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Baokun Tang, Minglei Tian & Kyung Ho Row

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DETERMINATION OF TERPENOIDS IN CHAMAECYPARIS OBTUSA LEAVES BY HEADSPACE SINGLE-DROP MICROEXTRACTION WITH GAS CHROMATOGRAPHY DETECTION

Baokun Tang, Minglei Tian, and Kyung Ho Row
Department of Chemistry and Chemical Engineering, Inha University, Incheon, Korea

Headspace single-drop microextraction (HS-SDME) was used to extract five terpenoids (α-terpinene, γ-terpinene, linalool, α-terpineol, and terpinyl acetate) from powdered Chamaecyparis obtusa leaves. The terpenoids were determined by gas chromatography with flame ionization detection. Under the optimized conditions, oleyl alcohol efficiently extracted the analytes from the leaves within 20 min at 100°C. The results showed that the amounts of α-terpinene, γ-terpinene, linalool, α-terpineol, and terpinyl acetate extracted from the samples were 2.19, 3.68, 0.11, 0.87, and 22.35 μg/g, respectively. Compared to microextraction of the compounds from the sample solution, the new technique had fewer steps. The method also had similar extraction properties as heat reflux extraction, but required a smaller sample volume and less time.

Keywords: Chamaecyparis obtusa; Gas chromatography; Headspace single-drop microextraction; Optimization; Terpenoids

INTRODUCTION

Chamaecyparis obtusa (Co) is a member of the family Cupressaceae that is cultivated in many parts of Asia including Korea, China, and Japan. The essential oils are used widely in many industries, such as environment, food, cosmetics, pesticides and pharmacology, because of the large amount of biologically active compounds in its essential oils (Hong et al. 2004). The biologically active compounds include terpenoids, which have antimicrobial (Lee, Back, and Han 2001), antioxidant (Marimuthu et al. 2008), antitumor, and antimalarial activities (Barrero et al. 2004; Okasaka et al. 2006). Therefore, a simple method for the extraction and analysis of the terpenoids in the essential oils of Chamaecyparis obtusa is important.
Among the many extraction methods, liquid phase microextraction (LPME) (S. Liu et al. 1995; H. Liu et al. 1996; He and Lee 1997; Jeannot and Cantwell 1997) has the advantages of a simple operation, speed, and low cost (Mahungo-Santana et al. 2001). Przyjazny, Austin, and Essenmacher (2000) first reported headspace single-drop microextraction (HS-SDME) mode, which has since attracted increasing attention as a solvent-minimized preconcentration technique (Psillakis and Kalogerakis 2002; Theis et al. 2001; Xu, Basheer, and Lee 2007). In HS-SDME, a solvent drop is suspended at the tip of a microsyringe needle, and exposed to the headspace of a sample matrix. The solvent drop adsorbs the target compounds volatilized from the sample matrix. Normally, the sample matrix is in a liquid phase (Jiang et al. 2008; Jiang et al. 2009; Ravelo-Perez et al. 2009; Moinfar and Milani Hosseini 2009; Hu et al. 2009), but in the present study, HS-SDME extracted targets compounds directly from the Co powders. After extraction, the suspended drop is retracted back into the microsyringe and injected into the gas chromatography (GC) apparatus for further analysis.

In this study, five terpenoids in Chamaecyparis obtusa leaves were extracted by HS-SDME and detected by GC with a flame ionization detector (GC-FID). The parameters relevant to the extraction efficiency of the terpenoids, such as an efficient alcohol extractant, time and temperature, were examined systematically. The optimized HS-SDME method was compared with a solution procedure and heat reflux extraction (HRE) for terpenoids from Co leaves.

EXPERIMENTAL

Chemicals and Materials

Methanol, ethanol, n-lauryl alcohol, and n-hexane (HPLC grade) were supplied by Duksan Pure Chemical Co., Ltd. (Ansan, Korea). N-butyl alcohol (Extra pure) was purchased from Oriental Chemical Industries (Incheon, Korea). Oleyl alcohol (GC grade) was obtained from Daejung Chemical and Metals Co., Ltd. (Siheung, Korea). N-octyl alcohol (GC grade) was purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan). α-Terpine, γ-terpinene, linalool, α-terpineol, and terpinyl acetate (GC grade) were acquired from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Double distilled water was filtered through a vacuum pump (Waters, Milford, MA, USA) and a filter (HA-0.45, Waters, Milford, MA, USA) prior to use.

The leaves of Chamaecyparis obtusa were collected from San Samtae-ri, Jeollanam-do, Korea. The plant samples were identified by the Nano Bio Research Center, Korea, and sealed at room temperature in the laboratory.

HS-SDME of Chamaecyparis obtusa Powders

A MS-300HS hot plate and magnetic stirrer (Misung Scientific Co., Ltd, Korea) was used to assist in the HS-SDME process. The maximum stirring rate of the apparatus was 1,500 rpm, and the maximum temperature was 380°C. A SDT-25 digital thermometer (Summit, Korea) was used to measure the temperature of the sample vial.
The fresh *Chamaecyparis obtusa* leaves were dried naturally in the laboratory and powdered in a popular shredder. Because of the lengthy time for natural drying, a fine powder was obtained easily. The leaf powder (0.5 g) was placed in a sample vial (20 mL), which was then sealed with a rubber plug. The sample vial was heated on a hot plate with different temperatures and stirred with a magnetic bar (0.5 cm). The temperature of the sample vial was measured with a digital thermometer. When the temperature of the sealing vial was increased to the required temperature, the needle of a GC syringe with 6 to 8 μL of the extractant was inserted into the sample vial through the sealing plug. After inserting the needle, 1 to 3 μL of the extractant in the syringe was pushed to the point of the needle forming a droplet. The droplet was approximately 1 cm above the surface of the leaf powder. When the extraction process was complete, the droplet at the needle point was drawn into the syringe which was removed from the sample vial. The extraction solution in the syringe was then injected into the GC for analysis.

**HS-SDME of *Chamaecyparis obtusa* Solutions**

HS-SDME of the *Chamaecyparis obtusa* solutions was performed according to the program reported by Moinfar and Milani Hosseini (2009). 0.5 g of the dried and powdered Co leaves was mixed with 5.0 mL of n-hexane in sealed flasks and the suspensions were extracted by heating (40°C, 60 min). The turbid solutions were placed in a sample vial (20 mL) and microextracted directly for 20 min with the extractant (oleyl alcohol) using the HS-SDME method.

**Heat Reflux Extraction**

The 20.0 g of the powdered *Chamaecyparis obtusa* leaves and 200 mL double distilled water were added to a 250-mL distillation flask. The distillation temperature was set to 100°C, and held at that temperature for 6 h. The volatile fraction in the plant was collected. When the process was complete, mixtures of the distillation oils were extracted in n-hexane and diluted to 100.0 mL with n-hexane. Subsequently, 2.0 μL of the n-hexane solutions was injected into the gas chromatograph and analyzed.

**GC Analysis**

GC was performed on a Yong Lin Instrument (Korea) GC-6100 with a DB-1701 capillary column (30 m × 0.320 mm × 1.00 μm) (Agilent Technologies) and detected with a FID detector. Ultra-high purity helium (purity 99.999%) was used as the carrier gas with a flow rate of 1.80 mL/min. The oven temperature was programmed as follows: initial temperature of 50°C increased to 100°C at 5°C/min, increased further to 250°C at 10°C/min, and held at that temperature for 5 min. The total time for a single GC run was 30 min. The injector temperature was 280°C, and the injection was performed in split mode at a rate 42:1.

To prepare the standard solutions, an accurate concentration of a α-terpinene, γ-terpinene, linalool, α-terpineol, and terpinyl acetate solution was obtained for analysis (0.0001, 0.0005, 0.001, 0.005, 0.01, and 0.1 mg mL⁻¹, respectively).
2.0 µL of each standard solution was injected and run to produce the calibration curves. Table 1 lists the parameters for each compound calibration curve.

### RESULTS AND DISCUSSION

#### Screening of the Alcohols

Few studies have reported the application of a series of alcohols in HS-SDME of the powder samples. This study optimized the experimental parameters, such as the extractant, temperature (27 to 120°C) and time (5 to 30 min).

Methanol, ethyl alcohol, n-butyl alcohol, n-octyl alcohol, n-lauryl alcohol, and oleyl alcohol were optimized as extractants. To select the appropriate extractant during these alcohols, only the extractant was changed in the experiments with the other parameters kept the same.

Figure 1 shows the effect of the different alcohols on the microextraction of the five targets in the powdered *Chamaecyparis obtusa* leaves for 5 min at 27°C. A small amount of linalool and α-terpineol was extracted from all solvents. Therefore, the amounts of the two targets were similar in these alcohols. On the other hand, the

<table>
<thead>
<tr>
<th>Analyte</th>
<th>R²</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
<th>LOD (ng/mL)</th>
<th>LOQ (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Terpinene</td>
<td>0.9981</td>
<td>5.2</td>
<td>90.1</td>
<td>1.845</td>
<td>7.142</td>
</tr>
<tr>
<td>γ-Terpinene</td>
<td>0.8874</td>
<td>4.3</td>
<td>88.4</td>
<td>1.577</td>
<td>6.548</td>
</tr>
<tr>
<td>Linalool</td>
<td>0.9968</td>
<td>3.8</td>
<td>81.3</td>
<td>1.975</td>
<td>6.876</td>
</tr>
<tr>
<td>α-terpineol</td>
<td>0.9932</td>
<td>5.4</td>
<td>90.2</td>
<td>2.942</td>
<td>9.946</td>
</tr>
<tr>
<td>Terpinyl acetate</td>
<td>0.9981</td>
<td>4.6</td>
<td>95.6</td>
<td>1.939</td>
<td>6.957</td>
</tr>
</tbody>
</table>

**Figure 1.** Effect of the extractants on the targets microextracted for 5 min at 27°C.
amounts of extracted $\alpha$-terpinene, $\gamma$-terpinene, and terpinyl acetate were different when the solvent was changed. The figure shows that the amount of the three targets increased with the number of carbon atoms in the solvent molecule. Figure 2 showed a similar trend when the experiment was performed at 60°C for an extraction time of 5 min. On the other hand, neither methanol nor ethyl alcohol was used because both volatilized rapidly at 60°C, meaning that they had insufficient time to extract the targets. All the trends revealed oleyl alcohol to be the best extractant among the alcohols tested. The volatility of oleyl alcohol was lower than the other alcohols, but its viscosity was higher. Therefore, it is advantageous in extracting the targets.

**Effect of Time**

According to the aforementioned results, oleyl alcohol was selected as the extractant in the following experiments. Time is also an important parameter. Therefore, the role of time in this HS-SDME method must be considered. Figure 3 shows the effects of time at 27°C in microextraction. Each amount of extracted $\alpha$-terpinene, $\gamma$-terpinene, linalool, and $\alpha$-terpineol in microextraction reached equilibrium within 10 min but there were many changes in the amount of terpinyl acetate extracted during this time. Microextraction is generally a short and simple process. Therefore, the amount of terpinyl acetate might reach equilibrium during the study time by changing the temperature.

Figure 4 shows the amounts of terpinyl acetate extracted for different times at 40, 60, 80, 100, and 120°C. The amounts of terpinyl acetate reached equilibrium rapidly when the temperature was increased; 20 min and 10 min at 80°C and 100°C, respectively. On the other hand, the amount of terpinyl acetate at 120°C during the

![Figure 2. Effect of the extractants on the targets microextracted for 5 min at 60°C.](image)
The experimental time was apparently lower than that at 80 or 100°C except for the measurement at 30 min, which was similar to that observed at 80°C. Overall, the amount of terpinyl acetate extracted for the same time increased with increasing temperature. The reason for the abnormal appearance was that the color of the powdered *Chamaecyparis obtusa* leaves changed rapidly to black with heating, indicating some deterioration of the targets compounds. The time to reach equilibrium for the five targets was 20 min, which is a relatively short extraction time for HS-SDME.

**Figure 3.** Effect of time on the targets microextracted by oleyl alcohol at 27°C.

**Figure 4.** Effect of time on terpinyl acetate microextracted by oleyl alcohol at different temperatures.
Effect of Temperature

The amounts of terpinyl acetate extracted increased when the temperature was increased from 27 to 120°C. Therefore, this study examined the other targets at a temperature at which the highest amount of terpinyl acetate was achieved. Accordingly, the amounts of each of the five targets extracted for 20 min at 20, 40, 60, 80, 100, and 120°C were measured, as shown in Figure 5. The highest amount of the targets extracted was obtained at 20 min and 100°C. Overall, the optimal conditions of the HS-SDME for the five targets in the leaves were oleyl alcohol as the solvent for 20 min at 100°C.

Analytical Performance

Under the optimized conditions, a series of samples were tested to check the developed method. The reproducibility and recovery was tested by carrying out five parallel experiments at 5.0 μg for each compound added to a blank vial. Figure 6 shows the chromatograms of the standard compounds and HS-SDME of the Co leaves. Based on the chromatographic signal-to-noise ratio (S/N = 3), the LODs of α-terpinene, γ-terpinene, linalool, α-terpineol, and terpinyl acetate were 1.845, 1.577, 1.975, 2.942, and 1.939 ng/mL, respectively. Based on a S/N = 10, the LOQs of α-terpinene, γ-terpinene, linalool, α-terpineol, and terpinyl acetate were 7.142, 6.548, 6.876, 9.946, and 6.957 ng/mL, respectively. The precision obtained was 3.9–5.2% (RSDs, n = 5) for standard solutions with three different levels of the three compounds (Table 1).

Comparison with HS-SDME of a Chamaecyparis obtusa Solution

Only one study of HS-SDME with tea (Moinfar and Milani Hosseini 2009) reported the transfer of organophosphorus pesticides from tea to n-hexane, followed

![Figure 5. Effect of temperature on the targets compounds microextracted by oleyl alcohol for 20 min.](image-url)
by analysis of the n-hexane solutions by HS-SDME. Therefore, this study compared HS-SDME with the previous method using powdered \textit{Chamaecyparis obtusa} leaves under the same conditions as that reference (extractant was oleyl alcohol, micro-extraction temperature was 40°C, microextraction time was 20 min) (Moinfar and Milani Hosseini 2009). The results are listed in Table 2. The amounts of the five targets from HS-SDME of the \textit{Co} leaves were apparently higher than those from the sample solutions because the latter involved more steps than the former. In HS-SDME of the sample solutions, the targets were first transferred from leaves to n-hexane, and then from the n-hexane solutions to oleyl alcohol as the extractant. On the other hand, with HS-SDME, the targets were transferred directly from the leaves to the extractant. In addition, the targets compounds were more difficult to volatilize from n-hexane solutions than from the powders under the same experiment conditions. Therefore, HS-SDME is a better method for extracting the five targets than in solution.

\begin{table}[h]
\centering
\caption{Amounts of the targets extracted from \textit{Chamaecyparis obtusa} leaves by HS-SDME with powders and solutions, and by heat reflux extraction} \label{tab:target}
\begin{tabular}{lccc}
\hline
Target & HS-SDME with powders \hspace{1cm} (n = 3) (µg/g) & HS-SDME with solutions \hspace{1cm} (n = 3) (µg/g) & HRE \hspace{1cm} (n = 3) (µg/g) \\
\hline
$\alpha$-Terpinene & 2.19 & 2.08 & 2.43 \\
$\gamma$-Terpinene & 3.68 & 2.97 & 3.16 \\
Linalool & 0.11 & 0.09 & 0.19 \\
$\alpha$-Terpineol & 0.87 & 0.64 & 0.89 \\
Terpinyl Acetate & 22.35 & 20.01 & 24.05 \\
\hline
\end{tabular}
\end{table}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Gas chromatograms of the targets in a standard sample solution (black curve) and HS-SDME sample (blue curve). (Figure available in color online.)}
\end{figure}
Comparison with Heat Reflux Extraction

Heat reflux extraction (HRE) is based on the transfer of analytes from a sample to a solvent, and is used widely to extract the targets. Therefore, HS-SDME was compared with HRE. Table 2 lists the amounts of the five targets extracted by HS-SDME and HRE. The results by HS-SDME were similar to those by HRE under similar conditions but HS-SDME required less time, less solvent, and was a simpler operation than HRE. In addition, after extraction of HS-SDME, the samples were analyzed directly by GC.

CONCLUSIONS

A new application of HS-SDME extracting targets from Chamaecyparis obtusa leaves is reported. The comparisons of HS-SDME with conventional methods demonstrated its effectiveness in extracting the five terpenoids from the leaves. The process was rapid and simple, with less consumption of organic solvent. The advantages of HS-SDME were obvious, but there were some limitations regarding the samples and targets. The targets of the samples in HS-SDME should be stable at the extraction temperature. Therefore, more study of HS-SDME is needed.

REFERENCES


