Rapid Analysis of Food and Fragrances Using High-Efficiency Capillary GC Columns

Application

Food, Flavors and Fragrances

Authors

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Abstract

An analysis of various essential oils/flavors was performed using both polar and nonpolar high-efficiency 0.18-mm id GC columns. Agilent's GC method translation software was used to translate existing 0.25-mm id column methods to 0.18-mm id columns. The ease with which this software can be used allowed for simple method development. Elution times were compared between the standard 0.25-mm id columns and the high-efficiency 0.18-mm id columns. The benefits of using hydrogen carrier gas for shorter analysis time will also be illustrated.

Introduction

There are many misconceptions about what it means to perform fast gas chromatography (GC) and what the term fast GC implies. Fast GC is often associated with the use of hydrogen as a carrier gas, and although this is certainly a good approach, it is not always necessary in order to shorten the analysis time. A second misconception is that changing column dimension results in time-consuming method development. Utilizing 0.18 mm id high-efficiency GC columns can greatly reduce the analysis time. When coupled with Agilent's GC method translation software, the time spent on method development can be greatly minimized.

Efficiency is often related to the number of theoretical plates (n) that a column has and is expressed as plates per meter. It follows that the longer the column, the more plates you have, and thus the more efficient the column. One way to measure column efficiency is to calculate the height equivalent of a theoretical plate (HETP = h). Following Equation 1, the lower the value of h is, the greater the value of n and therefore the efficiency. The shorter the plate is, the larger the number of plates that can be "stacked" in a given length of column. By reducing the column id, the plate height is reduced, which results in more plates per meter (see Equation 2). A more efficient, smaller id column can be used to obtain the same number of plates in a shorter length of column. The shorter the column, the less time the analytes take to travel that length of column, which equates to shorter analysis times without the loss of efficiency or resolution.

$$h = \frac{L}{n}$$

Equation 1: Height Equivalent to a Theoretical Plate

h = height equivalent to a theoretical plate
L = length of the column in millimeters
n = number of theoretical plates

Equation 2: Height Equivalent to a Theoretical Plate in Relation to Column Diameter

$$h_{\min} = r\sqrt{\frac{(11k^2 + 6k + 1)}{3(1+k)^2}}$$

h_{min} = height equivalent to a theoretical plate
r = radius of column
k = capacity factor (partition coefficient) of an analyte

The high-efficiency GC columns are designed to maintain the same phase ratio as the more commonly used 0.25-mm id columns, making for easy method translation, as will be illustrated. Phase ratio is a unitless measure of the relationship between the column radius and the stationary phase thickness. If this calculated number changes when changing from one dimension column to another, there is a change in the retention of a particular solute (k). Equation 3 illustrates that even though a shorter column means a shorter time that the analyte takes to elute from the column, k will remain constant because the unretained compound will also elute more quickly.

Equation 3: Partition Ratio

$$k = \frac{t_r - t_o}{t_o}$$

k = partition coefficient of an analyte
t_r = retention time of analyte
t_o = retention time of an unretained compound

During the chromatographic process, the resulting chromatogram and its associated resolution are the product of the thermodynamics of the system. If the dimensions of the column are changed, then the thermodynamics of the system also change. A new temperature program must be developed to match the new column dimensions. This is why most analysts avoid trying to go faster; the time and energy that goes into developing a new method just isn't worth it. One solution to this problem is to utilize the GC method translation software that is available online at the Agilent Web site http://www.chem.agilent.com/cag/servsup/usersoft/files/GCTS.htm (see Figure 1). This free software takes the guesswork out of developing a new temperature program. This assumes that the same column phase type and same phase ratio are being used between the two methods. It is not imperative to use the same phase ratio; however, if the phase ratio is not maintained, the elution order should be confirmed. An additional option for faster analysis is to utilize a more efficient carrier gas. When changing carrier gas types from one to another, the method translation software takes into account the efficiencies of the four most commonly used carrier gases (argon, nitrogen, helium, and hydrogen) and adjusts the method parameters accordingly.

In this application the benefits of using high-efficiency columns to shorten run times will be illustrated. Two commonly used columns for food/fragrance analysis are the DB-1 and DB-WAX. A comparative analysis will be performed between a more commonly used dimension (30 m × 0.25 mm × 0.25 µm) and that of the high-efficiency column dimension (20 m × 0.18 mm × 0.18 µm). In addition, the ability of the GC method translator to minimize time spent performing method development will be demonstrated. The benefits of using hydrogen as a carrier gas in conjunction with the high-efficiency columns will also be addressed.

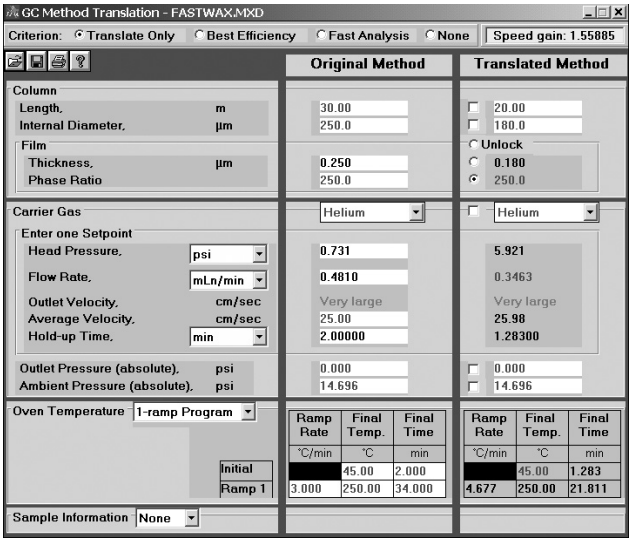

Figure 1. Method translation input screen.

Experimental

All analyses were performed using an Agilent 6890 GC with a 5973 MSD, equipped with a split/splitless inlet. The analytical conditions are summarized in Table 1 (DB-1 columns) and Table 2 (DB-WAX columns). Original method parameters were not optimized for each compound, but rather developed to accommodate a wide range of essential oils and fragrances. Method parameters used for the high-efficiency columns were translated directly from the Agilent method translation software. Spearmint and ylang-ylang samples were prepared by dilution of neat oils with acetone at roughly 40:1.

Table 1. Method Conditions for DB-1 Columns

Method A	
Column	30 m × 0.25 mm × 0.25 µm DB-1 p/n 122-1032
Carrier	Helium 25 cm/sec measured at 40 °C
Injector	250 °C, Split 40:1, 1-µL injection
Oven	40 °C hold 1 min
	5 °C/min to 290 °C
Method B	
Column	20 m × 0.18 mm × 0.18 µm DB-1 p/n 121-1022
Carrier	Helium 26 cm/sec measured at 40 °C
Oven	40 °C hold 0.64 min
	4.67 °C/min to 290 °C
Method C	
Column	20 m × 0.18 mm × 0.18 µm DB-1 p/n 121-1022
Carrier	Hydrogen 47 cm/sec measured at 40 °C
Oven	40 °C hold 0.38 min
	13 °C/min to 290 °C hold 13.09 min

Table 2. Method Conditions for DB-WAX columns

Method A	
Column	30 m × 0.25 mm × 0.25 µm DB-WAX p/n 122-7032
Carrier	Helium 25.4 cm/sec measured at 45 °C
Injector	250 °C, Split 30:1, 1-µL injection
Oven	45 °C hold 2 min
	3 °C/min to 250 °C hold 34 min
Method B	
Column	20 m × 0.18 mm × 0.18 µm DB-WAX p/n 121-7022
Carrier	Helium 26.3 cm/sec measured at 45 °C
Oven	45 °C hold 1.28 min
	4.68 °C/min to 250 °C hold 21.81 min
Method C	
Column	20 m × 0.18 mm × 0.18 µm DB-WAX p/n 121-7022
Carrier	Hydrogen 44.3 cm/sec measured at 45 °C
Oven	45 °C hold 0.77 min
	7.79 °C/min to 250 °C hold 13.09 min

Results and Discussion

Typical chromatograms are presented here for all three GC methods on both the DB-1 and DB-WAX columns. Peak identities can be found in Tables 3A and 3B. Significant speed gain is achieved by simply switching to the high-efficiency column and continuing to use helium as the carrier, without a loss of resolution. Spearmint was tested on the DB-1 column. The elution time for the last-eluting compound for spearmint is viridiflorol, which decreased from 27.41 minutes to 17.73 minutes, as illustrated in Figures 1 and 2. This represents a speed gain of approximately 35%. Resolution between three close-eluting compounds remained nearly identical as is illustrated in Table 4. Elution time of the last compound for ylang-ylang oil tested on the DB-WAX column, benzyl salicylate, decreased from 63.47 to 41.07 minutes. This is illustrated in Figures 3 and 4. This represents a speed gain of approximately 35%. Resolution between two pairs of compounds remained essentially unchanged (see Table 5).

Using hydrogen as a carrier gas in conjunction with the high-efficiency columns resulted in additional speed gains. Due to its small molecular size, hydrogen can be used at higher velocities without loss of efficiency. These additional benefits are illustrated in Figures 5 and 6 as well as in Tables 4 and 5. The overall speed gain from the original method was found to be 61% for both the DB-1 and DB-WAX methods. The method translation software allowed for essentially plug-and-play method development. The results that were obtained were used without modification to the values provided by the translation software.

Table 3A. Component List for DB-1 Chromatograms

	Compound List for Spearmint Oil Chromatogram				
1	α-Pinene	12	γ-Terpinene	23	cis-Carvyl acetate
2	Sabinene	13	trans-Sabinene hydrate	24	cis-Jasmone
3	ß-Pinene	14	Terpinolene	25	ß-Bourbonene
4	3-Octanol	15	Linalool	26	α-Bourbonene
5	Myrcene	16	3-Octyl acetate	27	ß-Caryophylene
6	α-Terpinene	17	Isomenthone	28	α-Copaene
7	ρ-Cymene	18	Terpinen-4-ol	29	trans-ß-Farnesene
8	1,8-Cineol	19	Dihydrocarvone	30	Germacrene-d
9	Limonene	20	trans-Carveol	31	Viridiflorol
10	cis-Ocimene	21	l-Carvone		
11	trans-Ocimene	22	trans-Dihydrocarveol acetate		

Table 3B. Component List for DB-WAX Chromatograms

	Compound List for Ylang-Ylang Oil Chromatogram				
1	α-Pinene	7	Methyl benzoate	13	Geranial acetate
2	Methyl-ρ-cresol	8	α-Caryophyllene	14	trans-Cinnamyl acetate
3	α-Copaene	9	Germacrene-d	15	ß-Bisbolene
4	α-Gurjunene	10	Benzyl acetate	16	Farnesyl acetate
5	Linalool	11	Farnescene	17	Benzyl benzoate
6	ß-Caryophyllene	12	δ-Cadinene	18	Benzyl salycilate

Table 4. Resolution of Closely Eluting Compounds by Column ID and Carrier Gas

	DB-1 ID and Carrier Gas Type		
Compound(s)	0.25 mm Helium	0.18 mm Helium	0.18 mm Hydrogen
Sabinene β-Pinene	1.52	1.59	1.56
α-Terpinene p-Cymene	1.61	1.73	1.86
Speed gain	N/A	35%	61%

Table 5. Resolution of Closely Eluting Compounds by Column ID and Carrier Gas

	DB-WAX ID and Carrier Gas Type		
Compound(s)	0.25 mm Helium	0.18 mm Helium	0.18 mm Hydrogen
α-Farnesene δ-Cadinene	2.16	2.14	2.13
δ-Cadinene Geranial acetate	1.67	1.66	1.64
Speed gain	N/A	35%	61%

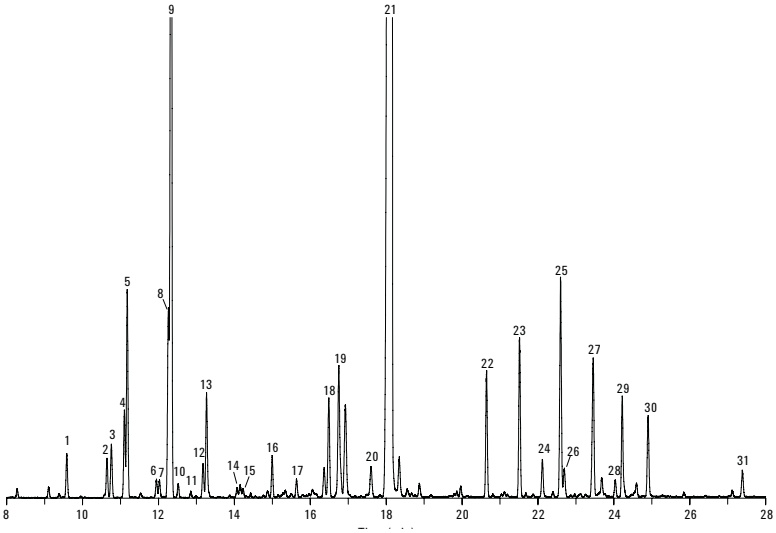

Figure 1. Spearmint oil sample on a DB-1, 30 m x 0.25 mm x 0.25 µm column and He carrier. (See Table 1, Method A, for experimental parameters.)

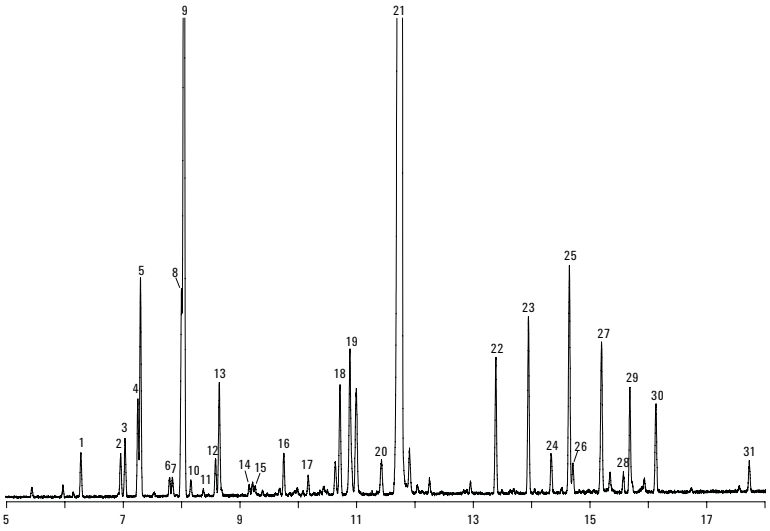

Figure 2. Spearmint oil sample on DB-1, 20 m x 0.18 mm x 0.18 µm column, He carrier. (See Table 1, Method B, for experimental parameters.)

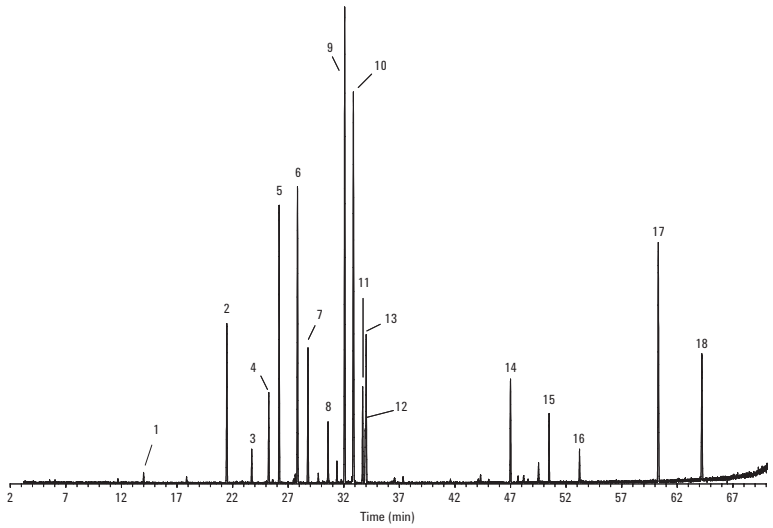

Figure 3. Ylang-ylang oil sample on a DB-WAX, 30 m x 0.25 mm x 0.25 µm column and He carrier. (See Table 2, Method A, for experimental parameters.)

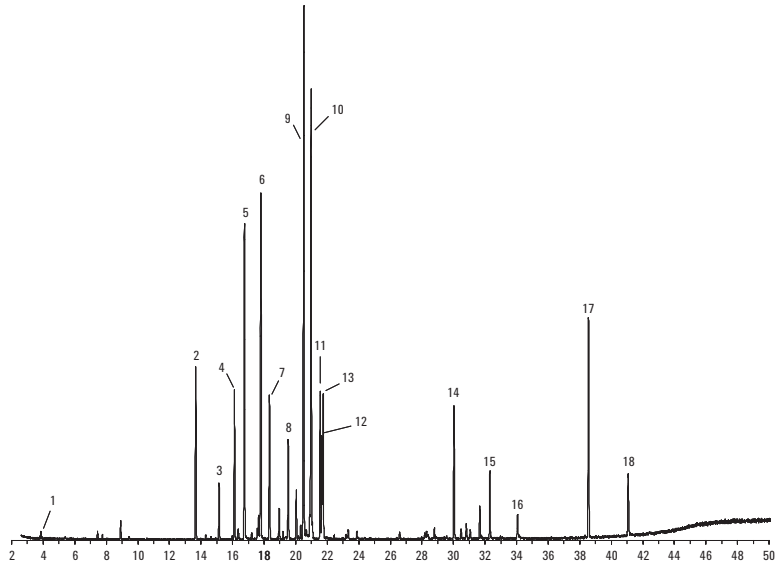

Figure 4. Ylang-ylang oil on DB-WAX, 20 m x 0.18 mm x 0.18 µm column, He carrier. (See Table 2, Method B, for experimental parameters.)

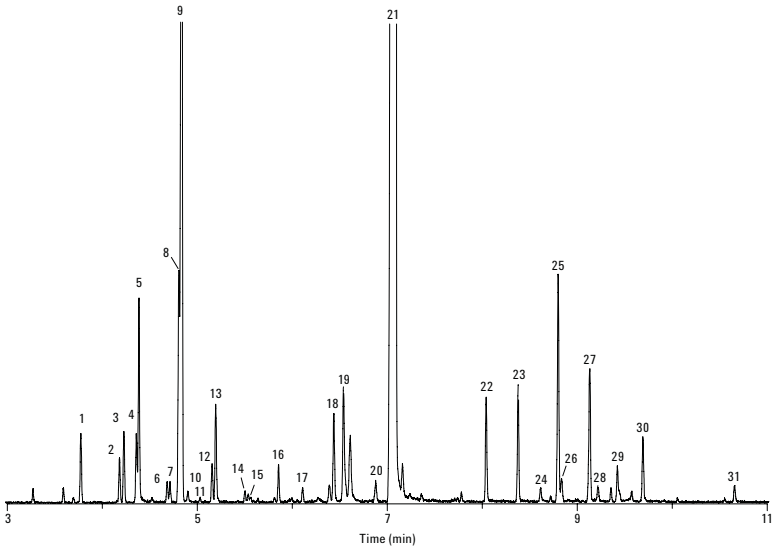

Figure 5. Spearmint oil sample on DB-1, 20 m x 0.18 mm x 0.18 µm with H_2 carrier. (See Table 1, Method C, for method parameters.)

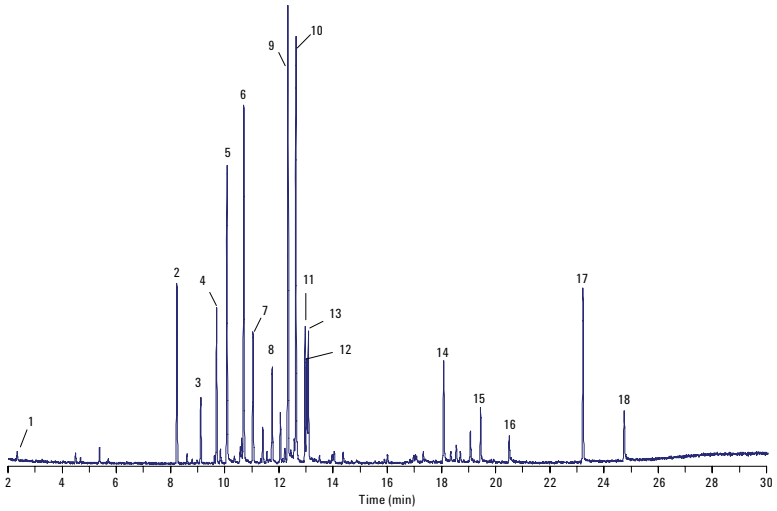

Figure 6. Ylang-ylang oil sample on a DB-WAX, 20 m x 0.18 mm x 0.18 µm column with H_2 carrier. (See Table 2, Method C, for method parameters.)

Conclusions

The use of high-efficiency columns has many benefits, as illustrated here. Shorter analysis times were achieved without significant loss of resolution. Time spent in method development was kept to a minimum through the use of the GC method translation software and the fact that the high-efficiency columns were phase ratio matched. While there are additional benefits for using hydrogen as the carrier gas, significant speed gain can be realized by simply using the high-efficiency columns while maintaining helium as the carrier.

*Although this application only depicts two oils, several additional flavors/fragrances were analyzed. Please contact Agilent Technologies Application Support for additional information.

Reference

D. Rood, *The Practical Guide to the Care, Maintenance and Troubleshooting of Capillary Gas Chromatographic Systems*, Huthig, Heidelberg, 1991

For More Information

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