

Solid-Phase Microextraction–Gas Chromatographic–Mass Selective Detection Analysis of Selected Sources of Menthol

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Abstract

Manual solid-phase microextraction (SPME) coupled with gas chromatography–mass selective detection has been shown to be a rapid, precise, and reliable method for testing the origin and purity of menthol samples. Exposure of a 65- μm carboxenopolydimethylsiloxane fiber to the headspace above a menthol sample held at 50°C in a sealed vial provides for excellent signal-to-noise responses for a variety of volatile components. Detection differences in menthol compositions are based on documenting predictable changes in the ratios of identified and/or unknown components adsorbed onto the SPME fiber. Differences in composition can be detected as a function of manufacturing site using this approach. In addition, the presence of added synthetic menthol can be detected in the natural samples employing the same principle, thus assisting in determining the purity of the natural menthol.

Introduction

Essential oils are complex mixtures of fragrance and flavor substances originating in plants. Almost all of these natural oils are very complex mixtures and are used in the perfume and flavor industries. The oils have traditionally been isolated by a variety of means from components of the plants such as blossoms, roots, seeds, leaves, stems, bark, or plant excretions. (–)-Menthol, the main component of peppermint essential oils, is among a group of aroma chemicals used extensively worldwide. It has been shown that the major types of peppermint oil are different with respect to flavor profiles and chemical composition (1–6) and contain many volatile compounds other than menthol. Cornmint oil still serves as the main source of (–)-menthol, though synthetic processes for menthol exist on commercially viable production scales.

Several countries such as China, India, Brazil, and Paraguay, as well as commercial manufacturers, supply the current menthol demand of approximately 6000 tons per year. The origin of an oil has been found to influence the distribution of components

within the oils (7,8). For example, the difference among natural essential oils containing menthol is distinguished by differences in the amounts of menthol isomers and menthylesters. On the other hand, synthetic menthol does not contain these naturally occurring materials and therefore is readily distinguishable from natural menthol using such analytical techniques as gas chromatography (GC).

Solid-phase microextraction (SPME) is a convenient and very efficient extraction procedure that involves the use of a thin, polymer-coated, fine silica fiber for the adsorption of analytes of interest from a sample matrix. SPME is relatively inexpensive, solvent-free, and a reliable approach which is currently being employed in the analysis of an array of matrices for a diverse group of analytes (9). Some of these matrices and analytes include chemical warfare agents in water (10), chlorobenzenes in soils (11), low molecular weight compounds in human breath (12), and Maillard reaction products in water (13). SPME is also found in applications in the field of essential oil analyses (14–16). These reports on the use of SPME for analysis of essential oils have demonstrated the attempt to provide a rapid analytical method with no intermediate sample preparation. In some cases, the limits of detection have approached the nanogram and picogram levels. Coupling the proven performance of SPME fibers with the documented differences in component distribution of essential oils containing (–)-menthol, it seemed feasible to pursue the potential of SPME for the rapid and precise speciation of the origin of the (–)-menthol, the influence of manufacturing, and any possibility of adulteration of natural (–)-menthol with synthetic menthol.

Experimental

Sample sources

One of the natural menthol samples was obtained from Aldrich Chemical Company (Milwaukee, WI). Additional natural menthol samples, verified to have been produced from cornmint oil at various manufacturing sites in China, were obtained from Feurst Day Lanson (London, England). Synthetic menthol was obtained from Haarmann and Reimer (Springfield, NJ). All samples were

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used as received.

Instrumental Conditions

A 30-m DB 1701 column (J&W Scientific, Folsom, CA) with a diameter of 0.25 mm and a 1- μ m film thickness was used in a Hewlett-Packard 5890 GC (Palo Alto, CA). The initial oven temperature was set at 50°C and programmed to 100°C at 5°C/min, then programmed to 240°C at 10°C/min. The effluent of the column was directed into a Hewlett-Packard mass selective detector (MSD) operating in the electron impact mode at 70eV. The GC-MSD interface and injection port temperatures were set at 250°C. Total ion chromatograms from m/z 33 to m/z 250 were collected.

Manual SPME approach

Manual SPME fibers were obtained from Supelco (Bellefonte, PA) and employed strictly following the manufacturer's directions for activation and use.

Laboratory-milled (Aldrich), finely ground, selected menthol

samples (5.0 g) were placed in 20-mL vials, capped, and placed in a water bath at 50°C for 10 min. Within a short time, the menthol powders melted. The selected SPME fiber was manually inserted into the vials for 20 s, removed, and then immediately inserted into the GC injection port. Six injections of the same sample were made to check the accuracy of the methodology. Six identical 95% natural samples were then prepared by manually mixing 4.75 g of a powdered natural menthol sample with 0.25 g of the synthetic menthol. The six individual samples were treated as previously described and analyzed to determine the accuracy of the sample preparation.

Laboratory-prepared mixtures of natural and synthetic menthols were prepared at selected percent compositions by gravimetrically weighing known amounts of each sample from a supply of laboratory-milled finely ground samples.

Results and Discussion

Theoretical studies have shown that the adsorbed quantity of material on an SPME fiber can be predicted from a simple adsorption model based on diffusion-controlled mass transfer process (17). As such, the degree of adsorption will also be a function of the chemical characteristics of the fiber materials. Thus, an evaluation of the available array of fibers, as well as fiber exposure time, was necessary to clearly establish the optimum set of experimental parameters which might differentiate the menthols of interest. Figure 1 contains the total ion chromatograms (TIC) resulting from the exposure of the selected fibers to a sample of synthetic menthol using the conditions described in the Experimental section. Exposure of the carboxenpolydimethylsiloxane (CARBOXENPDMS) fiber to the headspace above the synthetic menthol sample clearly yielded a TIC having a greater number of responses (peaks) than the other fibers. Figure 1 does not adequately represent a full description of the presence of minor components in the menthol

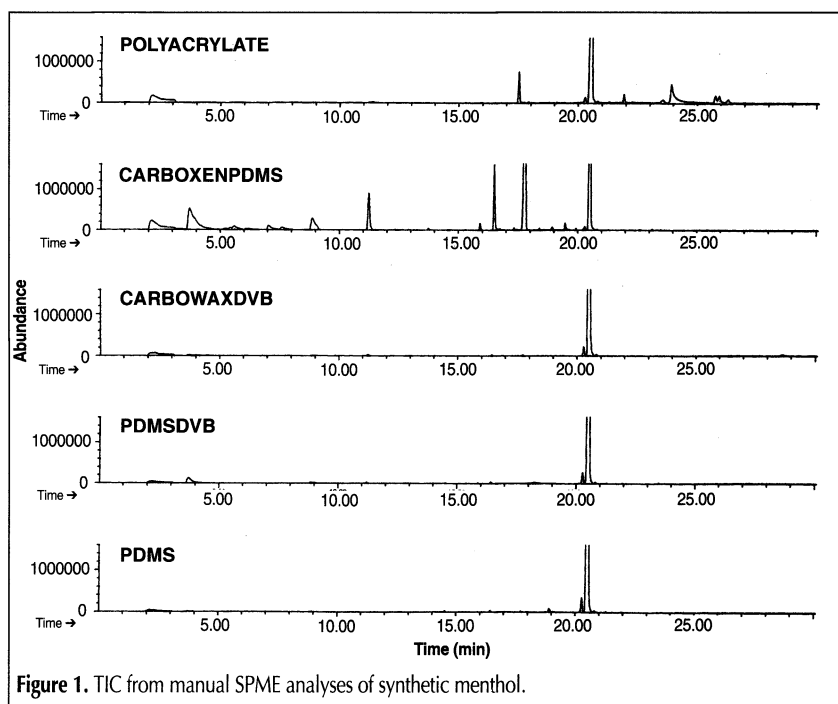


Figure 1. TIC from manual SPME analyses of synthetic menthol.

Table I. Precision Experiments Based on a Manual SPME Approach

Within-sample precision				Sample-to-sample precision			
Run	Area %*			Sample	Area %*		
	15.97 min	19.93 min	21.94 min		15.97 min	19.93 min	21.94 min
1	4.74	2.18	4.83	1	4.72	2.13	5.02
2	4.86	2.14	5.01	2	5.04	2.40	4.34
3	4.76	2.25	5.03	3	5.26	2.14	4.82
4	4.67	2.19	4.95	4	5.28	2.21	5.06
5	4.71	2.14	5.03	5	4.74	2.41	4.03
6	4.74	2.20	4.96	6	5.16	2.36	3.94
Average	4.75	2.18	4.96	Average	5.03	2.28	4.53
Standard deviation	0.06	0.04	0.08	Standard deviation	0.25	0.13	0.50
%RSD	1.34	1.84	1.60	%RSD	4.96	5.73	11.04

* Area percent of 95% natural-5% synthetic menthol.

sample. The main purpose of Figure 1 is to communicate the substantial differences in behavior of the fibers toward the headspace above a selected menthol sample.

Further investigations into the performance of the CARBOX-

ENPDMS fiber revealed that the fiber had the capability of providing discriminating power between a synthetic and natural menthol sample (Figure 2). In Figure 2, the presence of the minor components of the menthol samples are readily evident. Thus, the CARBOXENPDMS fiber was selected as the most promising candidate fiber for the studies.

Previous work with SPME fibers showed that they possess the potential of providing experimental results with excellent precision (13–15). This trend was found to exist with the approach described here. Contained in Table I are the area percent values obtained for selected peaks in the TIC from a 95% natural–5% synthetic menthol sample prepared in the laboratory. The percent relative standard deviation (%RSD) was excellent and consistently less than 2%. The %RSD obtained for sample-to-sample precision most probably reflects slight differences in sample composition.

The initial SPME experiments were conducted on the authentic and laboratory-prepared mixtures at room temperature. The results using this approach were promising; however, heating the vial of ground menthol for 10 min in a 50°C water bath was attempted in order to examine the potential for releasing even more compounds into the headspace of the sealed vial. For example, the amount of material in the headspace above the heated synthetic menthol sample increased as expected. The best curve fitting the data (i.e., the fit with the greatest correlation coefficient r^2 value) was found to be an exponential. The lines depicted in Figure 3 were those drawn by Excel software (Microsoft, Redmond, WA). Both minor components of the menthol sample (isopulegol at 19.92 min and the unknown at 20.24 min) follow the same trend with increasing White Cat 0044 percentage. In addition, as might be reasonably expected, the TIC area ratio of selected components to menthol changed because of the 50°C sample preparation (Table II).

Menthol manufacturing site variations

Based on the premise that the distribution of components within the menthol sample could differ because of slight differences in manufacturing processes, one might expect the area percent of one of the minor components (isopulegol, retention time 19.9 min) to be sample-specific. The area percent data in Table III would indicate this to be true. The sample identifiers SNP and Glacier denote menthol samples prepared at the same site. Each manufacturing location, as well as the natural sample from Aldrich, possesses a unique percent contribution from isopulegol at 19.9 min. Based on these results, one interpretation would be that the natural menthol sample from Aldrich was not manufactured at the sites

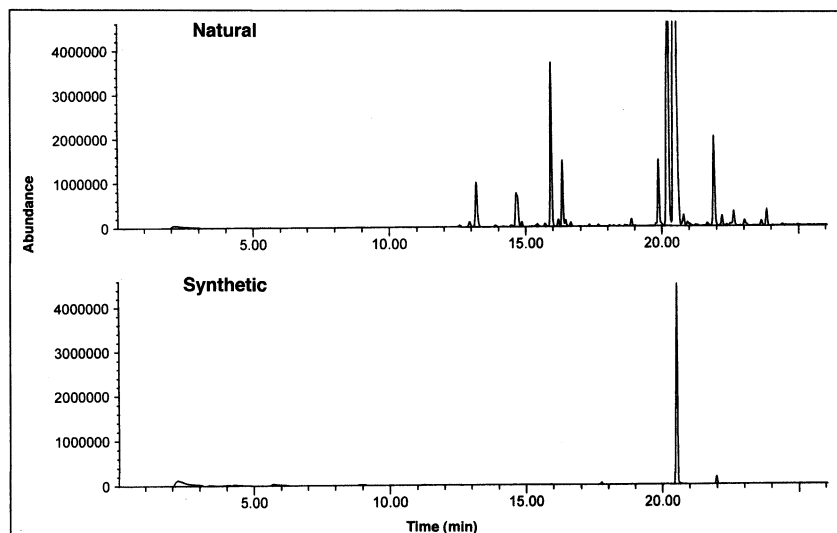


Figure 2. TIC of headspace above synthetic and natural menthol samples using a CARBOXENPDMS SPME fiber.

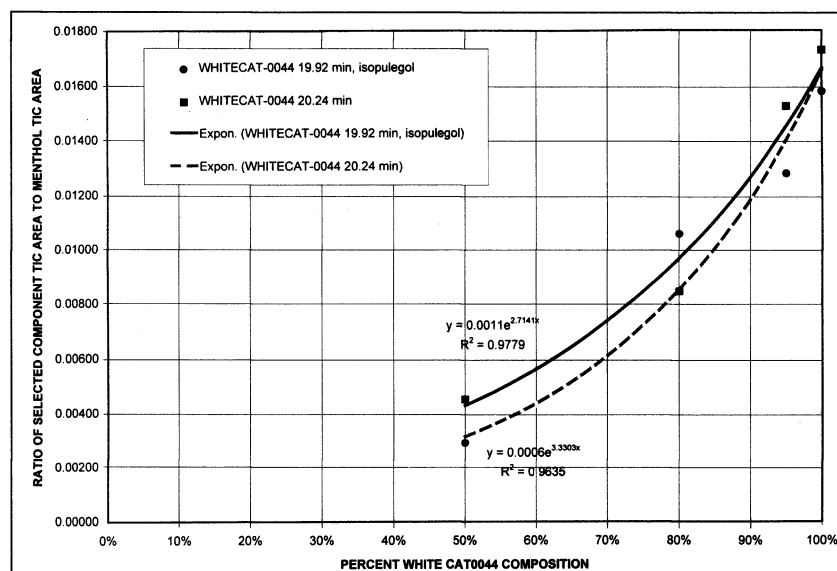


Figure 3. Influence of synthetic menthol addition on the ratio of selected components in an artificial synthetic–natural menthol sample made from SNP-0122.

Retention time (min)*	Solid TIC area	Ratio	Melted TIC area	Ratio
19.92	189,054	0.00036	585,444	0.00029
20.24	1,387,380	0.00262	4,788,858	0.00241
21.92	16,605	0.00003	203,552	0.00010
Menthol	528,660,850		1,987,496,303	

* Retention time of 100% synthetic menthol.

represented by the other samples and must have been obtained from another source. An alternative interpretation of the data obtained from samples as a function of manufacturing sites could be that there are subtle differences in the cornmint samples as a function of agricultural regions or seasonal climatic variations. The data does indicate that rapid and precise identification of menthol manufacturing sites within a country is a distinct possibility when using SPME–GC–MSD.

Authentication of all-natural menthol

The ability to accurately determine and substantiate the

authenticity of a natural sample is essential in situations where consumers are involved. The presence of any additive precludes the possibility of employing the material as an all-natural ingredient. For example, supplementing natural menthol with synthetic menthol would be viewed as removing the all-natural characteristics of the menthol. The ability to detect such an addition would be essential to substantiate a claim of “all-natural menthol”. Based on these SPME–GC–MSD results, the ability to detect the presence of synthetic menthol in a sample of “all-natural menthol” seems plausible. The reasoning rests with a postulated change in the ratio of the menthol TIC area to that of the other minor components. For example, the addition of pure synthetic menthol to a mixture of menthol and other minor components should produce a change in the ratio of components. To test the feasibility of the postulate, samples containing various ratios of the synthetic menthol from Harrmann and Reimer and natural menthol from Aldrich were prepared from finely ground powders of both samples. The artificial mixtures were analyzed by SPME–GC–MSD and the ratio of the TIC area of three selected peaks to that of the menthol were calculated (Table IV). The data relating to the ratio of the component at retention time 19.9 min (isopulegol) to that of menthol versus percent natural composition clearly indicated that detection of synthetic menthol in natural menthol was plausible.

Source	Area % at 19.9 min
China, SNP-0122	2.66
China, SNP-0084	3.09
China, White Cat-0044	2.05
China, Anhui-0092	3.08
China, Glacier-0052	3.60
China, Glacier-0248	3.65
USA, Aldrich Chemical	3.42

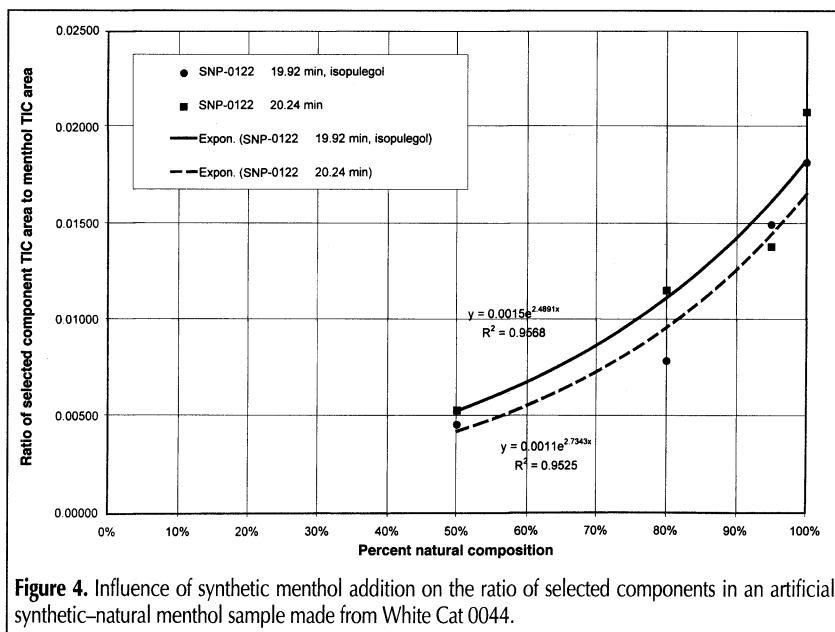


Figure 4. Influence of synthetic menthol addition on the ratio of selected components in an artificial synthetic–natural menthol sample made from White Cat 0044.

Based on this performance, artificial mixtures of the natural menthols and the synthetic menthol were prepared gravimetrically from the finely ground samples, followed by SPME–GC–MSD analysis of the mixtures. Figures 3 and 4 display a portion of the data collected on the artificial mixtures and convincingly substantiate that addition of synthetic menthol does alter the distribution of components. With r^2 values averaging greater than 0.95, this systematic alteration follows a consistent pattern. Moreover, very similar trends were observed for the other synthetic–natural mixtures. One important observation of this approach for menthol analysis was that identification of the minor components was not essential. For example, the unknown component (at retention time 20.24 min) was as effective as the known component, isopulegol (at retention time 19.9 min), in substantiating the analytical approach.

Table IV. Component-to-Menthol Ratios for Selected Menthol Compositions: Heated Samples

Component retention time (min)	100% Synthetic		50% Natural		80% Natural		95% Natural		100% Natural	
	Area	Ratio	Area	Ratio	Area	Ratio	Area	Ratio	Area	Ratio
19.92	585,444	0.00029	9,664,023	0.00497	18,822,662	0.0084	45,116,582	0.02215	45,637,139	0.02282
20.24	4,788,858	0.00241	17,822,140	0.00917	33,375,462	0.0149	44,879,189	0.02203	36,589,744	0.01830
21.92	203,552	0.00010	734,947	0.00038	1,977,839	0.0009	2,912,801	0.00143	1,893,416	0.00095
Menthol	1,987,496,303		1,943,116,534		2,244,185,404		2,036,889,616		1,999,688,133	

Conclusion

Manual SPME with a 65- μ m CARBOXENPDMS fiber coupled with GC-MSD is a precise and reliable method for testing the origin and purity of menthol samples. Based on the TIC area of minor components relative to the TIC area of menthol, changes in sample composition can be predictably detected. Identification of the minor components is not required to determine the purity of the menthol. These results show, for example, that a natural menthol sample containing a small percentage of synthetic menthol by weight is clearly distinguishable from a 100% natural sample.

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