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:H₂O: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 cholegic 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.

R¹ = OH, R² = H, thymol
R¹ = H, R² = OH, carvacrol

Fig. 1. Structural formulas of thymol and carvacrol.
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$_3$CN-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$_2$O and MeOH:H$_2$O:THF eluents in various ratios were used to separate a model mixture of thymol and carvacrol. The chromatographic characteristics were determined using the formulas:

\[
R_S = \frac{2(t_{R_2} - t_{R_1})}{W_{R_1} + W_{R_2}}
\]

\[
\alpha = \frac{t_{R_2} - t_{R_0}}{t_{R_1} - t_{R_0}}
\]

A study of the chromatographic resolution in the binary system MeOH:H$_2$O 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>
<thead>
<tr>
<th>Eluent</th>
<th>$t_{R_1}$</th>
<th>$t_{R_2}$</th>
<th>$R_S$</th>
<th>$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH:H$_2$O, 62:38</td>
<td>13.9</td>
<td>14.8</td>
<td>0.69</td>
<td>1.07</td>
</tr>
<tr>
<td>MeOH:H$_2$O, 58:42</td>
<td>20.5</td>
<td>21.6</td>
<td>0.83</td>
<td>1.06</td>
</tr>
<tr>
<td>MeOH:H$_2$O:THF, 58:42:22</td>
<td>15.5</td>
<td>18.0</td>
<td>1.24</td>
<td>1.18</td>
</tr>
<tr>
<td>MeOH:H$_2$O, 54:46</td>
<td>28.6</td>
<td>29.7</td>
<td>0.87</td>
<td>1.04</td>
</tr>
<tr>
<td>MeOH:H$_2$O, 50:50</td>
<td>34.5</td>
<td>35.8</td>
<td>0.90</td>
<td>1.04</td>
</tr>
<tr>
<td>MeOH:H$_2$O:THF, 50:50:6</td>
<td>26.2</td>
<td>28.4</td>
<td>1.29</td>
<td>1.09</td>
</tr>
<tr>
<td>MeOH:H$_2$O:THF, 50:50:15</td>
<td>21.1</td>
<td>23.4</td>
<td>1.41</td>
<td>1.12</td>
</tr>
<tr>
<td>MeOH:H$_2$O:THF, 50:50:18</td>
<td>15.5</td>
<td>18.0</td>
<td>2.23</td>
<td>1.18</td>
</tr>
<tr>
<td>MeOH:H$_2$O:THF, 50:50:22</td>
<td>12.0</td>
<td>14.8</td>
<td>3.11</td>
<td>1.28</td>
</tr>
</tbody>
</table>

Note: $t_{R_1}$ is the retention time of carvacrol (min); $t_{R_2}$, of thymol (min).