Enantiomeric Composition of Essential Oils by Chiral GC/MS

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Overview

Purpose
Determine the chemical profile of bergamot, lavender, peppermint, spearmint, and rose oil by examination of the enantiomeric ratios using chiral capillary columns and GC/MS.

Methods
Essential oils were analyzed by GC/MS using a split injection onto a capillary column with a stationary phase containing derivatized cyclodextrin macromolecules, achieving optimum separations of enantiomers.

Introduction
The food and flavor industry has been working to establish standards which specify the preferred chemical profile of essential oils. The enantiomeric ratio of optically active components of essential oils is often specific to the species and origin of the material used in the production of the extracts and oils.1

The expected ratio values may be used to reject products if they do not conform to those for the natural oil. Some enantiomeric isomers were analyzed by injecting dilutions of purchased neat materials from Sigma Aldrich. Several oils were also purchased from a local health food store and analyzed. All of the oils tested were proven to be of natural origin.

A chiral carbon atom has four different functional groups. Molecules containing one or more chiral atoms are called chiral molecules. The nonsuperimposable mirror images of the chiral molecule are called enantiomers. Their physical properties will be identical, but they will have different aroma and flavor characteristics and have different toxicity and biological activity. They are also optical isomers, because they rotate plane polarized light in different directions. If they rotate polarized light to the right, they are termed dextrorotary (d) or (+); to the left, levorotary (l) or (-). Another annotation is designated by the priority of the atomic number of the first bonded atom. If the configuration is clockwise around the asymmetric carbon, it is denoted as (R). If the configuration is counterclockwise, it is denoted as (S).2

Methods
Before analyzing the various oils, the chromatography was optimized. The parameters were selected based on the information provided in the “Restek Guide to the Analysis of Chiral Compounds by GC”. The guide recommends higher linear velocities (80 cm/sec), slower temperature ramps (1 – 2 °C/min), an appropriate initial operating temperature (40 to 60°C), and on-column concentrations of 50 ng or less. The Trenzzahl values increase with an increase in linear velocity, improving resolution. As the oven temperature ramp decreases below 3°C/min, the Trenzzahl values increase with enantiomeric resolution factors.3 A split/splitless injector was configured for a split injection at 200°C with a 3 mm id silanized glass split liner. A 1 µL injection was made with an AS 2000 fully programmable autosampler. The oils were diluted to 1% in methylene chloride. The analytical column was a Restek® Rt-βDEXse 0.32 mm x 30 meter, 0.25 micron film with a carrier gas flow of 5 cc/min helium or linear velocity of 80 cm/sec and a split flow of 50 cc/min (Split ratio of 10/1). The oven was programmed at 2°C/min during the elution time of the enantiomers (Figure 1). The Finnigan™ TRACE™ DSQ quadrupole mass spectrometer from Thermo Electron was autotuned for a classical tune, and default values from the Autotune file were used. The mass spectrometer was able to handle the high carrier gas flow by configuring with the 250 liter pump option. A Full Scan analysis was set to scan from 35 to 300 m/z at a scan rate of 1,000 amu/sec. The source was set at 200°C.

Figure 1: TIC of Split Injection of standard at 100 ng/uL in methylene chloride (Split ratio of 10/1)
Results

Resolution

Chiral compounds contain an asymmetric carbon center. The mirror images, called enantiomers, are not superimposable. Resolution of the enantiomers is critical because they have identical mass spectra. The enantiomers were purchased from Sigma-Aldrich and analyzed individually. Resolution was defined as the separation of 2 peaks in terms of their difference in elution times divided by the average peak width at baseline. The resolution for linalool and alpha ionone are shown in Figures 2 and 3. Figure 4 shows the spectra and structures of some of the analyzed enantiomers.

Precision

Since identification is made by retention time, precision is critical for this analysis. A series of replicates of the standard mixture at 100 ng/μL at a Split of 10/1 was injected 25 times, and the retention time drift was tabulated. The results are shown in Table 1. The difference between the mean retention time and each replicate injection was less than one second for all enantiomers, except (R) & (S)–citronellol, which were not baseline resolved.
Table 1: Retention Time Drift of 25 Replicate Injections of Standard

<table>
<thead>
<tr>
<th>Retention Time (sec)</th>
<th>Component</th>
<th>Standard Drift</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>(+)-alpha-pinene</td>
<td>6.74</td>
<td>1.02</td>
</tr>
<tr>
<td>0.03</td>
<td>(-)-limonene</td>
<td>9.17 (1.3%)</td>
<td>0.02</td>
</tr>
<tr>
<td>0.04</td>
<td>(+)-limonene</td>
<td>9.61 (98.7%)</td>
<td>0.03</td>
</tr>
<tr>
<td>0.05</td>
<td>alpha-terpinene</td>
<td>11.43</td>
<td>0.01</td>
</tr>
<tr>
<td>0.06</td>
<td>(-)-linalool</td>
<td>14.12</td>
<td>0.05</td>
</tr>
<tr>
<td>0.07</td>
<td>(R)-linalyl acetate</td>
<td>17.31</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Figure 5: Bergamot Oil – Predominant Isomer was (+)-limonene

Figure 6: Lavender Oil – Predominant Isomer was (-)-linalool

Figure 7: Spearmint Oil – Predominant Isomer was (-)-limonene

Figure 8: Peppermint Oil – Predominant Isomer was (-)-limonene

Figure 9: Lavender Oil – Predominant Isomer was (-)-linalool

Figure 10: Peppermint Oil – Predominant Isomer was (-)-limonene
Figure 9: Rosewood Oil – Predominant Isomer was (S) Citronellol

1. rose oxide 11.76
2. (-)-linalool 14.11
3. (+)-linalool 14.66
4. Rose oil 20.16
5. (S)-citronellol 21.69
6. nerol 21.89

Figure 10: NIST Library Spectra Matching of Enantiomers in Bergamot Oil

1. (-)-limonene
2. (-)-linalool
3. linalyl acetate
Predominant Optical Isomers

An experiment was set up to determine if essential oils purchased at a local health food store were authentic. The following oils were analyzed using the method: bergamot, lavender, spearmint, peppermint, and rose oil. All of the analyses showed that the samples were from natural origins because a predominant optical isomer was identified, as seen in Figures 5, 6, 7, 8, and 9.

Identification by Spectra

The composition of a natural oil is very unique. Several chemical groups are found, including terpenes, alcohols, ketones, lactones, esters, and epoxides. The mass spectrometer is a powerful tool for classifying structures of unknown compounds. NIST library matches are shown in Figures 10 and 11 for the enantiomers studied.

Conclusions

When analyzing compounds with identical mass spectra, chromatography must be used to provide separation so identification can be made. If the physical properties are different, the resolution can be achieved by the oven program rate or by selection of the appropriate stationary phase. With enantiomers, the only distinguishable properties are the optical characteristics. Stationary phases with derivatized cycloextrin macromolecules have been developed to provide separation of enantiomers. The Finnigan TRACE DSQ quadrupole mass spectrometer was used with a chiral column for the identification of enantiomers in bergamot, peppermint, spearmint, and rose oil. A predominant enantiomer isomer, characteristic of a natural oil, was found in each oil sample analyzed.

The retention time precision for replicate runs was within one second. All enantiomers studied showed baseline resolution, with the exception of (R) and (S)-citronellol, which may be resolved with a Restek Rt-βDEXsa column. The analysis may be run using a Flame Ionization Detector, but there would be no identification for coeluting compounds. The Finnigan TRACE DSQ was operated at the elevated linear velocity required for the resolution of enantiomers, providing mass spectral confirmation.

References

2. “A Guide to the Analysis of Chiral Compounds by GC”, Dr. Tabacchi, Dr. Saturnin, Claire-Lise Porret, Restek Corporation, cat# 59889