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SOLID-PHASE MICROEXTRACTION OF MENTHOL FROM PEPPERMINT

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SUMMARY

The present work presents for the first time the extraction of menthol with a new solid-phase microextraction method using as solid phase a helical solid sorbent [1,2] followed by introduction into a capillary column for a gas chromatographic analysis. The menthol was extracted from headspace sample and also a liquid solution sample and then was analyzed by gas chromatography. The quantitative evaluation was carried out with external standard method both for headspace and liquid solution.

Keywords: menthol; Mentha piperita; gas chromatography; solid-phase microextraction; helical solid sorbent; mint tea.

INTRODUCTION

Menthol is a monoterpenic alcohol found in a large amount (29-48%) [3] in the volatile oil extracted from the leaves of *Mentha piperita* and other species of mint. It is a crystalline, transparent substance with a strong fragrance and counterirritant and anesthetic properties, used in medicine, cosmetic and food industry. Menthol's ability to chemically trigger the cold-sensitive receptors in the skin is responsible for the well known cooling sensation that it provokes when inhaled, eaten, or applied to the skin. Menthol has eight possible stereoisomers due to the three asymmetrical carbon atoms in its molecule [4]. The main form of menthol occurring in nature is the (-)-menthol form which is assigned the (1R,

2S, 5R) configuration (Figure 1). Menthol is commonly determined by extraction followed by gas chromatography or liquid chromatography methods [5-7]. Liquid chromatography requires time and expensive or sometimes toxic solvents. The main advantages of gas chromatography (GC) are the reduced analysis time, the small amount of sample needed, the sensitivity and the precision of the method.

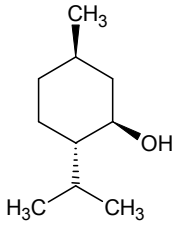

Figure 1. The menthol molecule

The isolation of organic compounds from a sample matrix by sorption on a solid sorbent and their desorption by the introduction of the sorbent into a GC injector for analysis implies the use of a sorbent material which is deposited on the surface of a small rod or tube [1]. The novelty of the method used in this study consists of a helical solid sorbent for solid phase dynamic extraction introduced by Ciucanu [2]. This reduces the extraction time necessary to reach equilibrium for the volatile compounds and improves the GC resolution. The thick polydimethylsiloxane coating of the sorbent improves the sensitivity of the method.

The aim of this study is the extraction of the menthol by solid-phase microextraction from mint using a helical solid sorbent. The paper is multidisciplinary because it treats both biological and chemical characteristics of menthol.

MATERIALS AND METHODS

Materials

(-)-Menthol (>99% purity) was obtained from Sigma-Aldrich. Mint tea is from Grande.

Apparatus

A helical solid sorbent made of polydimethylsiloxane was used for the microextraction.

The sample analysis was performed with a Carlos Erba Instruments HRGC 5300 gas chromatograph. The capillary column was an DB-XLB (30 m x 0.25 mm x 0.25 μm

film thickness) from J&W Scientific INC (USA) with hydrogen as carrier gas at 0.8 mL/min. The injector temperature was set at 220°C. Detection was performed with a flame ionization detector with the temperature set at 220°C. The flame uses a mixture of air and hydrogen, both at 25 mL/min. The oven temperature was initially held at 50^0C for 1 minute, then increased to 90^0C at 10^0/min, held for 5 minutes, followed by 40^0C/min to 220^0C, held for 2 minutes.

Solid-phase microextraction (SPME)

The helical solid sorbent can be a solid material with any geometrical shape arranged in helical line with sorption properties or coated physically or chemically with a sorbent material. The sorbent used in this research was handmade. Figure 2 shows the helical solid sorbent.

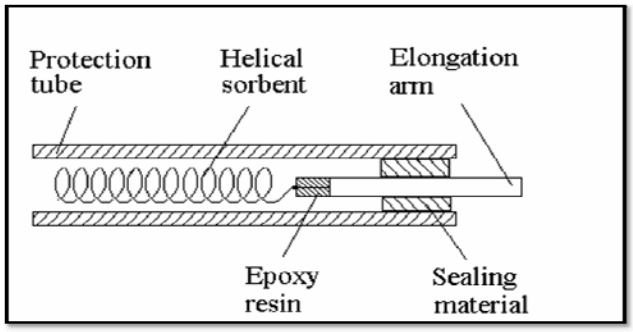

Figure 2. Transverse section of the helical solid sorbent

Samples

Qualitative analysis was performed for five samples: pure menthol crystals headspace (S1), peppermint leaves headspace (S2), boiled tea headspace (S3), room temperature tea headspace (S4) and liquid extraction from tea (S5). Menthol crystals were inserted into a vial and sealed with a septum. The peppermint leaves sample was prepared by inserting the leaves in a vial which was sealed. The boiled mint tea sample was obtained by introducing the content of a tea bag (2.5 g) into a vial, followed by the addition of hot water and immediate sealing of the vial. The sample was magnetically stirred at 800 rpm. The fourth sample was obtained after the previous one cooled down until room temperature and it was also magnetically stirred at 800 rpm. These four samples were submitted to headspace extraction for 15 minutes each. The last analysis consisted of a liquid extraction at room temperature from the same tea. This sample was also magnetically stirred at 800 rpm and the time of extraction was 15 minutes.

Quantitative analysis was made to establish the area-concentration calibration curve. Three menthol solutions were made and extraction was conducted both from

headspace and from solution for each sample. The first solution consisted of 12.38 mg of menthol weighed at an analytical scale and diluted with 50 mL of water. The calculated concentration of this sample was 247.6 μg/mL. The second sample was represented by 2.97 mg of menthol diluted in 50 mL of water therefore the obtained solution had a concentration of 59.4 μg/mL. The third sample was obtained by the dilution of the second sample with water at a ratio of 1:10 which resulted in a concentration of 5.4 μg/mL. All three samples were magnetically stirred at 800 rpm until the menthol has dissolved completely and they were extracted for 15 minutes at room temperature for each analysis.

RESULTS

Figure 3 represents the gas chromatogram obtained from the headspace analysis of menthol crystals. Menthol crystals headspace analysis was performed to determine menthol's retention time in these chromatographic conditions and was used as a standard for the following analysis.

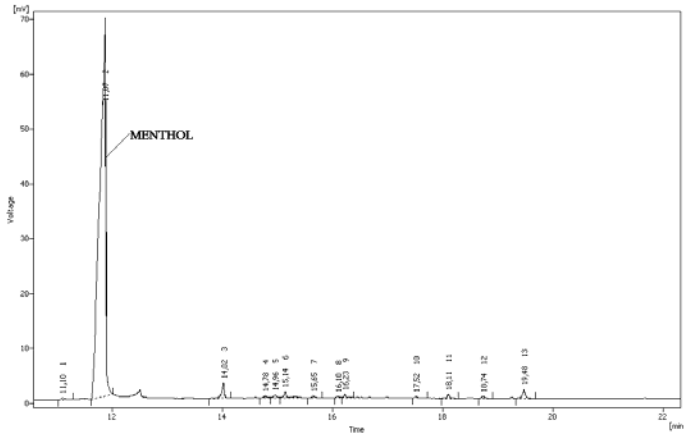

Figure 3. Gas chromatogram of the headspace analysis of menthol crystals. For the conditions, see the "Apparatus" section

Figures 4-7 represent the chromatograms obtained from the other samples taken in study. The boiled mint tea sample (Figure 5) was the only one influenced by temperature.

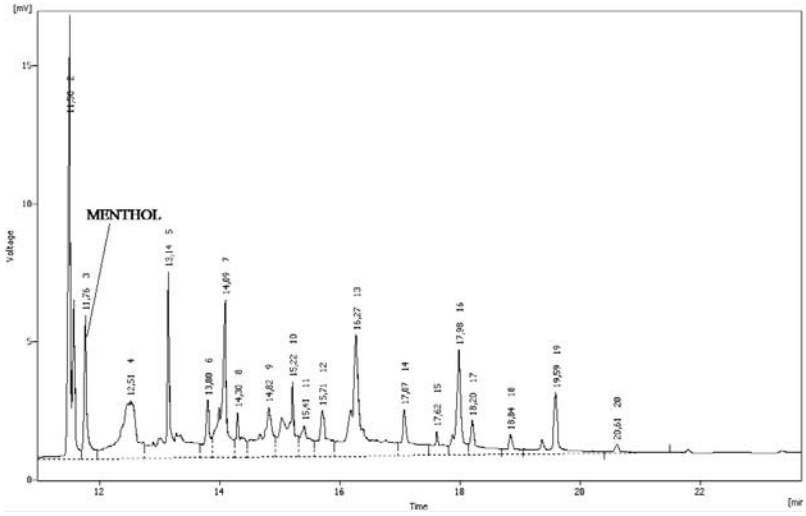

Figure 4. Gas chromatogram of the headspace analysis of peppermint leaves. For the conditions, see the “Apparatus” section

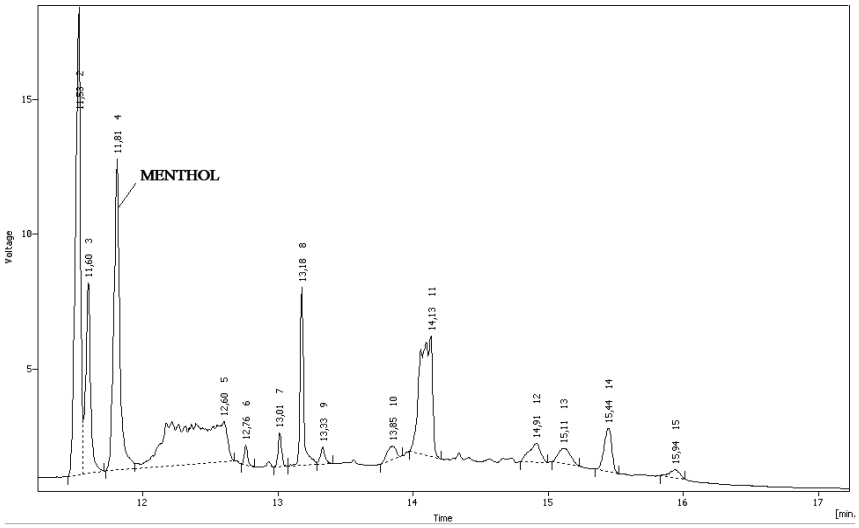

Figure 5. Gas chromatogram of the headspace analysis of boiled mint tea. For the conditions, see the “Apparatus” section

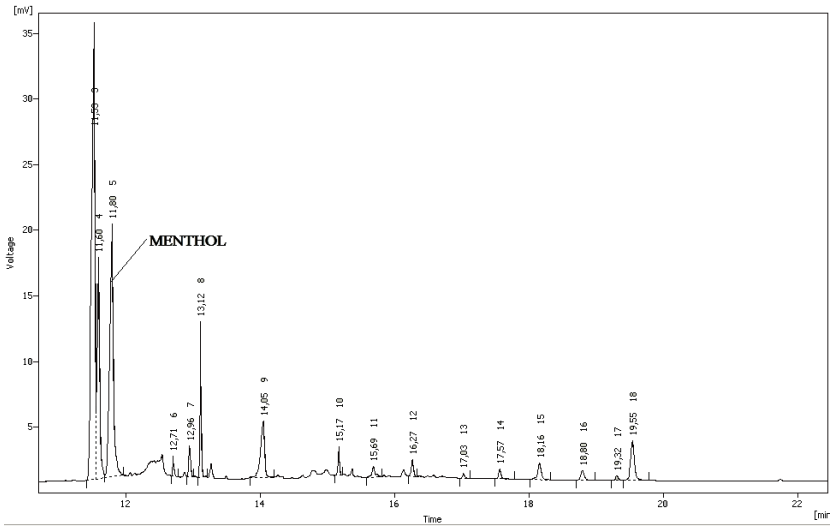

Figure 6. Gas chromatogram of the headspace analysis of room temperature mint tea. For the conditions, see the “Apparatus” section

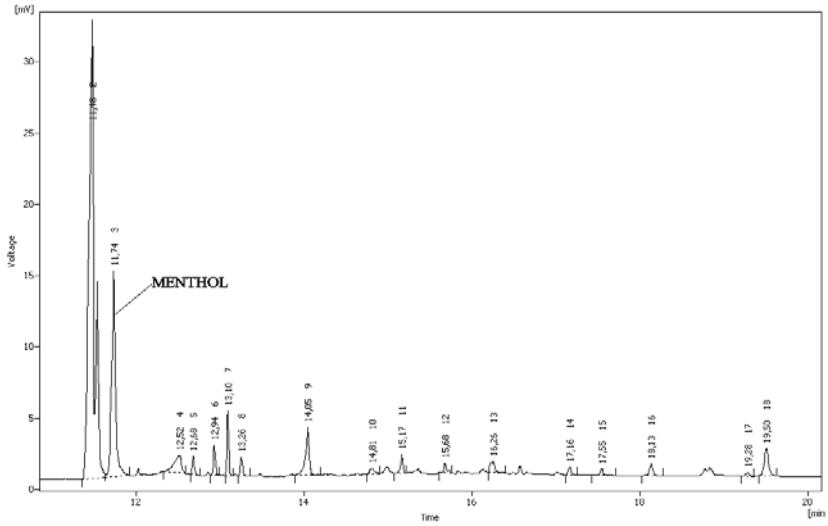

Figure 7. Gas chromatogram of the tea liquid extraction. For the conditions, see the “Apparatus” section

In Table I are contained all the data of the menthol peaks from the performed analysis.

Table I. Menthol peak dimensional characteristics

Nr.	Sample	Menthol peak			
		Area (mV·s)	Height (mV)	Area (%)	Height (%)
1	Menthol crystals headspace extraction	506.969	68.925	93.9	88.3
2	Peppermint leaves headspace extraction	15.245	5.033	6.6	9.1
3	Boiled tea headspace extraction	34.996	11.517	16.8	20.7
4	Room temperature tea headspace extraction	68.672	19.231	21.9	18.6
5	Tea extraction	44.553	14.379	18.4	20.9

The quantitative analysis showed that the technique had a precision of approximately 99% in both cases.

Due to the fact that the substance used was only 99% pure there were other peaks in the chromatograms beside the menthol peak. Therefore the concentration of the menthol peak is lower than the concentration of the menthol solution.

The menthol peak`s concentration was calculated by applying the percentage of the area to the total concentration of the solution. This step was necessary to create the calibration curve.

Table II synthesizes the values of the menthol peak areas obtained in the quantitative analysis from headspace and from solution and the calculated concentrations of the menthol peaks in each case.

The quantitative analysis shows that the dimensions of the peaks are proportional with the concentration of the analyzed solutions. The precision of the method indicates that it was correctly executed and it is suited for determining menthol from peppermint.

Figures 8-9 present the calibration curves resulted from the headspace and solution microextractions.

Table II. Values of the menthol peaks areas as a function of concentration

Nr.	Concentration (mg/mL)	Area of the menthol peak (mV·s)	
		Headspace analysis	Solution analysis
1	247.6	602.914	1386.987
2	59.4	190.278	432.071
3	5.4	22.829	50.969

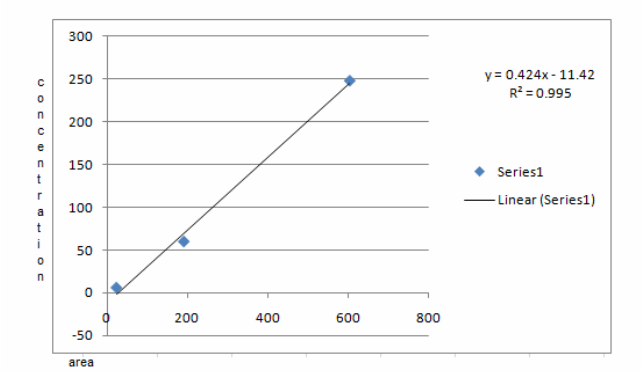

Figure 8. The calibration curve of the quantitative analysis from headspace

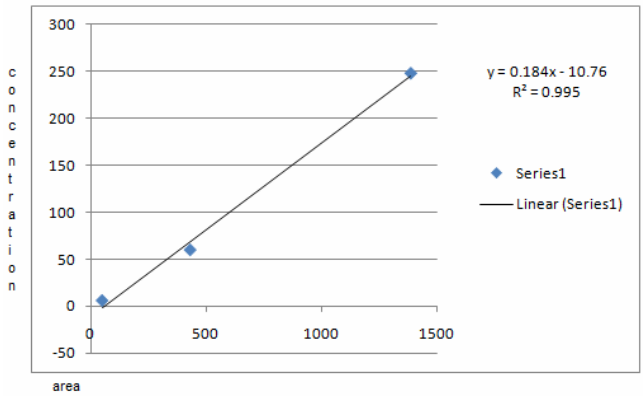

Figure 9. The calibration curve of the quantitative analysis from solution

The calibration equations were used to determine the concentrations of the menthol peaks obtained in the qualitative analysis. This step meant to establish a corelation between the qualitative and quantitative analysis. In Table III are shown the concentrations of the peaks calculated using the calibration equations.

Table III. The determination of the menthol concentration based on the calibration curves

Sample	Area (u.a.)	Extraction phase	Calibration equation	Menthol concentration (mg/mL)
S1	506.969	headspace	y=0.424x-11.42	203.534
S2	15.245	headspace	y=0.424x-11.42	4.956
S3	34.996	headspace	y=0.424x-11.42	3.418
S4	68.672	headspace	y=0.424x-11.42	17.696
S5	44.553	liquid	y=0.184x-10.76	2.562

DISCUSSION

Headspace-SPME is an equilibrium technique. During extraction the analyte migrates among the three phases, the matrix, the headspace above the sample, and the fiber coating, until equilibrium is reached. Therefore, analytes are not completely extracted from the matrix.

By comparing Figure 1 with Figures 4-7 it's easy to notice that in the first case menthol's peak, which appeared at a retention time of approximately 11.8 min, has the largest area and height. The dimensions of the menthol peak in the menthol crystals headspace analysis are considerably larger compared to the other peaks that appear in the chromatogram. In the other samples the menthol peak's dimensions are not highlighted when compared to the peaks of the other compounds. Table III confirms the results deduced by comparing the areas of the menthol peaks of the five samples. This table brings completions because the calibration curves and equations show exactly the concentration of the peaks in the solutions that were utilized. The first sample is the most concentrated in menthol both in the qualitative and quantitative conclusions. The difference is in the other samples, so if in the qualitative analysis the second most concentrated sample seemed to be S5, in the quantitative analysis the place is taken by the fourth sample. Next is S2, followed by S3 and finally S5. We can conclude that the quantitative analysis brought more precision

and accuracy to the results of the experiment.

Nevertheless, the other peaks in the chromatograms have similar retention times, forms and values in all the samples considered even in the first sample case. This fact indicates that the menthol used was slightly contaminated with compounds found in mint probably as a manufacturing process consequence. The dimension of the peaks varies as a function of concentration. The temperature influenced sample showed less peaks than the other samples due to the fact that this sample was immediately sealed so that the compounds were forced to remain in the liquid phase. The peppermint leaves headspace analysis expressed the minimum menthol peaks of all the samples.

CONCLUSION

1. Extraction of flavor compounds with sorbents is highly efficient, very fast and selective and has the advantage of maintaining the integrity of the samples.
2. Mint tea's flavour is given by a mixture of compounds of which menthol has a similar contribution to the others.
3. Mint tea has a more powerful odor that taste.

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