Quantitative Analysis of Menthol Isomer Distributions in Selected Samples

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Abstract

Menthol occurs naturally in oils of the Mentha species in the (1*R*, 3*R*, 4*S*)-(–) form (*l*-menthol), whereas synthetic menthol is available either in the same form or as a racemic mixture (*d*- and *l*-menthol). Quantitative analysis of the presence of the (1*S*, 3*S*, 4*R*)-(+)- form (*d*-menthol) is achieved by using gas chromatographic analysis on a chiral capillary column with selective ion monitoring detection. Detection of the presence of as little as 0.01% *d*-menthol in the total menthol concentration is possible with relative standard deviation values averaging around 7%. Minimal sample preparation with short sample analysis times of 30 min provide for a rapid sample turn around. This method should be applicable to the speciation of menthol in a wide variety of menthol-containing products, including cigarettes.

Introduction

Menthol (*l*-menthol or [–]-menthol) is the major byproduct of the *Mentha* species, which has been used since the beginning of recorded history. For example, the remains of *Mentha* species have been found in Egyptian graves (1). It has also been described in ancient Chinese literature. Historically, the *Mentha* species were used as herbs for cooking and in preparations for illnesses.(2) Today, *M. arvensis* (cornmint) and *M. piperita* (peppermint) are the primary menthol-rich mint species in use. Menthol is isolated from *M. arvensis* oil and used in a wide variety of commercial applications, including, for example, pharmaceuticals, oral care products, tobacco products, confections, chewing gums, perfumed products, and lotions. The level of incorporation of menthol into these products ranges from about 0.03% to about 4.0% (3).

The popularity of mint is based not only on its pleasant taste and easy digestibility but also its increasing association with freshness, cleanliness, and hygiene. The estimated world consumption of mint in 1989 was estimated at about 10,000 tons (2). In 1993, the estimated total production of peppermint oils and dementholated cornmint oils was approximately 12,000 tons (4).

Dementholated cornmint oil and peppermint oil produce different characteristic flavor profiles. The taste profile of dementholated cornmint oil is characterized by pronounced earthy, mushroomy, phenolic, and bitter notes. However, positive taste characteristics such as impact, freshness, taste volume (fullness), etc., are lower in dementholated cornmint oil. Some of these differences can be corrected; however, the correction is very limited. Therefore, dementholated cornmint oil is used merely as an extender for peppermint oil.

Peppermint oils are significantly higher in quality and are therefore more valuable. Their flavor is sweeter and more well-rounded. The tea and herbal aroma components of peppermint oil are more pronounced and produce a fullness of taste. Their impression of freshness, and thus their cooling effect and impact, is more intense than the dementholated cornmint oils.

In most cases, rectified oils or "blends" of both dementholated cornmint oil and peppermint oil are actually employed. Although the oils are customarily subjected to a 6–9 month maturing process, both blending and maturing processes are an absolute necessity in order to compensate for fluctuations from harvest to harvest caused by weather conditions. However, blending and particularly the tedious maturing process represent tie-ups of capital that few companies can afford.

The largest and most important component of peppermint oil is *l*-menthol, which makes up 43–50% of the oil. It is an optically active substance in which eight stereoisomers or four racemic compounds exist. Because all development processes in nature occur in one direction, only one specific form is created in natural peppermint oil, namely *l*-menthol.

The characteristic flavor of *l*-menthol is dependent on its conformation. Only *l*-menthol imparts the well-known desired cooling effect. Another 20–25% of peppermint oil is composed of the menthol derivatives menthone and isomenthone (5). Menthyl acetate, neomenthol, and isomenthol make up 10%. The terpenes, alpha and beta pinene, limonene, sabinene hydrate, piperitone, and pulegone each account for about 1% of the composition of peppermint oil. Menthofuran and 1,8-cineole (eucalyptol) are also important to taste. Normally, pep-

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permint oils contain 1–3% menthofuran; however, the percentage can reach 10% depending on the origin of the oils.

The high optical purity of peppermint oil constituents is attributed to the stereoselectively controlled biosynthetic steps involved in their formation. Croteau et al.(6) and Hopp (4) have studied the biosynthesis of C_3- and C_6-oxygenated *p*-menthanes in *Mentha* species for several years and have proposed a pathway for the production of *l*-menthol or (–)-menthol. In a review article, Werkhoff et al. have shown that only (–)-menthol is present in natural menthol obtained from *M. arvensis* and *M. piperita* (7).

In deference to pure synthetically prepared (–)-menthol, the taste quality of natural (–)-menthol from cornmint oil varies depending on intrinsic factors (genetic or hereditability of the stolon, state of plant maturity, etc.) and extrinsic factors (sunlight, water, temperature, pressure, elevation, latitude, soil, etc.) affecting mint plant growth. Additionally, the conditions and type of equipment used in distillation are very important in determining the taste quality of menthol produced from cornmint oil. Thus, natural menthol samples from various countries can often be identified depending on their taste and odor quality. The small differences in quality often are due to very small trace impurities found in the menthol crystals. Synthetic menthol generally does not contain such impurities. As a result, it should be possible to distinguish between natural and synthetic menthol samples. Experiments using solid-phase microextraction (SPME) in concert with gas chromatography (GC) and mass selective detection (MSD) have shown that differentiation between natural and synthetic menthol samples is reasonably straightforward, and minimal sample preparation is involved (8).

Precise, accurate, rapid, automated methods for the characterization of natural products, including essential oils containing significant quantities of menthol, remains a constant goal. In the case of menthol, the objective is to clearly establish the menthol isomer distribution of the sample and hence provide useful information concerning the source of the menthol.

Numerous reports of separations based on chiral interactions have appeared in the past few years (9). For example, the most important chiral acids in wine have been effectively separated by enantioselective capillary GC using a cyclodextrin stationary phase. Simultaneous analysis of lactic, malic, and tartaric acids was possible. Askari and coworkers investigated the enantioselective chiral GC analysis of chiral monoterpenes in *Mentha* species (10). Faber et al. (11) employed direct enantioselective analysis of several monoterpenes in *Mentha* species achieved by using multidimensional GC with a column having a derivatized cyclodextrin chiral stationary phase.

Werkhoff and Hopp (7) described some of the earliest separations of menthol isomers. More recently, Werkhoff et al. (12) have described some chirospecific analysis of essential oils. In addition, by anchoring a chiral selector to a polysiloxane, a phase capable of analyzing mixtures of pharmaceutical product enantiomers has been demonstrated. After derivatization, enantioselective determination of menthol in pharmaceuticals has been reported (1). Armstrong et al. have discussed the relevance of enantiomeric separations in food and beverage analyses by describing the separation of chiral component isomers in samples such as coffee, tea, and cocoa (13,14).

A straightforward approach to the separation of menthol enantiomers using commercially available columns with particular attention to menthol that exploits the unique structure of the menthol molecule will be described. More particularly, chiral separation of menthol isomers followed by detection via selected ion monitoring (SIM) –MSD will be described as a viable approach for the determination of the isomer distribution characteristics of selected menthol samples.

Experimental

Sample sources and preparation

Natural *l*-menthol as well as *d*-menthol samples were obtained from Aldrich Chemical (Milwaukee, WI). Additional natural menthol samples, verified to have been produced in various manufacturing sites in China from cornmint oil, were obtained from Furst Day Lanson (London, UK). Synthetic menthols were obtained from commercial sources such as Haarmann and Reimer (Springfield, NJ). Samples of mouthwash, toothpaste, after-shave lotion, creme de menthe, and skin-cleaning pads were obtained locally. All samples were used as received.

Samples of menthol crystals were prepared by dissolving known amounts of each menthol sample in a known volume of methylene chloride (Burdick & Jackson Labs, Muskegon, MI). Sequential dilutions of a stock solution were prepared fresh as necessary for analysis.

Pure, all-natural *l*-menthol samples were fortified with known small amounts of *d*-menthol by accurately adding microliter quantities of a *d*-menthol standard in methylene chloride via a 10-µL syringe (Hamilton, Reno, NV) to a known quantity and concentration of the natural *l*-menthol sample in methylene chloride.

The mouthwash, after-shave lotion, and creme de menthe samples were prepared by extraction of 10 mL of each sample with 10 mL of methylene chloride in a 60-mL separatory funnel.

Table I. *d*-Menthol Percentage of Total Menthol in Selected Commercial Products

Product	*d*-Menthol (%) in total menthol
Mouthwash A	0.154
Mouthwash B	50.77
Toothpaste	0.045
After-shave lotion	0.155
Creme de menthe	0.043
Skin-cleaning pad	< 0.01
100% synthetic menthol	0.149
100% all-natural menthol (Aldrich Chemical)	< 0.01
Chinese natural sample A	< 0.01
Chinese natural sample B	< 0.01
Chinese natural sample C	< 0.01
Chinese natural sample D	< 0.01

The toothpaste sample was prepared by suspending 1 g of toothpaste in 10 mL of water followed by extraction of the water suspension with 10 mL of methylene chloride as described above. The skin-cleaning pad sample was prepared by suspending 2 pads in 10 mL of water followed by extraction of the pad–water sample with 10 mL of methylene chloride. After layer separation, the methylene chloride from each extraction was dried over anhydrous sodium sulfate. In some cases, layer separation was facilitated by centrifuging the layers at 3000 rpm for 15 min.

Instrumental conditions

A 30-m Cyclodextrin column (J&W Scientific, Folsom, CA) with a diameter of 0.25 mm and a 0.25-µm film thickness was used in a Hewlett-Packard (Palo Alto, CA) 6890 GC. Samples were automatically injected into the GC using a Hewlett-Packard 6890 series autosampler. The oven temperature was set at 100°C. The samples were injected in the split mode with a split ratio of 25:1. The flow was held constant at 1.0 mL/min. The effluent of the column was directed into a Hewlett-Packard 5973 MSD operating in the electron-impact mode at 70 eV. Based on the electron-impact mass spectrum of *d*- and *l*-menthol, the MSD was operated in the SIM mode at *m/z* 71. The GC–MSD interface and injection port temperatures were set at 230°C. Instrument reproducibility data was collected using the average, standard deviation, and relative standard deviation (RSD [%]) resulting from a minimum of five injections of each sample. The RSD values for all of the injections were less than ± 10%, typically averaging around 7%.

Instrument calibration

To establish the range of concentration(s) that would be close to the limits of detection, a calibration curve was constructed by adding microliter amounts of methylene chloride solutions containing relatively low amounts of *d*-menthol to methylene chloride solutions of *l*-menthol made from natural menthol samples. The response curve was linear over a concentration range of 0.05–0.45% *d*-menthol with an excellent correlation coefficient (r^2 = 0.9997). Response curves were constructed using three other natural menthol samples, and comparable results were discovered for each sample. Thus, detection of *d*-menthol in *l*-menthol at approximately 0.05% was reasonably straightforward using this approach.

Furthermore, by adjusting the voltage on the MSD multiplier to a higher value, the detection limit for *d*-menthol was lowered considerably. For example, the average SIM area counts for the *d*-menthol in *l*-menthol at 0.057% was approximately 500. When the multiplier voltage was increased by 200, the average SIM area counts for the *d*-menthol in the same sample increased to approx-

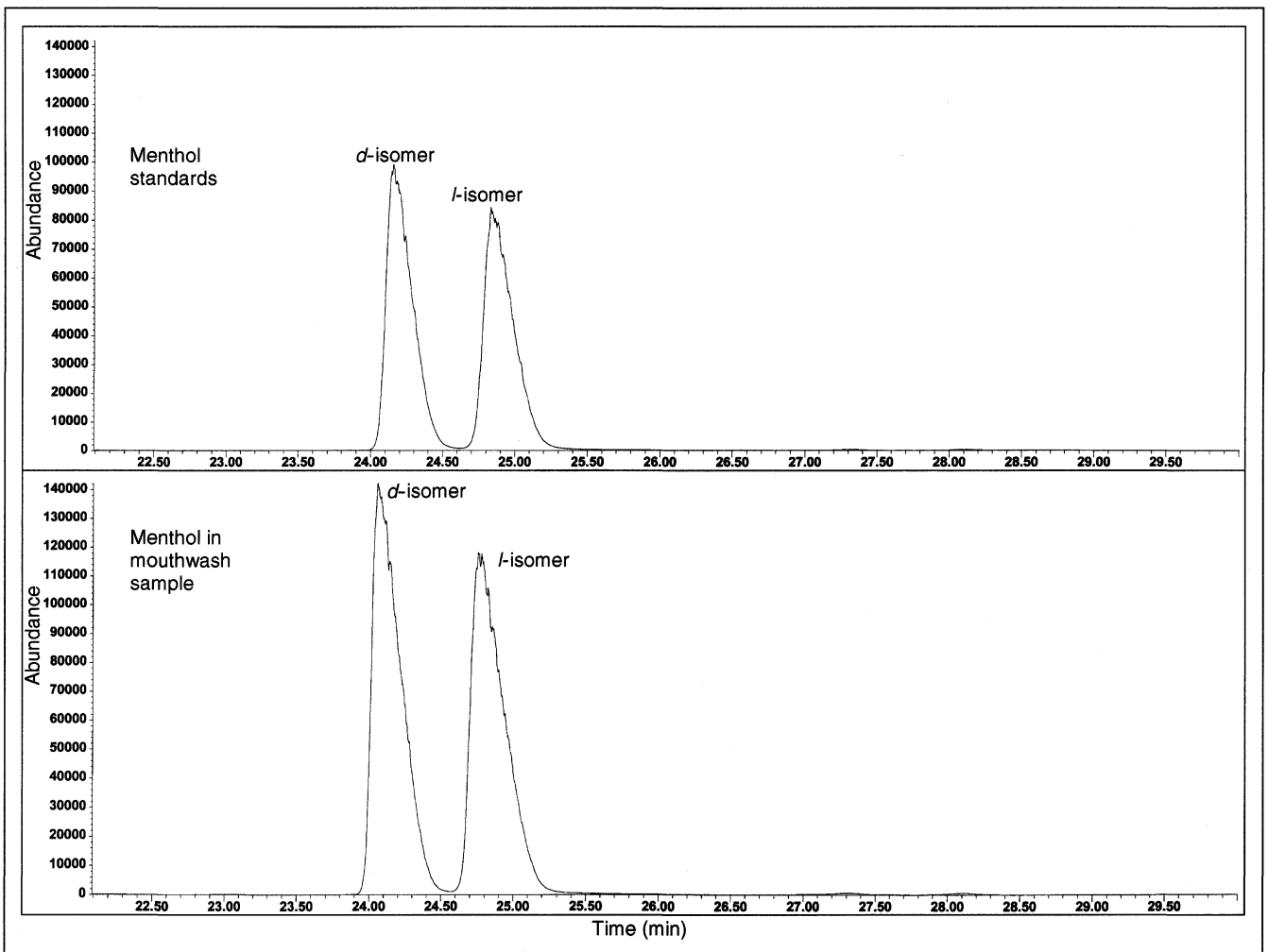

Figure 1. Selected ion chromatograms of menthol standards and menthol in mouthwash sample B.

imately 5000 without a comparable increase in the background noise level. For example, for the lowest standard measured at the higher multiplier voltage, the signal-to-noise ratio was 9:1. The response curve at this higher voltage setting was also linear over a concentration range of 0.01–0.05% *d*-menthol with an excellent correlation coefficient (r^2 = 0.9990). Thus, the detection limit for the quantitation of *d*-menthol in a predominately *l*-menthol methylene chloride solution was lowered to approximately 0.01% by weight. Stated in another way, the enantiomeric purity of the natural menthol in the samples under examination here could be determined at ≥ 99.99% based on the isomer distribution.

Results and Discussion

This report describes the direct determination of the *d*- and *l*-menthol isomer distribution of menthol-containing samples with minimal sample preparation employing separation on a chiral phase with detection and quantitation by SIM–MSD.

Naturally occurring menthol from cornmint oil and peppermint oil exists only in the *l*- form, *vide supra*. The other optical isomer (*d*) does not exist in these species but can be prepared and employed as an alternative or supplement to the natural material. Thus, separation, speciation, and quantitation of the isomers present in a menthol-containing sample is critical to establishing the true character of the menthol-containing sample under investigation. The separation of the *l*- and *d*- isomers of menthol using the conditions described above was very similar to that described previously (12), although the means of quantitation and sample collection were significantly unique.

Table I reveals that all of the natural samples obtained from Chinese sources had less than 0.01% *d*-menthol. Likewise, the natural *l*-menthol sample from Aldrich Chemical contained less than 0.01% *d*-menthol. These observations were consistent with the previous literature; however, the detection limits reported here were approximately an order of magnitude lower than those previously reported (7,12).

Analysis of the commercial samples reported to contain menthol produced a range of responses in terms of *d*-menthol content. The Chinese samples and the skin-cleaning pad appeared to contain all-natural menthol with *d*-menthol percentage levels less than 0.01%. The toothpaste and creme de menthe samples also appeared to contain mostly all-natural menthol. However, the after-shave lotion and mouthwash A appeared to most probably contain 100% synthetic menthol, based on the *d*-menthol percentage in the 100% synthetic samples at 0.149% *d*-menthol. Mouthwash B contained a mixture of *d*- and *l*-menthol in an approximate 50:50 ratio (see Figure 1). Thus, this mouthwash was at least partially formulated with unnatural menthol.

Conclusion

By taking advantage of the distribution of optical isomers in naturally occurring menthol, documentation of the origin of menthol employed in the production of selected menthols and menthol-containing commercial products has been possible. Specifically, separation of the optical isomers via a GC equipped with a chiral column followed by detection with SIM–MSD was able to provide qualitatively specific and quantitative determination of the amount of *d*-menthol in samples at approximately 0.01% of the total menthol burden. This approach improved on previous approaches using non-specific flame-ionization detectors. Determination of the menthol isomer distribution in commercial and natural menthol samples using this method was rapid, precise, and accurate. The analysis time was 30 min per sample, and the RSD values were consistently around 7%. The chiral separation coupled with detection by SIM provided improved method specificity and accuracy.

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