Determining Peppermint Oil Authenticity by Chiral Gas Chromatography Mass Spectrometry

Julie Kowalski, Rick Lake, Gary Stidsen, and Neil Mosesman
Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823-8812, US

Abstract

Essential oils have a long history as flavor additives and herbal remedies. Today, essential oils are widely used in foods, candies, cosmetics, personal care products, and perfumes. Modern research has demonstrated health benefits of some essential oils widely used as herbal remedies. The use of essential oils in commercial goods and the rising demand for all-natural products drive a need for more rigorous testing. Often, a product is adulterated to increase desirable properties of the natural oil or to avoid costly manufacturing of all-natural products. Adulteration of essential oils provokes concern over compromised quality, but also introduces health safety issues.

Reliable, sensitive analytical methods are vital to detect complex manipulation of synthetic materials to mimic natural materials. Because some naturally occurring stereoisomers show greater biological activity than other isomers, reliable methods of analyzing specific chiral components can be used to monitor product quality.

This work demonstrates a robust chiral GC/MS method that can be used with confidence to differentiate among oil formulations. We investigated selectivity for chiral components using two cyclodextrin-based columns: RT-γDEXsa™ and RT-βDEXsm™. Upon analysis of peppermint oil, for example, the selectivity of the RT-βDEXsm™ phase (A) is well suited to separating minor chiral components like pinene and limonene, while the RT-γDEXsa™ phase works well for distinguishing isomers of major components. Patterns of marker compounds for different oil formulations are produced. This demonstrates how marker compounds can be used as "fingerprints" to determine the authenticity of a sample. Chiral cyclodextrin-based stationary phases allow detection of both major and minor chiral components as marker compounds, providing manufacturers and buyers with consistent profiles with which to confirm product authenticity and quality. 1,2

Introduction

Peppermint oil is a commonly isolated component of the plants *Mentha piperita* and *Mentha arvensis*. Peppermint oil preparation begins with harvesting leaves and flowering tops from the plant. Although other techniques exist, steam distillation is the most commonly used method for extracting highly concentrated oil from the plant material. Oil from one pound of plant material will flavor approximately 135,000 sticks of gum.3

The use of peppermint oil in commercial goods and the rising demand for all-natural products drive a need for more rigorous testing. Often, a product is adulterated to increase desirable properties of the natural oil or to avoid costly manufacturing of all-natural peppermint. This usually is accomplished by adding individual components, adding a similar but cheaper oil, such as corn mint oil, or diluting the oil with various solvents. Adulteration of peppermint oil fuels concern over compromised quality, but also introduces health safety issues; for example, there is potential for an allergic reaction to an added un-natural compound or excess of a natural component.

Reliable, sensitive analytical methods are vital to detect complex manipulation of synthetic materials to mimic natural materials. Because some naturally occurring stereoisomers show greater biological activity than other isomers, reliable methods of analyzing specific chiral components can be used to monitor product quality. For example, (-)-menthol is the stereoisomer known to have the greatest cooling and scent effects. Also, standardizing the composition that defines a "natural" product can be difficult, due to natural variation among plants and plant varieties, variation in geographical and seasonal factors, and due to inconsistencies in the manufacturing process.

Analytical Challenges

Analysis of peppermint oil can be challenging for various reasons. Major and minor components exist at very different concentrations, and several components are chiral. Adulteration of a peppermint product most often involves major components menthol, methone, and methyl acetate, but each of these is represented by several stereoisomers. Menthol, for example, has three chiral centers, making for a total of eight stereoisomers, and making chromatographic separation difficult. Minor components include additional enantiomeric compounds, such as pinene and limonene. Several of these minor compounds can be used as markers for authenticity and standardizing.

Experimental Design

Here we show a robust chiral GC/MS method that can be used with confidence to differentiate among peppermint oil formulations. Numerous chiral GC column phases are available; we investigated selectivity for chiral components using two cyclodextrin-based columns: RT-γDEXsa™ and RT-βDEXsm™. We demonstrate the method with analyses of neat peppermint oils –standard mixtures of the major and minor components were injected onto the two columns. Differences in selectivity were seen for both major and minor components on the two columns.

Peppermint oil samples were purchased from six different commercial sources. Each sample was identified by harvest location, processing and year. Analyses were performed on a Shimadzu GC/MS; model GC-17A, MS-QP5000. One microliter of neat oil was injected at a split ratio optimized for major or minor components: 150:1 or 100:1, respectively. All analyses were performed in triplicate. The autosampler program included extensive rinsing with methylene chloride to prevent sample memory and the syringe from plugging.

Data were analyzed using Shimadzu LabSolution, Version 1.20. Identifications of pinene and limonene isomers were based on matching retention times to individual standards, and mass spectra were searched against NIST libraries and custom libraries created in LabSolution. Identifications of menthol, methone, and methyl acetate isomers were based on rough retention time comparisons to literature values and a mass spectra library search.4 Integration of chromatograms was consistent.

Chromatographic conditions:

- Restek RT-βDEX-sm (30 m x 0.25 mm x 0.25 µm)
- Restek RT-γDEX-sa (30 m x 0.25 mm x 0.25 µm)
- Inlet: 230°C
- MS transfer line: 200°C
- Column flow rate: helium, constant pressure, 35cm/second, 150:1 split injection
- Oven program: 40°C to 120°C at 5°C/min to 135°C at 3°C/min to 200°C at 5°C/min
- 1.0 µL of neat oils
- MS: 40-300 amu, scan, EI

Results: Minor Components

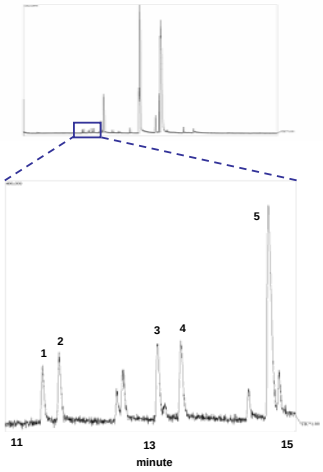

Figure 1: This chromatogram shows the selectivity of the Rt-βDEXsm™ phase is well suited to separating minor chiral components like pinene and limonene. Peaks identified based on retention time matching to standards. **1** and **2** (+/-) alpha pinene and **3** and **4** (+/-) beta pinene and **5** R-(+) limonene.

Results: Major Components

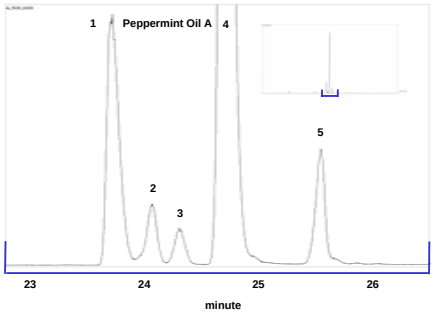

Figure 2: The chromatogram represents the common peak pattern in this retention window for peppermint oil, based on analysis of six different purchased samples. The samples can from a variety of geographic locations, different farming practices, and processing methods. Peaks identified based on retention time matching to standards and previous literature. **1** methone, **2** menthol, **3** methone **4** menthol and **5** menthyl acetate.

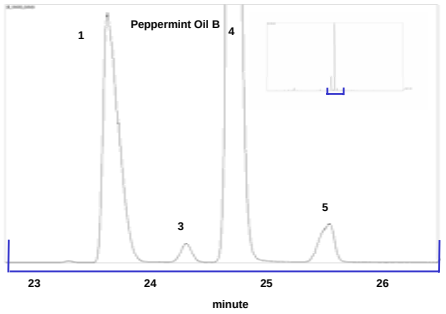

Figure 3: This chromatogram shows the peak pattern of one commercial sample that does not follow the expected pattern. This pattern is quite different due to the absence of peak **2** and different relative abundances of the remaining peaks. **1** methone, **3** methone, **4** menthol and **5** menthyl acetate.

Conclusion

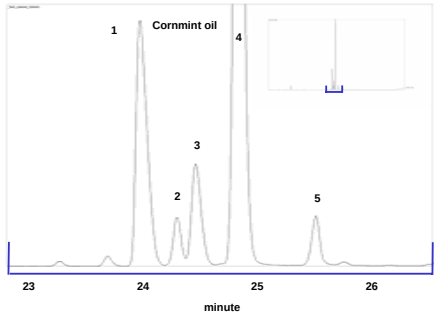

Figure 4: This is a chromatogram of cornmint oil, a common adulterant. It is common to see a reversal in abundance of peaks 2 and 3. **1** methone, **2** menthol, **3** methone **4** menthol and **5** menthyl acetate.

Table 1: Summary of general rules to distinguish between pure peppermint oil and cornmint oil.

Peppermint Oil	Cornmint oil
Peak 2 area ≥ Peak 3 area by as much as three times	Peak 2 area ≤ Peak 3 area
Peak 5 area > Peak 3 and/or Peak 2 area by as much as five times but typically one to two times greater	Peak 5 area < Peak 3 and/or Peak 2 area by as much as five times but typically one to two times greater

Conclusion

Natural products, like peppermint oil, are difficult to analyze and monitor quality. One approach would be to use marker compounds. The RT-γDEX and RT-βDEX chiral columns allow the detection of both minor and major chiral compounds. The RT-βDEX works best, allowing resolution of minor stereoisomers, like pinene. The ratios of these isomers is consistent between samples and can therefore identify oils of lesser quality. The RT-γDEX works best for the active components, like menthol. These biologically active compounds are useful to measure of oil potency and thus safety.

Peppermint oil have a characteristic pattern and isotope ratios (not published here) that is common to most samples. However, when this pattern is not observed, it is an indication that the oil is not peppermint oil or is adulterated.

Cornmint is a common adulterant and shows a consistent pattern for menthol and related compounds. This pattern seems to stay consistent between samples (data not shown here). From this, guidelines can be easily (direct sample injection) developed and used to determine if the ratios of these compounds fit with in specifications for product lot testing.

References

1.http://www.botanical.com/botanical/mgmh/m/mints-39.html#pep)

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3.http://www.geo.msu.edu/geo333/mint.html

4.J. Chromatogr. A, 1054: 87-93 (2004).