Application Note: 10019

Enantiomeric Composition of Essential Oils by Chiral GC/MS

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Key Words

- **Finnigan TRACE DSQ**
- **Chiral**
- **Enantiomers**
- **Essential Oils**
- **GC/MS**

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.

Results

Several oils were analyzed on a quadrupole mass spectrometer. The mass spectrometer was able to provide structural information, matching library spectra for identification. Retention times were stable to within one second, and the enantiomeric ratios of optically active components were shown.

Introduction

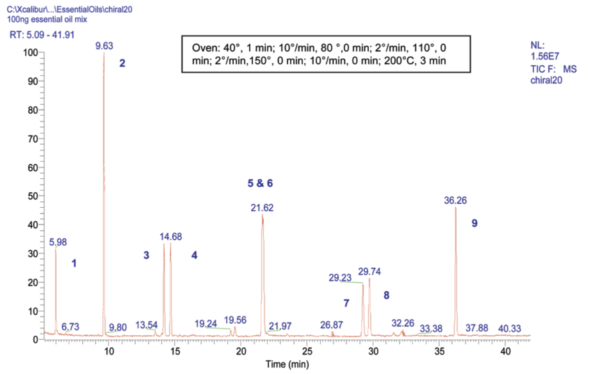
The food and flavor industry has been working to establish standards which specify the preferred chemical profile of essential oils. The enantiomeric ratio of optically active components of essential oils is often specific to the species and origin of the material used in the production of the extracts 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 Trennzahl values increase with an increase in linear velocity, improving resolution. As the oven temperature ramp decreases below 3°C/min, the Trennzahl values increase with enantiomeric resolution factors.[2] 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.



Oven: 40°, 1 min; 10°/min, 80 °,0 min; 2°/min, 110°, 0 min; 2°/min,150°, 0 min; 10°/min, 0 min; 200°C, 3 min

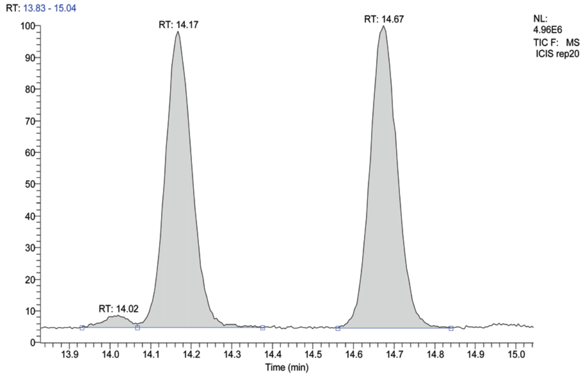
1. (+)-alpha-pinene	5.98	4. (+)-linalool	14.68	7. (R)-alpha-ionone	29.23
2. (+)-limonene	9.63	5. (R)-beta-citronellol	21.62	8. (S)-alpha-ionone	29.74
3. (-)-linalool	14.18	6. (S)-beta-citronellol	21.67	9. Beta-ionone	36.26

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

Results

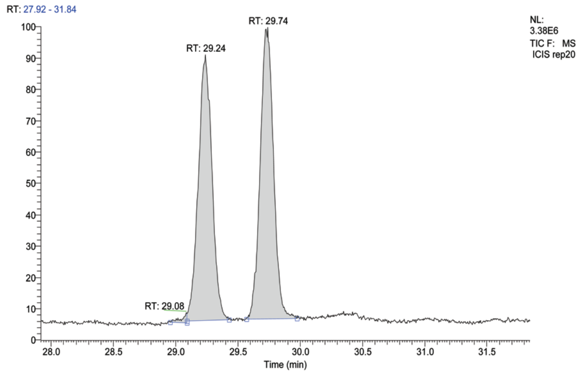
Resolution

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.[3] 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.



$$R = \frac{14.67-14.17}{0.275}$$

Figure 2: (-) & (+)-Linalool: Resolution = 1.8



$$R = \frac{29.74-29.24}{0.375}$$

Figure 3: (R) & (S)-alpha-ionone: Resolution = 1.3

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/uL 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.

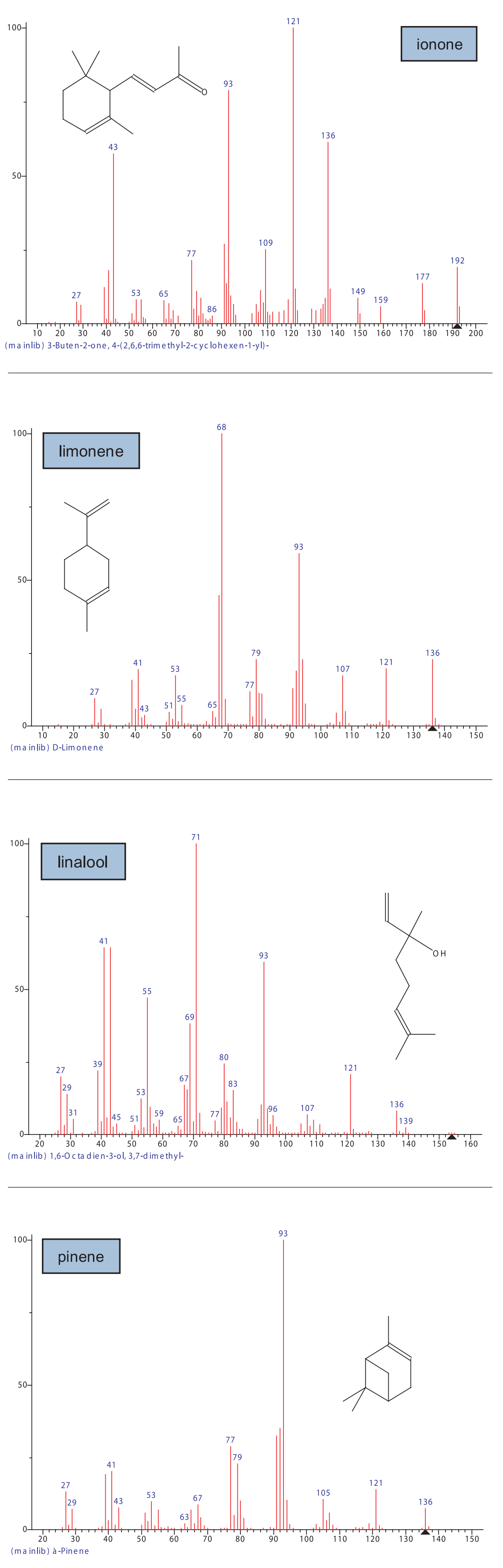
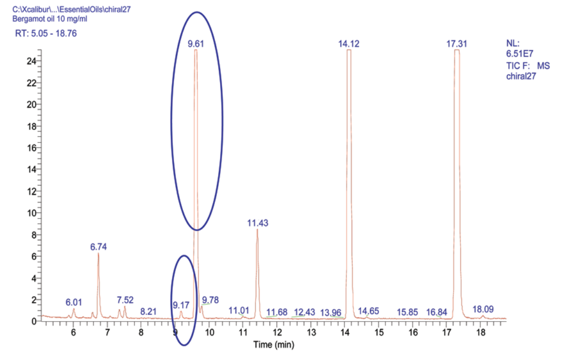


Figure 4: Chiral Compounds with Asymmetric Carbon Center (Enantiomers)

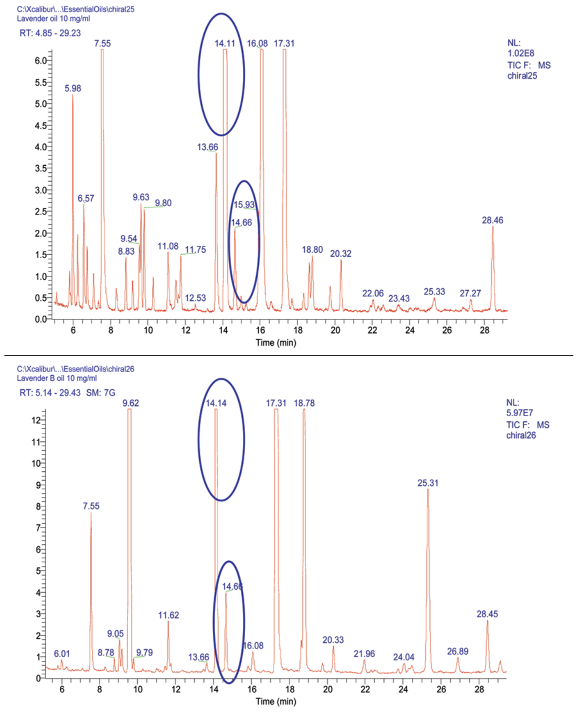
Filename	(+)-alpha-pinene	Diff (sec)	(+)-limonene	Diff (sec)	(+)-linaloo	Diff (sec)	R-beta-citronello	Diff-(sec)	S-beta-citronello	Diff (sec)	R-alpha-ionone	Diff (sec)	S-alpha-ionone	Diff (sec)	beta-ionone	Diff (sec)
rep02	5.986	-0.152	9.622	-0.052	14.164	-0.207	21.601	-0.389	21.667	0.296	29.232	0.678	29.725	0.616	36.257	1.022
rep03	5.986	-0.168	9.627	0.214	14.163	-0.239	21.600	-0.404	21.676	0.858	29.241	1.225	29.734	1.163	36.257	1.022
rep04	5.986	-0.168	9.627	0.214	14.163	-0.254	21.605	-0.123	21.676	0.843	29.236	0.912	29.729	0.85	36.256	0.975
rep05	5.991	-0.113	9.627	0.214	14.163	-0.238	21.605	-0.123	21.671	0.562	29.236	0.928	29.729	0.866	36.257	0.991
rep06	5.986	-0.168	9.622	-0.067	14.163	-0.254	21.595	-0.701	21.647	-0.876	29.231	0.615	29.729	0.85	36.251	0.678
rep07	5.986	-0.168	9.622	-0.068	14.158	-0.536	21.600	-0.404	21.657	-0.298	29.213	-0.494	29.710	-0.275	36.233	-0.431
rep08	5.986	-0.168	9.617	-0.349	14.154	-0.801	21.591	-0.967	21.657	-0.298	29.208	-0.776	29.696	-1.119	36.223	-0.994
rep09	5.986	-0.168	9.627	0.214	14.163	-0.254	21.666	3.565	21.666	0.265	29.208	-0.775	29.696	-1.134	36.223	-1.009
rep10	5.986	-0.168	9.617	-0.052	14.153	-0.817	21.596	-0.686	21.657	-0.298	29.208	-0.776	29.696	-1.119	36.223	-0.994
rep11	5.986	-0.168	9.622	-0.067	14.158	-0.535	21.596	-0.685	21.657	-0.282	29.213	-0.463	29.706	-0.525	36.224	-0.978
rep12	5.986	-0.168	9.622	-0.083	14.158	-0.551	21.590	-0.998	21.657	-0.314	29.212	-0.526	29.705	-0.587	36.227	-0.759
rep13	5.986	-0.168	9.617	-0.364	14.149	-1.098	21.586	-1.263	21.643	-1.157	29.208	-0.775	29.701	-0.837	36.223	-0.993
rep14	5.986	-0.168	9.622	-0.068	14.153	-0.817	21.586	-1.264	21.657	-0.298	29.208	-0.776	29.706	-0.556	36.223	-1.01
rep15	5.986	-0.168	9.612	-0.645	14.144	-1.41	21.643	2.143	21.643	-1.157	29.198	-1.354	29.696	-1.119	36.223	-1.009
rep16	5.987	-0.136	9.618	-0.317	14.154	-0.551	21.653	2.752	21.653	-0.548	29.208	-0.76	29.701	-0.821	36.233	-0.431
rep17	5.991	0.145	9.622	-0.067	14.158	-0.566	21.585	-1.31	21.656	-0.345	29.207	-0.853	29.704	-0.634	36.226	-0.822
rep18	5.991	0.114	9.617	-0.364	14.158	-0.566	21.651	2.659	21.651	-0.641	29.211	-0.572	29.709	-0.353	36.236	-0.259
rep19	5.991	0.113	9.626	0.198	14.163	-0.27	21.595	-0.732	21.671	0.515	29.226	0.287	29.719	0.225	36.245	0.319
rep20	5.996	0.395	9.626	0.199	14.167	-0.004	21.604	-0.185	21.680	1.062	29.240	1.313	29.728	0.788	36.255	0.897
rep21	5.996	0.41	9.626	0.198	14.167	0.026	21.609	0.126	21.676	0.811	29.231	0.615	29.729	0.834	36.256	0.943
rep22	5.996	0.41	9.627	0.214	14.167	0.027	21.600	-0.436	21.666	0.233	29.231	0.599	29.724	0.553	36.251	0.678
rep23	5.991	0.113	9.622	-0.067	14.158	-0.551	21.595	-0.701	21.666	0.265	29.232	0.631	29.724	0.569	36.252	0.694
rep24	5.996	0.395	9.631	0.495	14.167	-0.027	21.609	0.143	21.676	0.827	29.236	0.897	29.729	0.835	36.256	0.96
rep25	5.996	0.394	9.627	0.213	14.163	-0.165	21.595	-0.717	21.671	0.53	29.231	0.615	29.724	0.553	36.247	0.397

Table 1: Retention Time Drift of 25 Replicate Injections of Standard



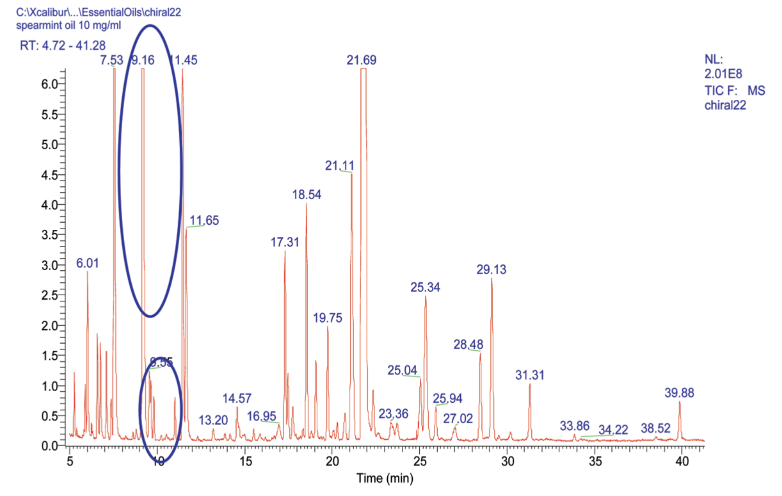
1. beta-pinene 6.74	3. (+)-limonene 9.61 (98.7%)	5. (-)-linalool 14.12
2. (-)-limonene 9.17 (1.3%)	4. alpha-terpinene 11.43	6. (R)-linalyl acetate 17.31

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



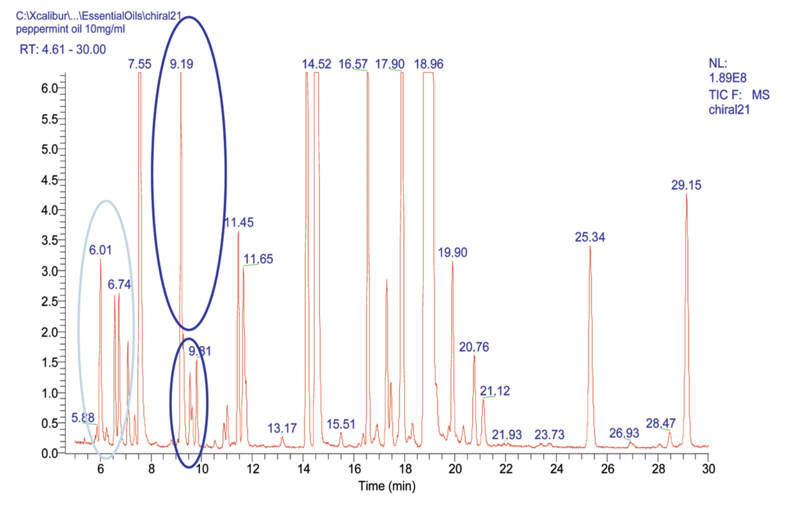
1. limonene oxide 7.55	3. (-)-linalool 14.11 (92.7%) Oil B	6. nerol-acetate 18.78
2. (+)-limonene 9.61	4. (+)-linalool 14.66 (7.3%) Oil B	7. caryophyliene 25.31
3. (-)-linalool 14.11 (98.3%) Oil A	5. (R)-linalyl-acetate 17.31	8. beta-farnesene 28.46
4. (+)-linalool 14.66 (1.7%) Oil A		

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



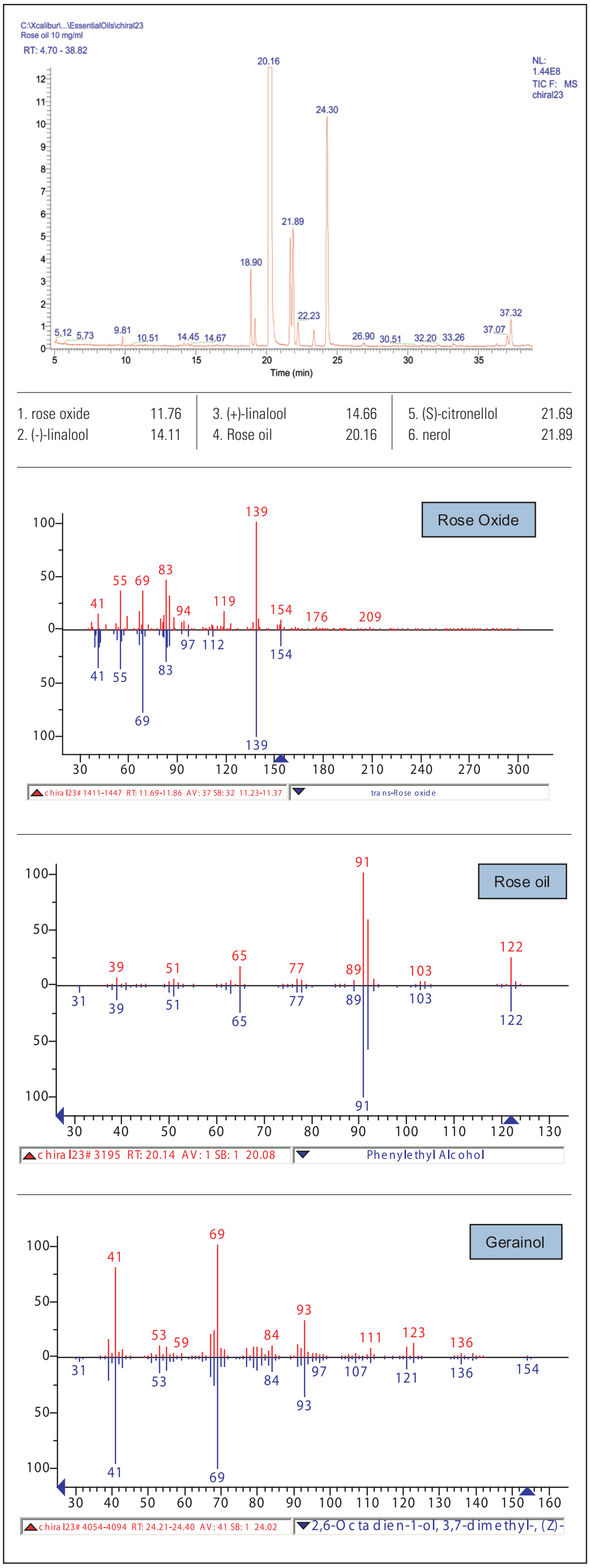
1. limonene oxide 7.53	3. (+)-limonene 9.63 (2.1%)	5. carvone 21.69
2. (-)-limonene 9.16 (97.9%)	4. Menthol 19.07	

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



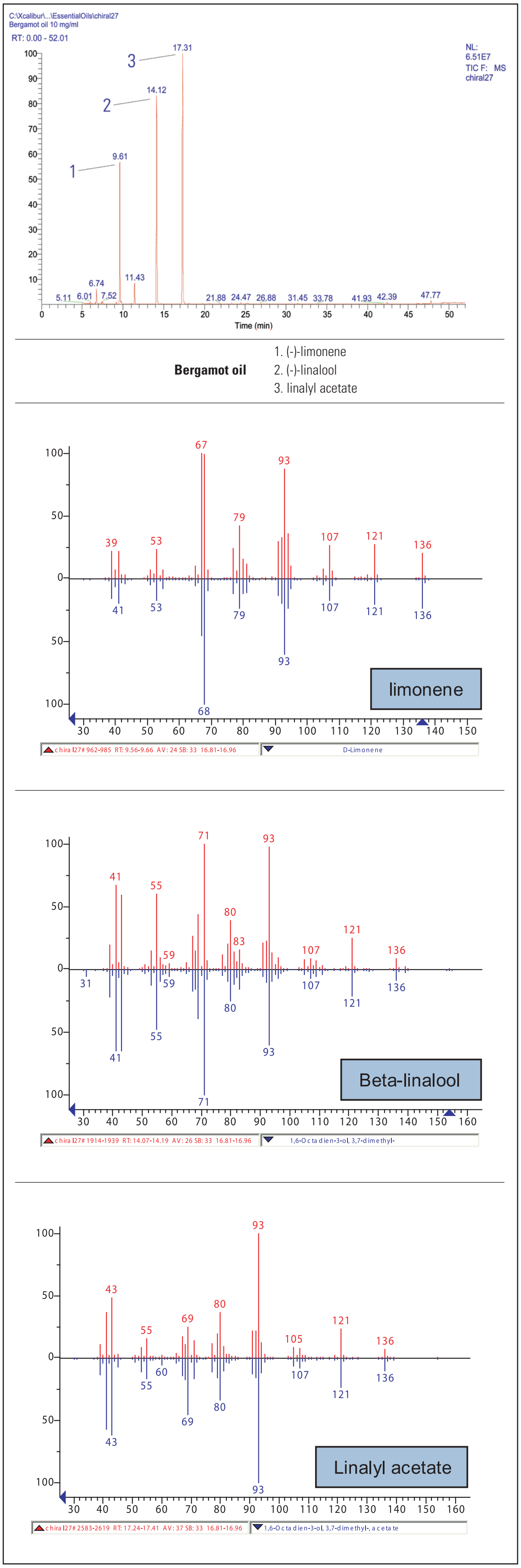
1. alpha-pinene 6.01	3. (-)-limonene 9.17 (91.0%)	5. Menthone 14.52
2. beta-pinene 6.74	4. (+)-limonene 9.61 (9.0%)	6. menthol 18.96

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



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

Figure 9: Rosewood Oil – Predominant Isomer was (S) Citronellol



Bergamot oil

1. (-)-limonene
2. (-)-linalool
3. linalyl acetate

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

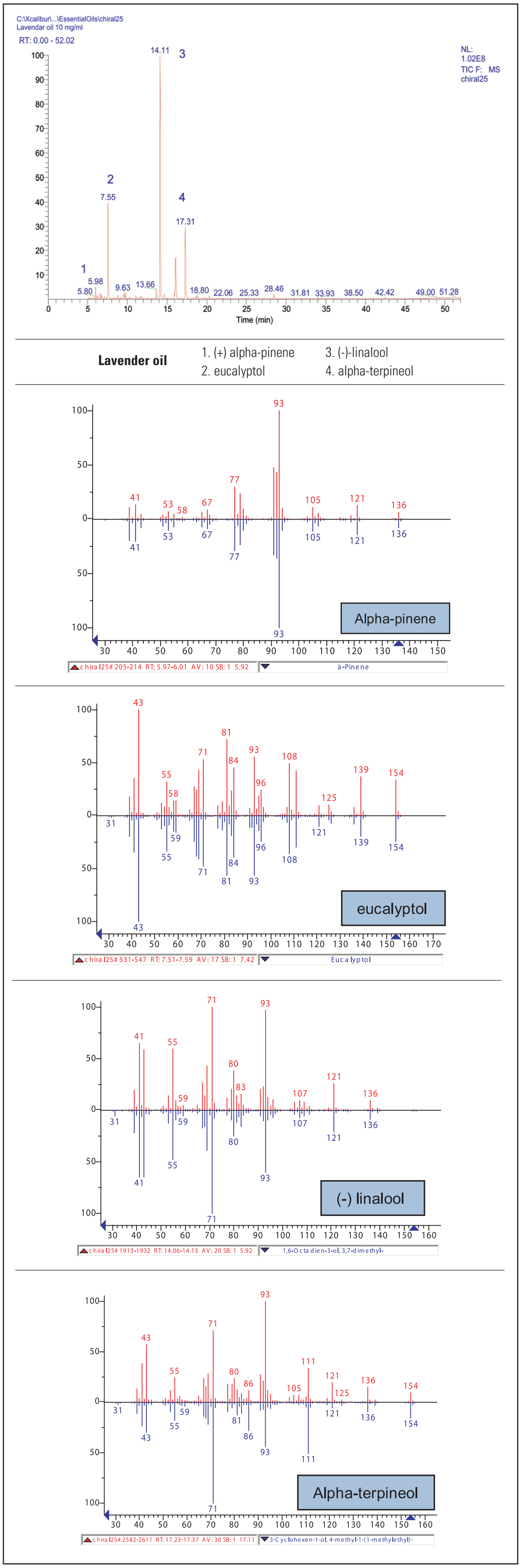


Figure 11: NIST Library Spectra Matching of Enantiomers in Lavender Oil

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 cyclodextrin 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.

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