic grade) were used to prepare the chromatographic systems. Prepared eluent was degassed under vacuum.



R^1 = OH, R^2 = H, thymol
R^1 = H, R^2 = OH, carvacrol

Fig. 1. Structural formulas of thymol and carvacrol.

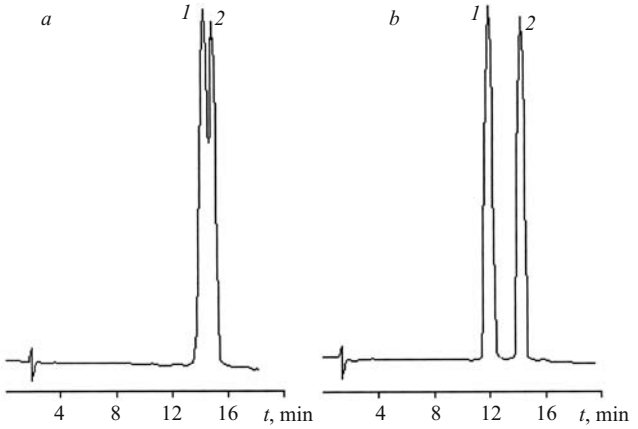


Fig. 2. Chromatograms of standard solutions of carvacrol (*1*) and thymol (*2*). Eluent MeOH:H_2O (62:38, *a*) and MeOH:H_2O:THF (50:50:22, *b*).

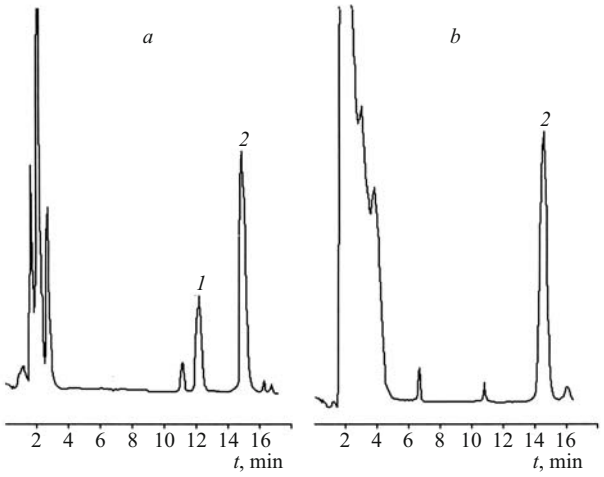


Fig. 3. Chromatograms of extracts of oregano (*a*) and wild rosemary runners (*b*). Eluent MeOH:H_2O:THF (50:50:22). Carvacrol (*1*) and thymol (*2*).

We used standard solutions of thymol and carvacrol (0.001 mg/mL) and plant raw material wild thyme (OOO Narodnaya Meditsina), wild rosemary runners (ZAO Zdorov(e), oregano (OOO Narodnaya Meditsina), and peppermint leaves (OOO Narodnaya Meditsina). A weighed portion of raw material (0.1 g) was extracted with alcohol (10 mL, 95%) for 1 h. The extract was filtered and diluted with MeOH.

RESULTS AND DISCUSSION

The quality of common thyme and wild thyme was determined by quantitative determination of the total content of essential oil obtained by steam distillation of the raw material with subsequent measurement of the volume of essential oil [11]. However, the biological activity of plants from the genus *Thymus* is determined not only by the total content of essential oil [11] but also the contents of thymol and carvacrol.

The study of the composition of essential oil by GC and GC–MS is complicated by the large amount of accompanying components in the essential oil and their similar retention times [8]. HPLC is a more popular analytical method. However, normal-phase HPLC using Silasorb SPH sorbent and CH_3CN:phosphate buffer eluent for analysis of plant extracts can determine only the total content of thymol and carvacrol because these isomers cannot be separated under those conditions [12]. It is known that reversed-phase HPLC using ODS, C18, and C8 as the stationary phase can be used in addition to normal-phase HPLC to determine methylphenols and phenols [13].

A reversed-phase column with Diasorb 130-C18T sorbent and MeOH:H_2O and MeOH:H_2O:THF eluents in various ratios were used to separate a model mixture of thymol and carvacrol. The chromatographic characteristics were determined using the formulas:

peak resolution $R_S = 2(t_{R2} - t_{R1})/(W_{b1} + W_{b2})$ and

selectivity $\alpha = (t_{R2} - t_{R0})/(t_{R1} - t_{R0})$.

A study of the chromatographic resolution in the binary system MeOH:H_2O showed that thymol and carvacrol were poorly resolved from each other. Decreasing the amount of MeOH in the eluent increased slightly the resolution of

TABLE 1. Chromatographic Separation Parameters for Thymol and Carvacrol

Eluent	t_{R_1}	t_{R_2}	R_S	α
MeOH:H_2O, 62:38	13.9	14.8	0.69	1.07
MeOH:H_2O, 58:42	20.5	21.6	0.83	1.06
MeOH:H_2O:THF, 58:42:22	15.5	18.0	1.24	1.18
MeOH:H_2O, 54:46	28.6	29.7	0.87	1.04
MeOH:H_2O, 50:50	34.5	35.8	0.90	1.04
MeOH:H_2O:THF, 50:50:6	26.2	28.4	1.29	1.09
MeOH:H_2O:THF, 50:50:15	21.1	23.4	1.41	1.12
MeOH:H_2O:THF, 50:50:18	15.5	18.0	2.23	1.18
MeOH:H_2O:THF, 50:50:22	12.0	14.8	3.11	1.28

Note: t_{R1} is the retention time of carvacrol (min); t_{R2}, of thymol (min).

TABLE 2. Thymol and Carvacrol Content in Plants

Plants and drugs	Content, mg/g	
	carvacrol	thymol
Thyme (OOO Narodnaya Meditsina)	0	0.080
Wild rosemary runners (ZAO Zdorov(e)	0	0.156
Oregano (OOO Narodnaya Meditsina)	0.035	0.132
Peppermint leaves (OOO Narodnaya Meditsina)	0.093	0.110

thymol and carvacrol but decreased the selectivity and increased the retention time (Table 1, Fig. 2).

Adding electron-donating additives such as THF decreased substantially the analysis time for compounds containing alcohol groups [14]. It was established that adding THF could decrease the retention time of thymol and carvacrol and increase their selectivity. The resolution R_S was 3.11; the selectivity, 1.28, for elution by MeOH:H_2O:THF (50:50:22). This indicated that thymol and carvacrol were effectively separated.

We used MeOH:H_2O:THF (50:50:22) to determine the contents of thymol and carvacrol in plants and drugs (Table 2, Fig. 3). Apparently the analyzed thyme was a nonphenolic chemotype. A high content of thymol was observed in wild thyme runners and a high content of thymol and carvacrol in oregano and peppermint leaves.

Thus, a reversed-phase HPLC technique for determining thymol and carvacrol in plants and drugs based on them was proposed. HPLC over a Diasorb 130-C18T column using MeOH:H_2O:THF (50:50:22) as mobile phase can separate isomers of carvacrol and thymol and be used for their quantitative analysis.

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