Analysis of Natural Oils and Extracts Using the Low Thermal Mass LTM Series II System

Application Note

Author

Roger L. Firor
Agilent Technologies, Inc.
2850 Centerville Rd
Wilmington, DE 19808

Abstract

The enantiomers of linalool and other important components of natural oils or natural extracts including cypress oil, geranium extract, balm leaves oil, and jasmin oil are separated on a multidimensional system using two separate GC ovens. The first oven is the conventional 7890 air bath oven equipped with a CFT deans switch and a 30 m x 0.25 mm x 0.25 µm DB5ms, and the second is a direct heated LTM II 5-inch module fitted with a 30 m x 0.25 mm x 0.25 µm CycloDex B chiral column. Use of independent temperature control for the chiral column allows for optimal separation conditions for linalool and other components in these natural materials. Full integration of LTM Series II in the Agilent 7890A GC system enables operation in constant flow mode. Both FID/MSD and FID/FID systems are described.

Agilent Technologies

Introduction

There are two principle use categories for Low Thermal Mass (LTM) technology. The first is simply to speed up separations and overall analysis cycle times while maintaining the desired separation. In this case, a method is usually translated to the use of a shorter and possibly smaller id column when going from an air bath oven to a LTM module. Method translation software is a powerful tool to help the method developer in this task [1]. The second category is use as a secondary oven in conjunction with the primary oven for increased flexibility to achieve a difficult
separation.

Linalool (3,7-dimethyl-1,6-octadien-3-ol) is an important compound in the perfume, food, and alternative medicine industries. Because it has an asymmetric carbon atom, it exists in optically active forms. The 3 (R) – form has a flowery fresh scent, while the 3 (S) + form has a slightly woody, less fresh scent. Separation of the two enantiomers is accomplished using a chiral column. Linalool from synthetic sources will have a nearly equal amount of each enantiomer. In natural products, usually one isomer will predominate. The chromatographic separation of the two isomers can be used to identify origin of the material and identify possible adulteration or counterfeit products. The addition of racemic synthetics to essential oils is a known problem. This adulteration will alter their physiochemical properties with a potentially undesirable outcome. This work is based on an earlier application showing the separation of Linalool enantiomers using the original LTM system. [2]

Deans switching or heat-cutting used in combination with two independent oven programs can be used to optimize separation of key compounds in both natural oils and synthetic formulations. Programming the chiral cyclodextin column to high temperatures that will result in bleed and column degradation can be avoided as well. In the configuration used in this work, it is not necessary to program the cyclodex column above 150 °C, well below its maximum temperature of 200 °C. The sample is injected into a DB5ms column for an initial separation. The deans switch is then used to cut specific sections of the DB5ms column to the LTM II module containing a cyclodextrin column. Linalool and other key compounds can be transferred in a single run to the LTM module for separation using its own optimized temperature program. Use of two independent ovens affords the analyst great flexibility to achieve the desired separation in optimal time.

The LTM Series II incorporates several significant improvements over the original LTM hardware/firmware/software. These include:

- Full integration and control by the Agilent 7890A GC system
- Complete integration in Chemstation
- True constant flow mode of operation
- Real time display of temperatures/pressures/flows

Experimental

Restrictor sizing and flow rate pairing of the primary and secondary columns necessary to balance the system was determined using the Deans Switch Calculator [3]. It is worth noting that the Deans Calculator assumes all columns and restrictor are in the same oven with the same temperature program. Obviously, LTM /7890 systems to not comply with this constraint. Since the starting temperature difference between the air bath oven and LTM module is only 5 °C, there is little impact on restrictor dimensions and the system will be perfectly balanced at the start of the run if restrictor size is based on air oven temperature. As the run proceeds and the temperature programs diverge (air oven and LTM), the restrictor flow and LTM flow may be different, but not enough to cause problems with the ratio of LTM column flow to primary column flow set at about 2 at initial conditions. In the system as described here, the restrictor flow starts at approximately 2.3 mL/min and decreases as the 7890 oven temperature rises to a flow of about 1.2 mL/min at 280 °C. This restrictor can be sized from 0.6 m to 0.7 m for this system.

Firmware and Software Requirements

Agilent 7890A GC system Firmware	A.01.12.1 or greater
Agilent GC ChemStation	B.04.03 DSP1, includes LTM II software control
Agilent MSD ChemStation	E.02.02

LTM Series II System

G6680A, 2 channel, two 5-inch format transfer line assemblies, and two power supplies. Columns ordered separately.

Deans Switch
G2855B or G3440A option #888

LTM Series II Column Configuration

Defining a typical LTM column assembly involves specifying each segment as shown in Figure 1. Both dimension and heat source must be specified. This is necessary for the system to operate in true constant flow mode.

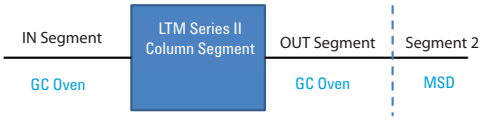

Figure 1. Terminology used to define an LTM Series II assembly.

Agilent 7890A Options

G3440A with options 112, 211, 309

System Configurations

Diagram MSD System

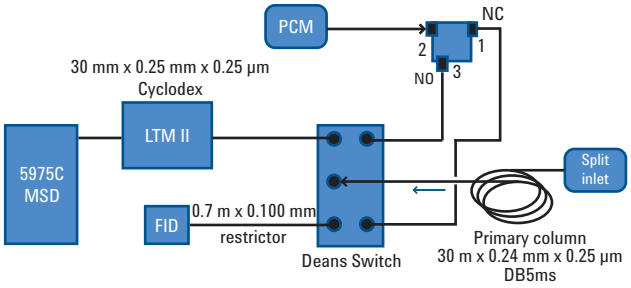

Figure 2. System diagram for the LTM Series II/ Deans/5975C system showing column and restrictor dimensions.

Method Parameters

Inlet	Split/splitless, 280 °C
Split ratio	200:1 to 400:1
Primary column	30 m x 0.25 mm x 0.25 µm DB5ms, 1.3 mL/min (constant flow mode)
7890 Oven program	70 °C (1 min) to 280 °C (17 min) @ 10 °C/min
LTM II Program	75 °C (7 min) to 130 °C (5 min) @ 2 °C/min
Deans switch restrictor	0.7 m x 0.100 µm deactivated fused silica
5975C MSD	scan 25 to 160 amu
Quad	175 °C
Source	230 °C
MSD transfer line	250 °C

LTM Module: Operated in constant flow mode, 2.5 mL/min

Column	30 m x 0.25 mm x 0.25 µm CycloDex
Insegment	0.5 m x .25 mm deactivated fused silica (heated by 7890 oven)
Outsegment	0.8 m x .25 mm deactivated fused silica (heated by 7890 oven)
Outsegment 2	0.17 m x .25 mm deactivated fused silica (heated by MSD transfer line)

FID System

Inlet	Split/splitless, 280 °C
Split ratio	200:1 to 400:1
Primary column	30 m x 0.25 mm x 0.25 µm DB5ms, 1.3 mL/min (constant flow mode)
7890 Oven program	70 °C (2 min) to 280 °C (16 min) @ 10 °C/min
LTM II program	70 °C (7 min) to 130 °C (1 min) @ 2 °C/min
Deans switch restrictor	0.5 m x 0.100 µm deactivated fused silica

LTM Module: Operated in constant flow mode, 2.5 mL/min

Column	20 m x 0.25 mm x 0.25 µm CycloDex, constant flow @ 2.4 mL/min
Insegment	0.5 m x .25 mm deactivated fused silica (heated by 7890 oven)
Outsegment	0.5 m x .25 mm deactivated fused silica (heated by 7890 oven)

Diagram FID System

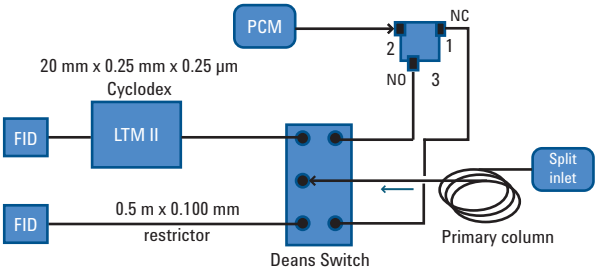

Figure 3. System diagram for the LTM Series II/Deans/FID system showing column and restrictor dimensions.

Samples

- Synthetic linalool standard, Fluka Chemical, Sigma-Aldrich
- Geranium extract (natural), cypress oil, jasmin extract (natural), balm leaf oil (natural), Sigma-Aldrich
- Detergent perfume additive, customer sample
- Orange oil 8X, customer sample

All samples were diluted in methylene chloride as appropriate except the detergent additive, which was injected neat.

Results and Discussion

MSD System

Individual isomers of linalool or other optically active compounds present in natural oils cannot be separated on non-chiral columns such as DB5ms. This is shown in Figure 4 for analysis of geranium extract on a 30 m DB5ms using the monitor FID channel with no heart cuts to the chiral column. Linalool isomers and probably other minor constituents co-elute around 8.3 minutes. Injecting the extract directly on the chiral column is also not a sound approach as elution of all components may require a temperature exceeding the 200 °C maximum of cyclodex. Bleed increases rapidly above 150 °C and will shorten column life as well.

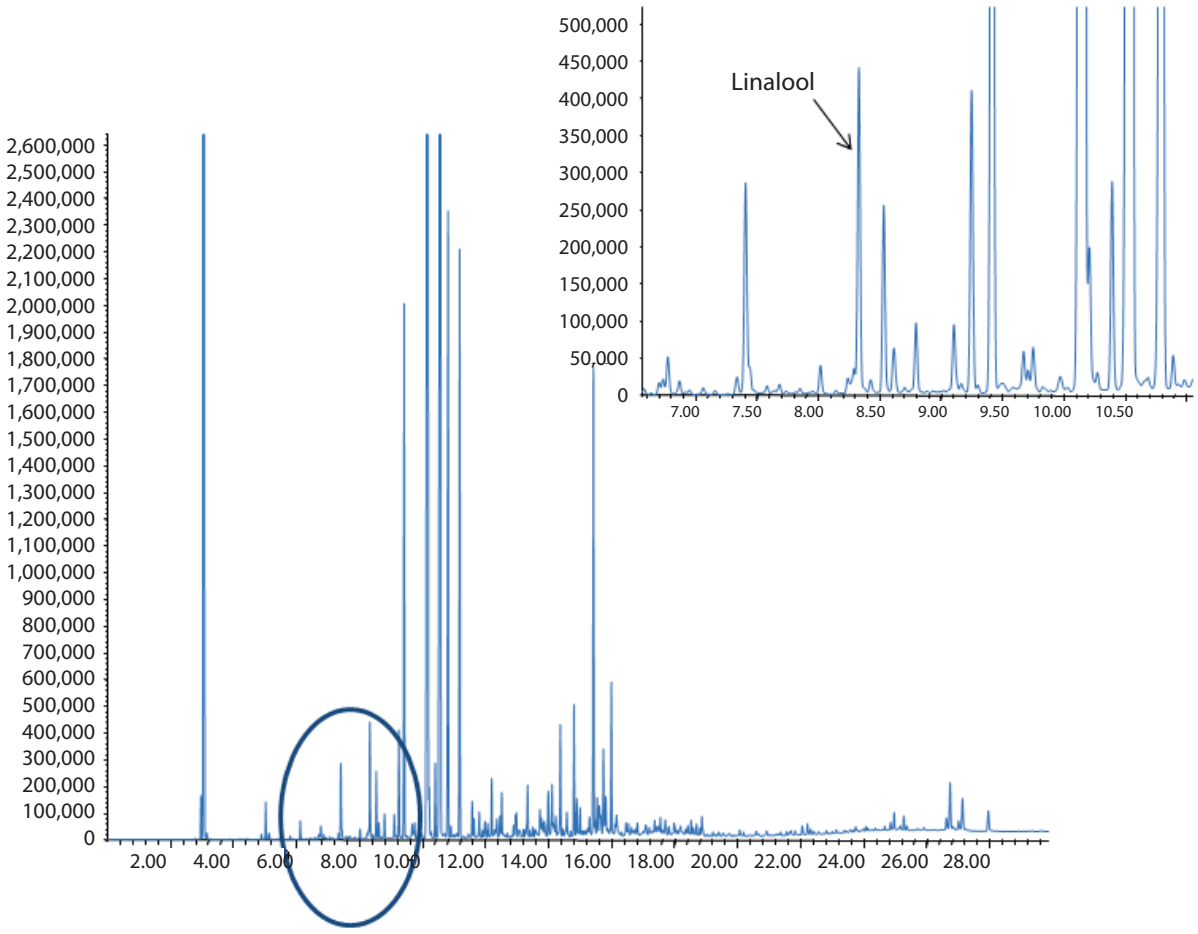

Figure 4. Geranium extract without heart cutting. Sample analyzed on the 30 m DB5ms with FID.

A sample of pure synthetic linalool was used to establish the cut time for analysis on the cyclodex column. Nearly equal amounts of each enantiomer are seen as expected for synthetic linalool when separated on the chiral column. Figure 5 shows distribution of the R and S isomers. Using the established cut window, results for geranium extract is shown in Figure 5 where near baseline separation is obtained using a 2 °C/min program rate on the LTM II chiral column. The absence of co-eluting compounds was confirmed with the MSD. Although this is a natural oil, nearly equal amounts of each linalool isomer is seen. The extraction process, perhaps hydrodistillation, may have caused racemisation to occur.

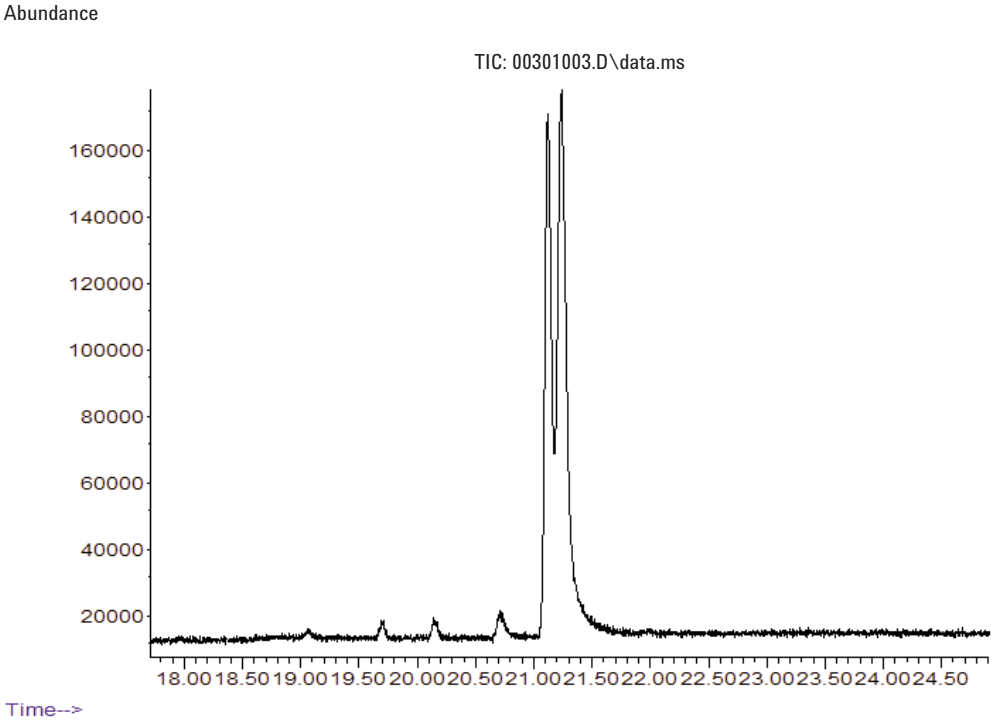

Figure 5. Heart cut to the chiral LTM column using the CFT deans switch. Cut times: 8.25 min to 8.38 min. Both enanitomers of linalool are separated.

Since there are a number of other important terpenoid compounds present in natural oils, it is possible to take advantage of the LTM II/Deans switch system for their analysis. For example, citronellol is of significant value and interest as a flavor and fragrance compound. It can be used for the synthesis of rose oxide, a powerful fragrance component with a strong fruity smell. Citronellol also exists as enantiomers. The S (–) -B-citronellol is present in geranium oil. Multiple retention time based heart cuts in a single run allow analysis of linalool, citronellol, and a few other compounds of interest. The third cut window is wide for transfer of a few major compounds. An example of this analysis is shown in Figure 6.

Linalool enantiomer distributions in other selected natural oils is given in Figures 7 through 10 for balm leaf oil, cypress oil, jasmin extract, and orange oil 8x, respectively. While linalool occurs in nature in both optical forms, all four of these oils show a prevalence of one enantiomer as is usually the case. Orange oil shows essentially enantiomerically pure linalool.

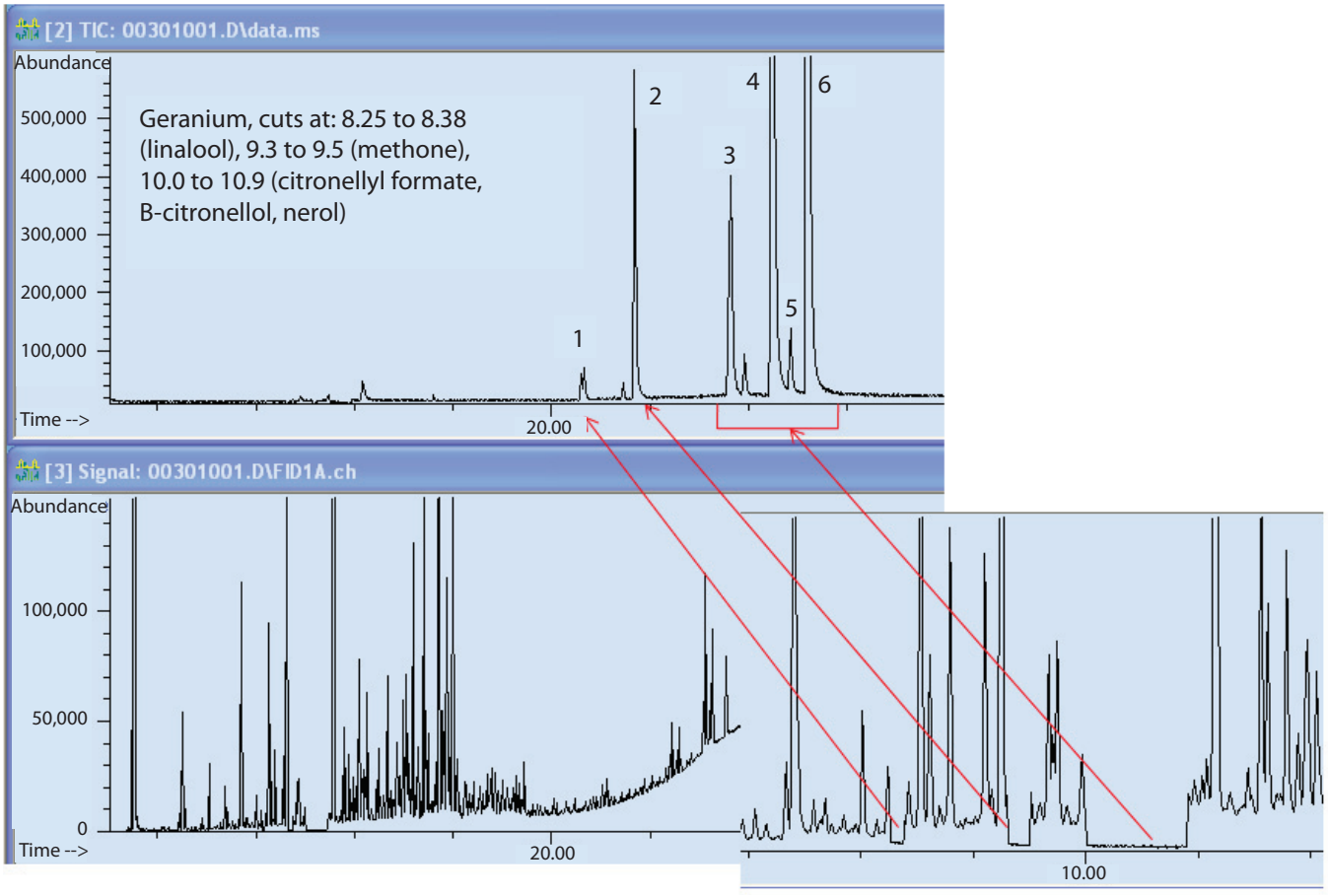

Figure 6. Three heart cuts in one chromatographic run used to separate linalool, methone, citronellyl formate, R (–) -B-citronellol, and nerol on the cyclodex column.

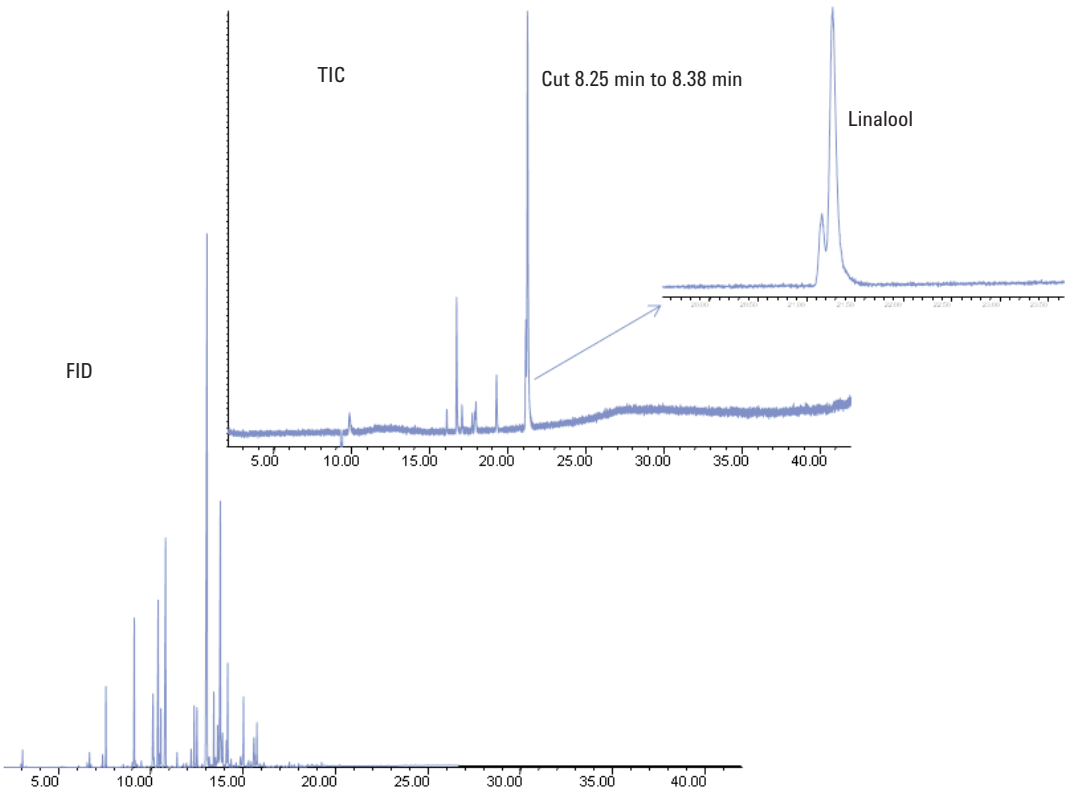

Figure 7. Balm leaf oil. FID, TIC, and zoom in on the linalool separation (upper right).

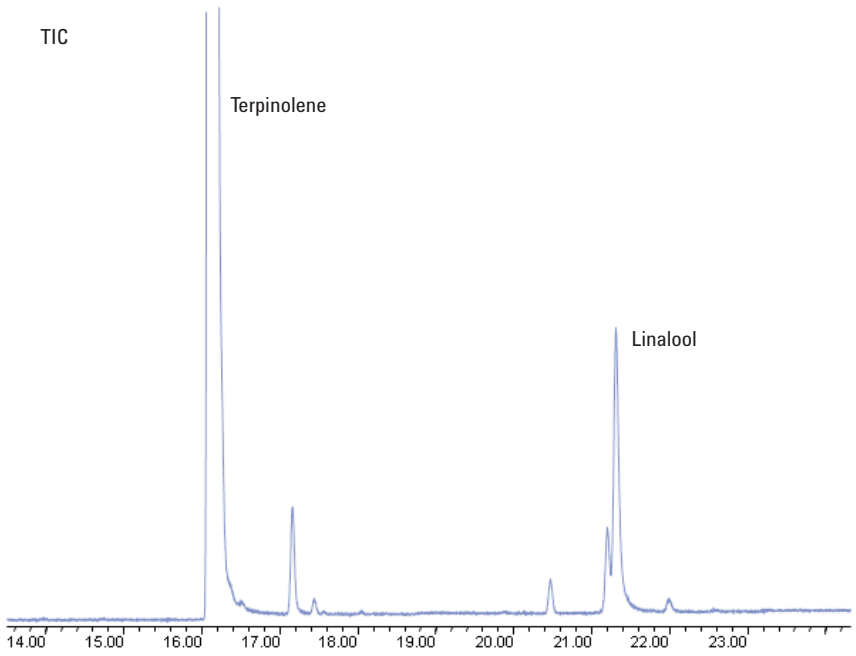

Figure 8. Linalool in cypress oil.

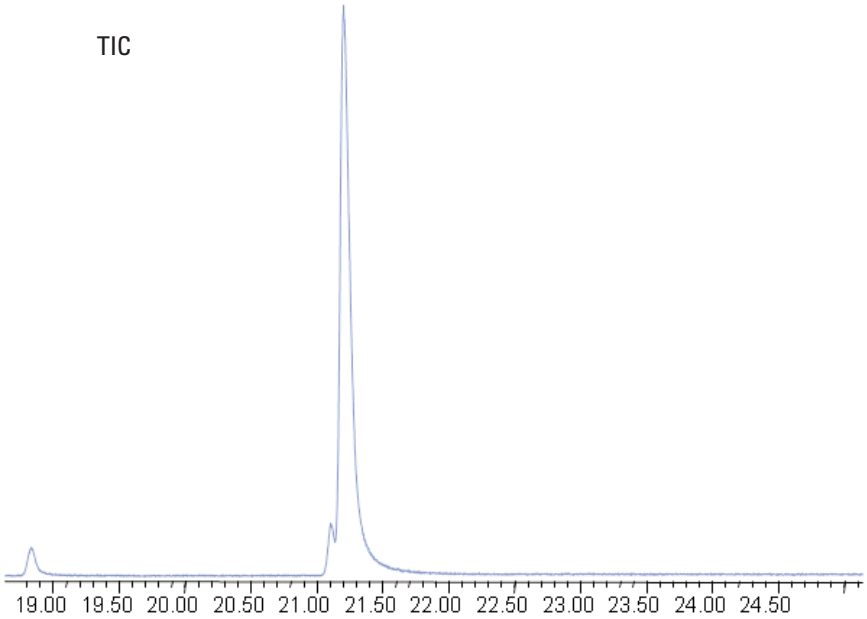

Figure 9. Linalool in jasmine extract showing greater than 95% of one optical isomer.

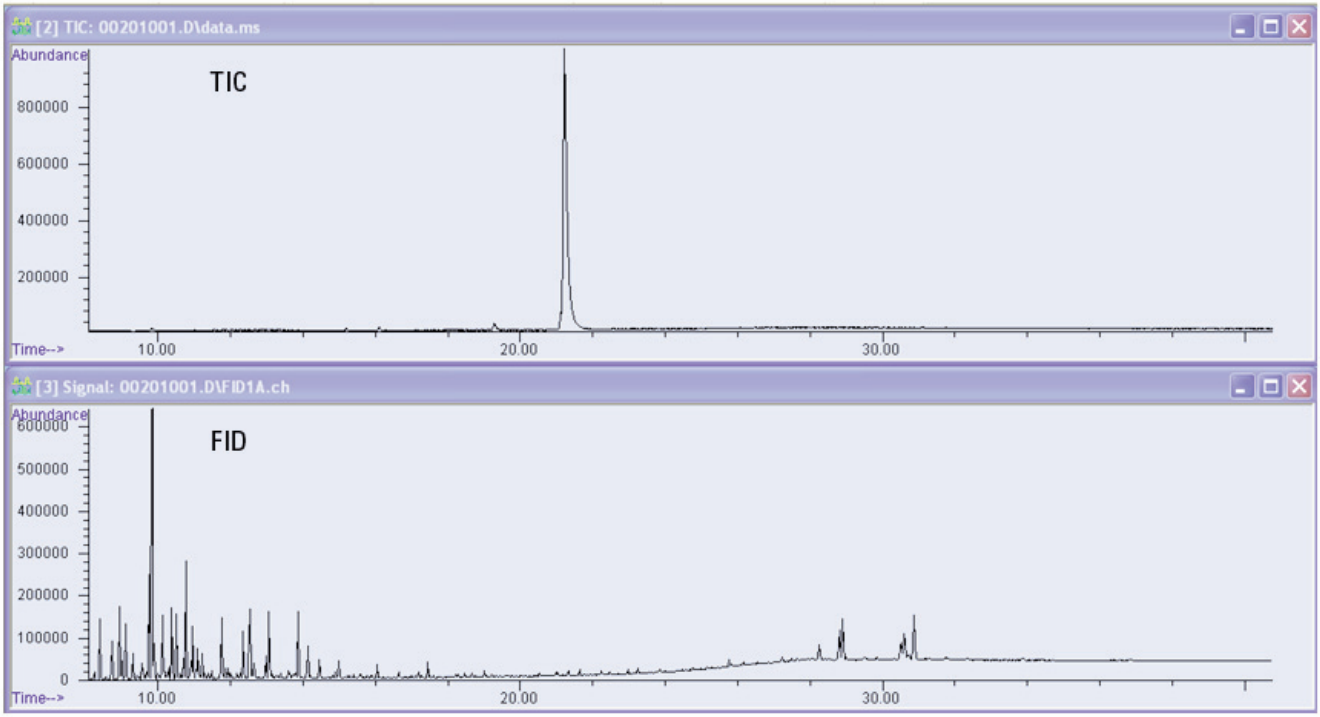

Figure 10. Orange oil 8x. TIC of linalool heart cut and FID monitor channels shown.

Finally, Figure 11 shows the heart cut from a complex detergent fragrance additive. Linalool appears to be of synthetic origin.

FID System

For more routine use, where peaks have already been identified, an FID only system can be used. In this configuration (Figure 3), a 20 m x 0.25 mm x 0.25 µm Cyclodex column was used. This allows for a faster analysis with a tradeoff of some loss of separation of the linalool enantiomers. Although in most cases, sufficient separation is still obtained to identify a material as containing natural or synthetic linalool. An example using cypress oil is shown in Figure 12 that can be compared to Figure 8 with the MSD/30 m LTM system. Elution occurs at about 14 minutes compared to 22 minutes with the 30 m cyclodex column.

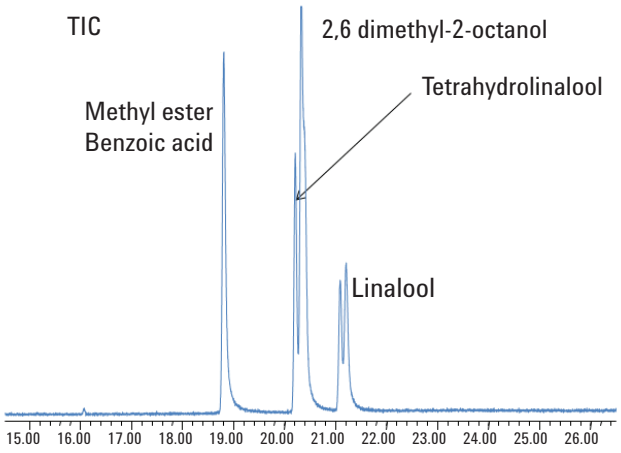

Figure 11. Analysis of linalool in a detergent fragrance.

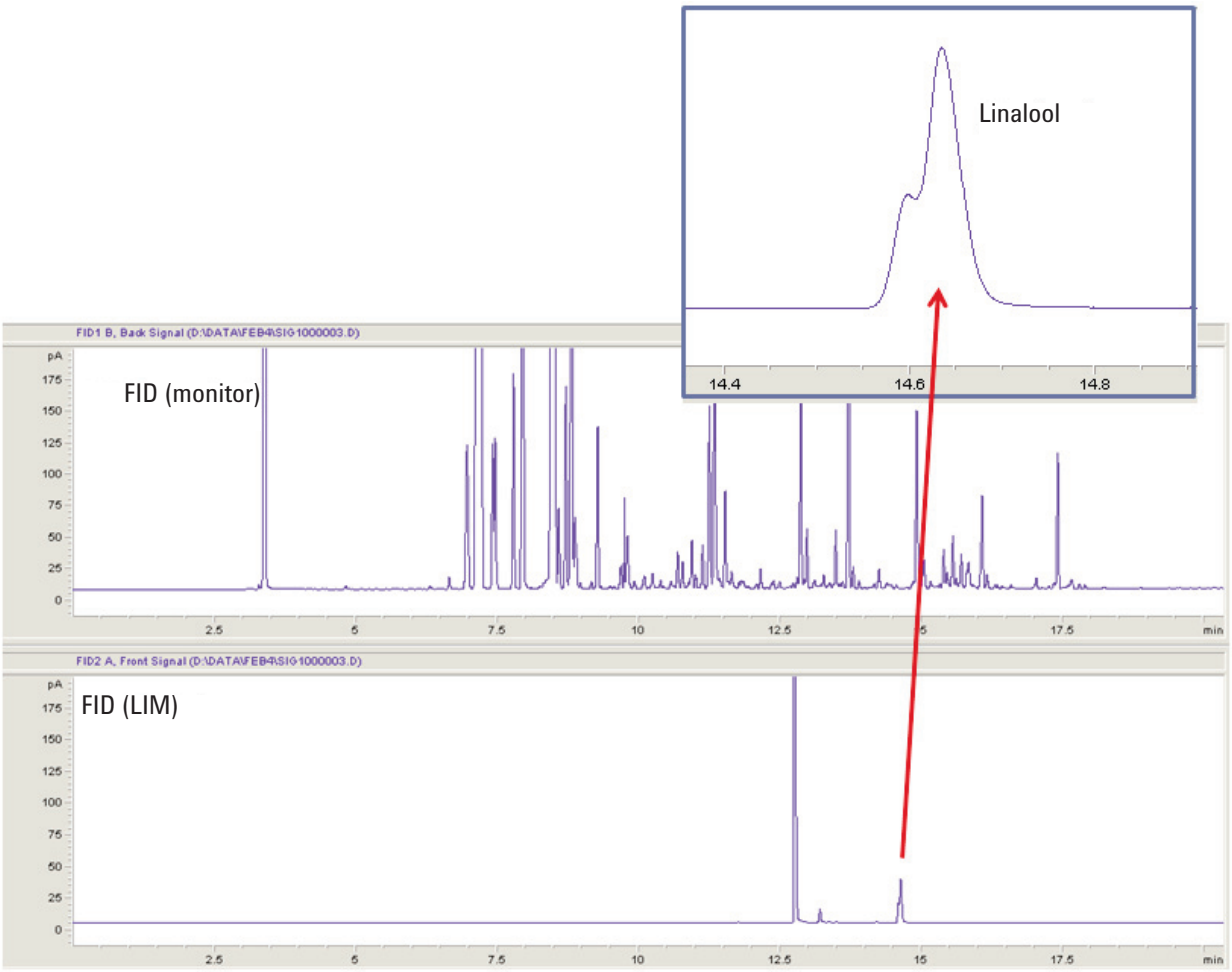

Figure 12. Analysis of linalool in geranium extract using the FID/Deans/LTM system. Cut times to the cyclodex column are 9.25 min to 10.05 min.

Conclusion

Various constituents of essential oils and extracts have considerable value in a number of industries including foods, flavors, natural and synthetic perfumes, alternative medicine, and aromatherapy. Because adulteration is also of concern in many high value formulations, a reliable analytical method is needed to provide positive identification and separation of optical isomers.

LTM series II incorporates a number of new features including full control integrated in Chemsttion, and operation in true constant flow mode that assists the analyst with development of new methods. This is especially true with more complex configurations involving the deans switch and the Agilent 5975C Series GC/MSD. The use of two independent ovens gives the analyst flexibility to optimize analysis of natural oils or synthetic formulations especially where chiral separations can be used for origin determination and authenticity. In many applications, analysis time will also be shorter since only compounds of interest are cut to the chiral column.

The systems described here can serve as valuable quality control tools for natural oils and perfumes. For routine QA, the simpler FID/FID can be considered where cost and ease of use is a priority.

The combination of LTM/CFT Deans/5975C can serve as the core for a wide variety of analytical problems beyond the chiral applications highlighted here. Only a change in the column sets is required.

References

1. Method Translation Software, downloadable from www.chem.agilent.com
2. “Independent Column Temperature Control using an LTM Oven Module for Improved Multidimensional Separation of Chiral Compounds”, Frank David and Matthew Klee, Application Note 5990-3428EN.
3. Deans Switch Calculator, available on the “Agilent Capillary Flow Technology Accessories” CD, included with CFT devices.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
June 8, 2011
5990-8151EN

Agilent Technologies