

Independent Column Temperature Control Using an LTM Oven Module for Improved Multidimensional Separation of Chiral Compounds

Application Note

Food and Flavors

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Abstract

An improved chiral separation of the enantiomers of linalool using a multidimensional capillary GC system with Deans switch heart-cutting and independent column temperature control is described. Linalool in a perfume sample and a lavender oil sample is initially separated on a non-chiral apolar column (Agilent J&W HP-5ms) installed in the standard GC oven. The linalool peak is heart-cut to a chiral cyclodextrin column woven with low thermal mass (LTM) components and installed in an LTM column module at lower temperature for better separation of enantiomers. Using the two-dimensional LTM configuration with independent column temperature control, better chiral separation, less cyclodextrin stationary phase bleed, and shorter analysis time were achieved. In addition, the chiral column is protected from low-volatility sample components that might contaminate the column, decreasing method performance and reducing column lifetime.



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Introduction

Linalool is an important flavor component in many essential oils, including lavender oil, geranium oil, and bergamot oil. It has a pleasant flowery odor. Proper characterization of linalool content in natural oils and perfumes includes determining the enantiomeric composition, especially when trying to uncover adulteration and counterfeiting. Chiral separation of the (+) and (–) enantiomers of linalool is possible with cyclodextrin-based capillary GC columns without the need for derivatization [1].

The gas chromatographic separation of enantiomers on a cyclodextrin-based column is strongly dependent on the column temperature: the lower the temperature, the better the separation. Of course this has to be balanced against excessive analysis times and ensuring elution of later-eluting sample components that might remain in the column at low temperatures, affecting subsequent runs. Cyclodextrin-based columns have a limited maximum allowable operating temperature (MAOT), exhibit high levels of bleed, and are less robust (more prone to oxidative degradation) than standard apolar capillary column phases, so heating to high temperatures for extended times to clean out highly retained sample components is not reasonable. Unfortunately, unlike most silicone-based stationary phases, cyclodextrin phases can also not be regenerated by solvent rinsing when contaminated.

For these reasons, a multidimensional GC configuration is preferred to exclude low-volatility components and reduce column exposure to high oven temperatures [2, 3]. To this end, a pre-separation on a nonselective column is used to isolate the peak(s) of interest from most other sample components. By heart-cutting target peak(s) to a chiral second-dimension column, enantiomers can be separated cleanly. For optimal results, both column temperatures are independently controlled. One way to accomplish this is to have two laboratory GCs side by side. Although effective, the cost and space requirements of this approach are often prohibitive. Another option is to use a low thermal mass (LTM) module integrated into the GC oven door, thereby minimizing impact on system footprint size, lowering cost of implementation while still providing completely independent column temperature zone [4].

In this application note, the separation of the enantiomers of linalool in perfume and lavender oil samples using two-dimensional capillary gas chromatography (CGC) with Deans switch heart-cutting and independent temperature control of columns using an LTM column module is described.

Experimental

Samples

Three samples were prepared: a reference sample containing 0.1% (synthetic) linalool in dichloromethane for calibration, a commercial perfume diluted to 5% in dichloromethane (50 mg diluted in 1 mL), and a natural lavender oil diluted to 2% in dichloromethane (20 mg in 1 mL).

Configuration

The analyses were performed on an Agilent 7890A GC system/Agilent 5975C Series GC/MSD combination. The GC was equipped with a split/splitless inlet, flame ionization detector (FID), pneumatics control flow module, a Capillary Flow Technology Deans switching system (p/n G2855B), Agilent LTM system controller bundle (p/n G6579A), and an Agilent J&W LTM column module (standard 5-inch size for fastest cooldown).

The primary column was a 30 m × 0.25 mm id × 0.25 μm Agilent J&W HP-5MS installed in the GC oven (from the split/splitless inlet to the Deans switch). A 30 m × 0.25 mm id × 0.25 μm Agilent J&W CycloDex-B (p/n 112-2532) chiral column was woven with LTM direct heating and sensing components heating then installed in the LTM column module (p/n 112-2532LTM column). A 0.5 m × 250 μm id deactivated fused silica tubing was used to connect the LTM column to the Deans switch, and a 1 m × 250 μm id deactivated fused silica tubing was used as a transfer line to connect the LTM column to the mass selective detector (MSD). A 63 cm × 100 μm deactivated fused silica capillary connected the Deans switch to a monitoring FID.

A configuration diagram is shown in Figure 1.

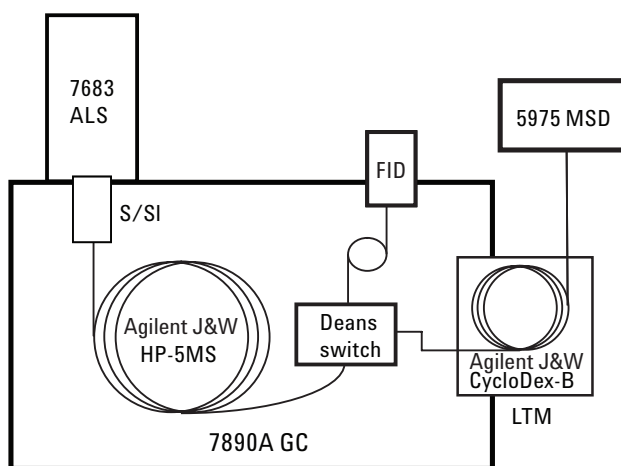


Figure 1. System configuration for two-dimensional heart-cut with independent column temperature control.

Analytical Conditions

Injection:	1 μ L, 280 $^{\circ}$ C, 1:100 split ratio
Carrier:	Helium, 1 mL/min constant flow mode (column inlet = SSL; outlet = PCM1) (179 kPa at 70 $^{\circ}$ C)
GC oven temp:	70 $^{\circ}$ C (1 min) 250 $^{\circ}$ C (11 min) at 10 $^{\circ}$ C/min
PCM 1:	2 mL/min constant flow mode (128 kPa at 70 $^{\circ}$ C)
LTM oven:	80 $^{\circ}$ C (9 min = end of heart-cut) 100 $^{\circ}$ C (1 min) at 1 $^{\circ}$ C/min
Total run time:	30 min
FID monitor detector:	300 $^{\circ}$ C, 30 mL/min H ₂ , 400 mL/min air
Deans switch valve:	Initially off, on at 8.5 min, off at 8.9 min (fraction from 8.5 to 8.9 min transferred to column 2)
MS:	Scan mode, 40 to 300 amu, Sampling rate = 2 ¹

Results and Discussion

The effect of temperature on resolution of linalool enantiomers on cyclodextrin columns can be demonstrated by the analysis of a reference sample containing synthetic linalool (both enantiomers present in 50/50 ratio). The chromatogram obtained with the cyclodextrin column using a temperature program (70 $^{\circ}$ C for 1 min \rightarrow 200 $^{\circ}$ C at 10 $^{\circ}$ C/min) is shown in Figure 2. From the chromatogram, it is clear that the enantiomers are not separated at the elution temperature of 165 $^{\circ}$ C. High bleed is also apparent above 190 $^{\circ}$ C.

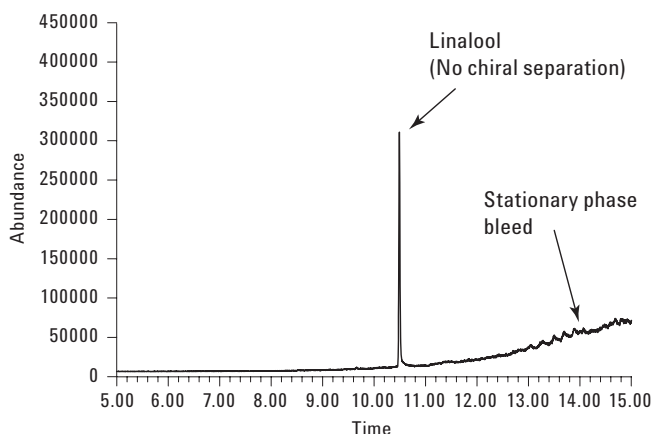


Figure 2. Reference linalool sample heart-cut to LTM module. The cyclodextrin secondary column was programmed at 70 $^{\circ}$ C (1 min) \rightarrow 250 $^{\circ}$ C (11 min) at 10 $^{\circ}$ C/min. Linalool enantiomers are not separated at the 165 $^{\circ}$ C elution temperature at 10.5 min.

Using a slower temperature program (80 $^{\circ}$ C for 9 min \rightarrow 100 $^{\circ}$ C at 1 $^{\circ}$ C/min), the enantiomers could be separated, as shown in Figure 3 (elution at 23 min = 94 $^{\circ}$ C elution temperature).

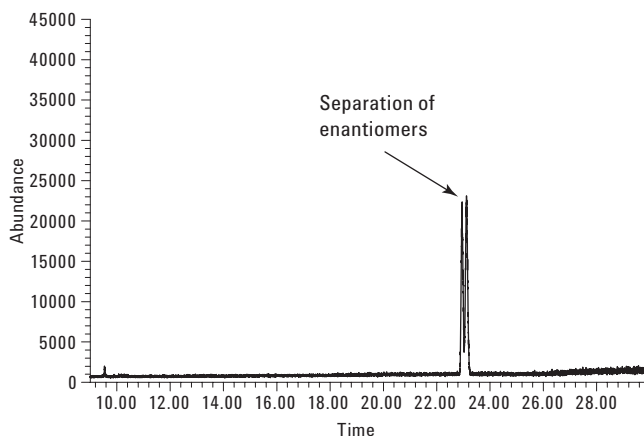


Figure 3. Reference linalool sample heart-cut to LTM module (cyclodextrin secondary column) operated with slower temperature program or 80 $^{\circ}$ C for 9 min \rightarrow 100 $^{\circ}$ C at 1 $^{\circ}$ C/min. Linalool enantiomers are separated at 94 $^{\circ}$ C elution temperature.

The chromatogram in Figure 4 shows why these conditions are unfortunately not suitable for analysis of real samples. The separation of a perfume sample directly on the chiral column using the original temperature program is shown. A hold of 16 minutes at the 200 $^{\circ}$ C ending temperature was needed to elute the complete sample. In addition, a very high bleed is seen, indicating that the column is being thermally stressed. It is also evident that many other sample components elute close to linalool, potentially complicating quantitation even of the unseparated enantiomers. If the slower temperature programs were used, analysis time would be excessively long and the potential for incomplete sample elution would increase.

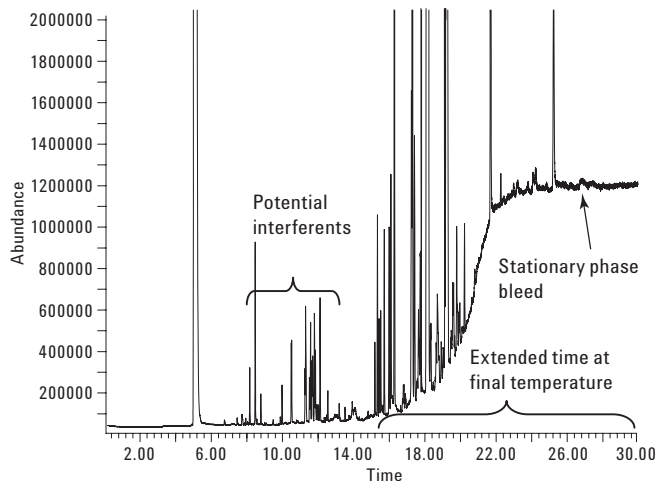


Figure 4. One-dimensional separation of perfume sample on chiral column only 70 $^{\circ}$ C (1 min) \rightarrow 200 $^{\circ}$ C (16 min) at 10 $^{\circ}$ C/min. Linalool enantiomers are not separated, considerable potential interferents are evident, and stationary phase bleed is high.

In order to effectively analyze linalool enantiomers while avoiding contamination of and stress to the chiral column, a two-dimensional GC separation was developed. Samples were initially separated on a nonselective, apolar, Agilent J&W HP-5MS primary column. This column separates sample components based on volatility, is much more rugged than the chiral column, and bleed is low. Unfortunately, no chiral separation is possible with this phase. The chromatogram shown in Figure 5a is from the FID monitoring primary column effluent when it is not being heart-cut. Linalool elutes at 8.7 minutes.

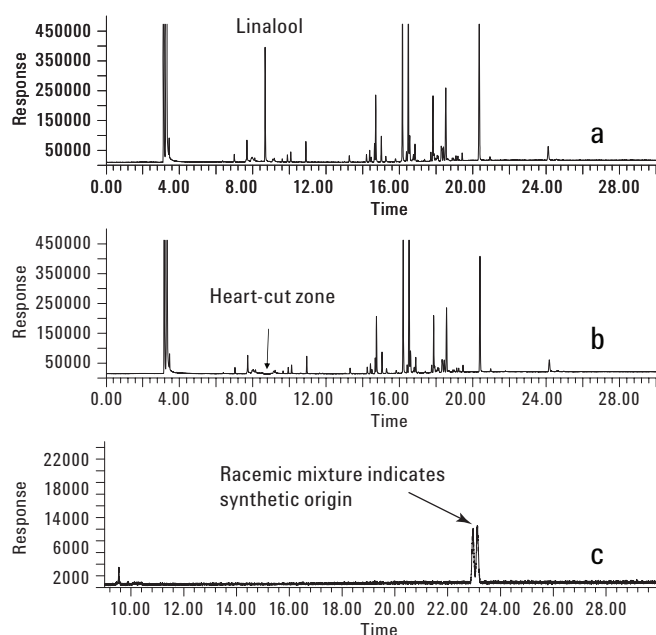


Figure 5. (a) Perfume sample on Agilent J&W HP-5ms primary column with no heart-cut. (b) Chromatogram of perfume sample with heart-cut from 8.5 to 8.9 min to secondary column. (c) Chromatogram (MSD) of linalool separated on cyclodextrin secondary column.

A heart-cut to the lower-temperature chiral column was then performed between 8.5 and 8.9 minutes (containing the linalool peak). One can see a flat part of the baseline in the FID monitor chromatogram of Figure 5b corresponding to the heart-cut zone. The linalool peak is thereby isolated from other sample components, including late-eluting solutes that are not introduced in the chiral secondary column.

The simultaneous separation of the linalool fraction on the chiral column is shown in Figure 5c. From this chromatogram, it is clear that at the lower temperature the two enantiomers are present, and there is an absence of co-eluting peaks. In this case, a racemic mixture (equal amounts of enantiomers) is clearly evident, indicating that the linalool is from a synthetic source.

The multidimensional analysis was repeated on a lavender oil sample. The chromatograms of bulk sample separation on the primary column and heart-cut chiral separation on the secondary column are shown in Figures 6a through 6c. In this case, the linalool peak nearly exclusively contains a single optical isomer, indicating that the linalool is from a natural source.

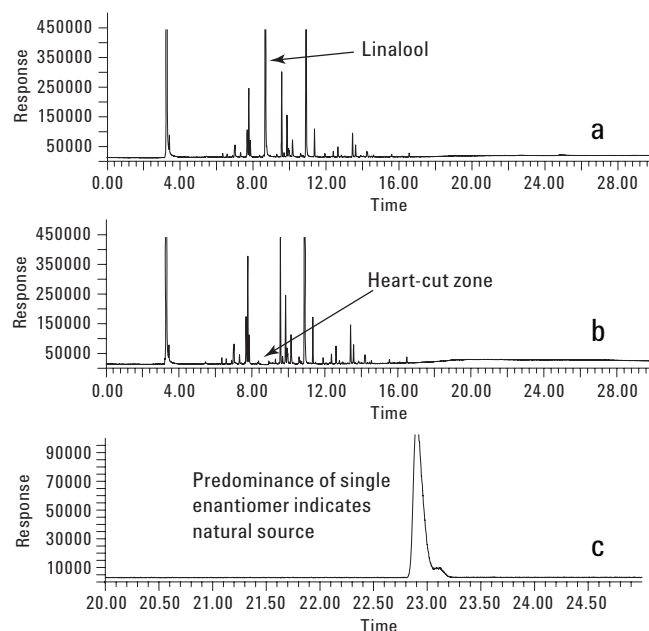


Figure 6. (a) Lavender sample on Agilent J&W HP-5ms primary column with no heart-cut. (b) Chromatogram of lavender sample with heart-cut from 8.5 to 8.9 min to secondary column. (c) Chromatogram (MSD) of linalool separated on cyclodextrin secondary column, showing predominantly one enantiomer.

Conclusions

This application demonstrates the benefits of independent column temperature control for two-dimensional GC separations. The control of column temperature is especially critical with chiral stationary phases such as cyclodextrins. Use of an LTM column module for the second temperature zone provides a cost-effective means of adding independent temperature zones to an Agilent 7890A GC system while taking up little incremental space.

Improved resolution of optical isomers of linalool using lower temperature and slow temperature ramps (isothermal conditions may also be effective) was demonstrated. Similar improvements can be expected for separation of other chiral compounds that are amenable to GC.

Samples of a commercial perfume and a natural lavender oil were effectively analyzed with the multidimensional system pairing Deans switch heart-cutting from a nonselective primary column to a chiral secondary column. Benefits of the approach include lower cost, better data quality (better separation of linalool peaks and isolation from potential interferents), and less thermal and chemical stress to the delicate chiral column.

References

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© Agilent Technologies, Inc., 2009
Published in the USA
January 5, 2009
5990-3428EN



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