Technical Note 20743

Optimizing GC Parameters for Faster Separations with Conventional Instrumentation

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Key Words
TraceGOLD fast GC analysis

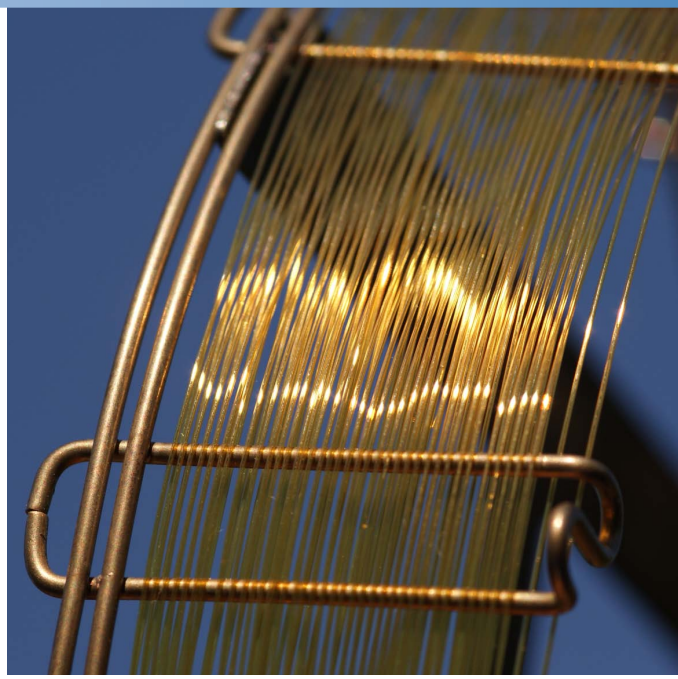
Abstract
Today's laboratories are increasingly looking for ways to speed up analysis time in order to increase sample throughput and reduce analysis costs, without compromising results. This article discusses the use of Thermo Scientific™ TraceGOLD™ Fast GC columns and demonstrates that methods can be transferred from conventional GC columns onto shorter, narrower I.D. columns with minimal method development and no compromise in the quality of results. The result was also up to 50% faster analysis time.

Introduction
The primary goal of developing a chromatographic separation is to resolve a mixture of analytes. From the general resolution equation (Equation 1), and the graph shown in Figure 1, it is evident there are three parameters that control resolution, namely efficiency (N), selectivity (α), and the retention (k') factor. Selectivity and the retention factor can be improved by changing the column chemistry, carrier gas linear velocity, and temperature ramp rate. The selectivity relates to the separation of two components. Although this is the most sensitive variable for resolution, for complex mixtures, efficiency is used to provide a wider chromatographic applicability.

Equation 1

$$R_s = \frac{1}{4}\sqrt{N}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'}{1 + k'}\right)$$

Chromatographic efficiency, and therefore resolution, can be increased by the use of a longer column, but this will result in a longer analysis time. The favored methods of increasing chromatographic efficiency are to reduce the column internal diameter (d_c) or reduce the film thickness (d_f). An advantage of using narrower columns and thinner films is that users of conventional GC methods can reduce the analysis time with minimal method development.



Significant improvements in the assay performance can be achieved without the need to make changes to the system setup using a fast GC column (20 m × 0.15 mm × 0.15 µm) compared to conventional column dimensions (30 m × 0.25 mm × 0.25 µm). Improved peak efficiencies can be obtained using a fast GC column without compromise in peak resolution, provided:

- The ratio of column length to I.D. is the same
- The column stationary phase remains the same
- The column phase ratio (β) is kept the same where possible

Further improvement in the productivity can be obtained by combining higher optimal linear velocity with an increase in the temperature ramp rate. The effects of different system parameter changes on chromatographic efficiency and speed of analysis are discussed in this note.

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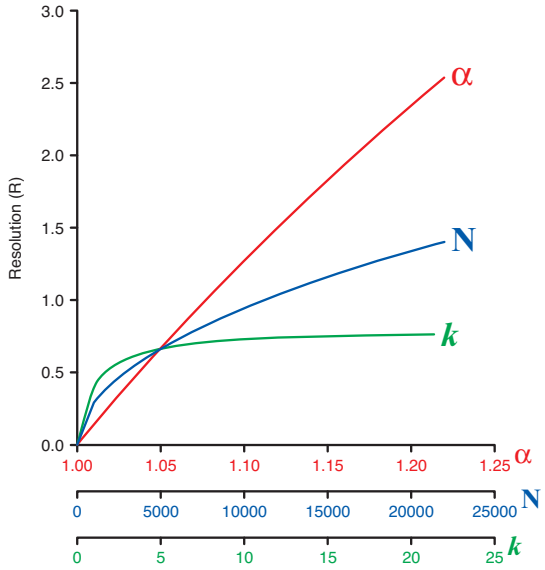

Figure 1: Graphical representation of the general resolution equation

Column Length

The separation of sixteen polyaromatic hydrocarbons (PAHs) on a Thermo Scientific™ TraceGOLD™ TG-5SilMS GC column under a gradient temperature program is demonstrated in Figure 2. By halving the column length and using the same chromatographic conditions, the speed of analysis was increased by 34%. Resolution of the critical pairs is lost as a result of decreased efficiency.

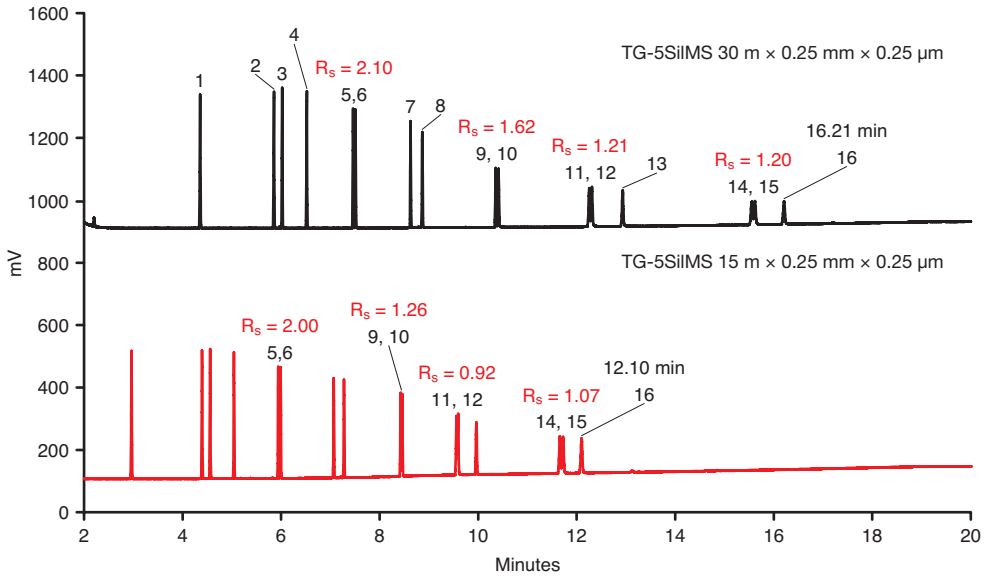

Figure 2: Column length effect on analysis time and resolution for the separation of 16 PAH standard mix

Experimental conditions: Inlet – SSL at 250 °C; Carrier gas – 1.2 mL/min helium, constant flow; Split injection – 30:1; Injection volume – 1.0 µL; Oven – 90 °C (1 min), 25 °C/min, 280 °C, 4 °C/min, 320 °C (5 min); Detector – FID at 280 °C

Analytes: 1. Naphthalene, 2. Acenaphthylene, 3. Acenapthene, 4. Flourene, 5. Phenanthrene, 6. Anthracene, 7. Fluoranthene, 8. Pyrene, 9. Benzo[a]anthracene, 10. Chrysene, 11. Benzo[b]fluoranthene, 12. Benzo[k]fluoranthene, 13. Benzo[a]pyrene, 14. Indeno[1,2,3-cd]pyrene, 15. Dibenzo[a,h]anthracene, 16. Benzo[g,h,i]perylene.

Column Internal Diameter (d_c)

Figure 3 illustrates that by reducing the column diameter, efficiency increases and as a consequence so does the resolution. The efficiency is always greater in the narrower bore column. Therefore, shorter columns can be used to reduce analysis time while offsetting the reduced efficiency coming from a shorter column length. Table 1 shows normalized efficiency for column length and diameter.

Another advantage of using a narrow bore column is that optimal linear velocity of the carrier gas also increases, which allows shorter analysis time. From the Golay plots for a fast GC column, the optimal linear velocity that provides the highest efficiency is 32.7 cm/s compared to 28.5 cm/s for the conventional GC column.

There are some practical considerations with the use of narrow bore columns, including lower sample loading capacity, which means that higher split ratios or reduced sample injection may be required to prevent column overload.

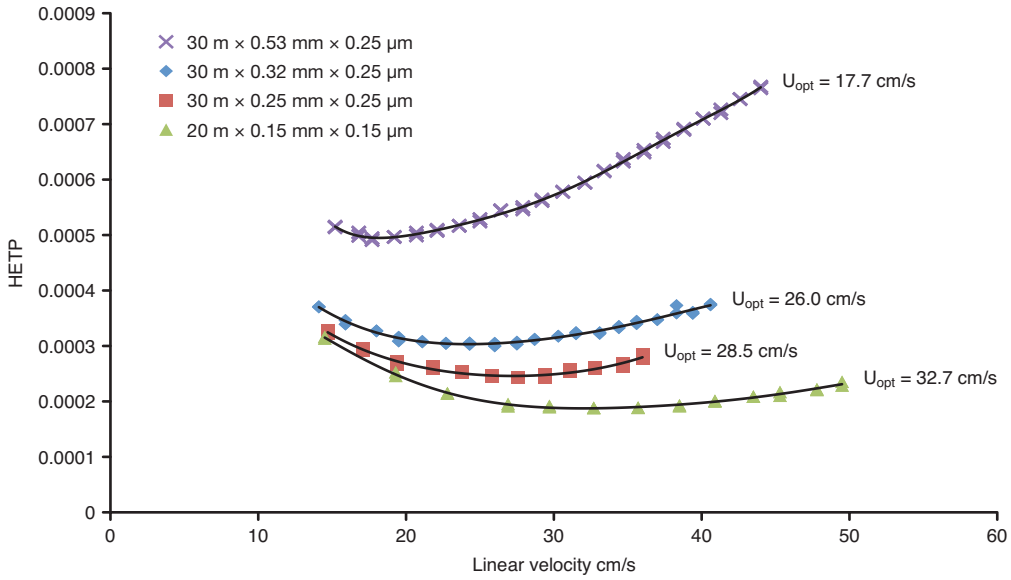

Figure 3: Influence of column diameter on optimal gas velocity and HETP

Column I.D.	Column length (m)				
	60	30	20	15	10
0.15	3.3	1.7	1.1	0.8	0.6
0.18	2.8	1.4	0.9	0.7	0.5
0.25	2.0	1.0	0.7	0.5	0.3
0.32	1.6	0.8	0.5	0.4	0.3
0.53	0.9	0.5	0.3	0.2	0.2

Table 1: Normalized efficiency relative to column length and diameter. Normalized efficiency is calculated using 30 m × 0.25 mm column dimension.

Phase Film Thickness (d_f) and Phase Ratio (β)

The separation of eleven compounds on a Thermo Scientific TraceGOLD TG-5MS GC column under a gradient temperature program is demonstrated in Figure 4. By reducing the film thickness from 0.50 µm to 0.25 µm and using the same chromatographic conditions, the speed of analysis increases by 13%. The resolution of the critical pairs increases considerably for these semi-volatile components as a result of increased efficiency.

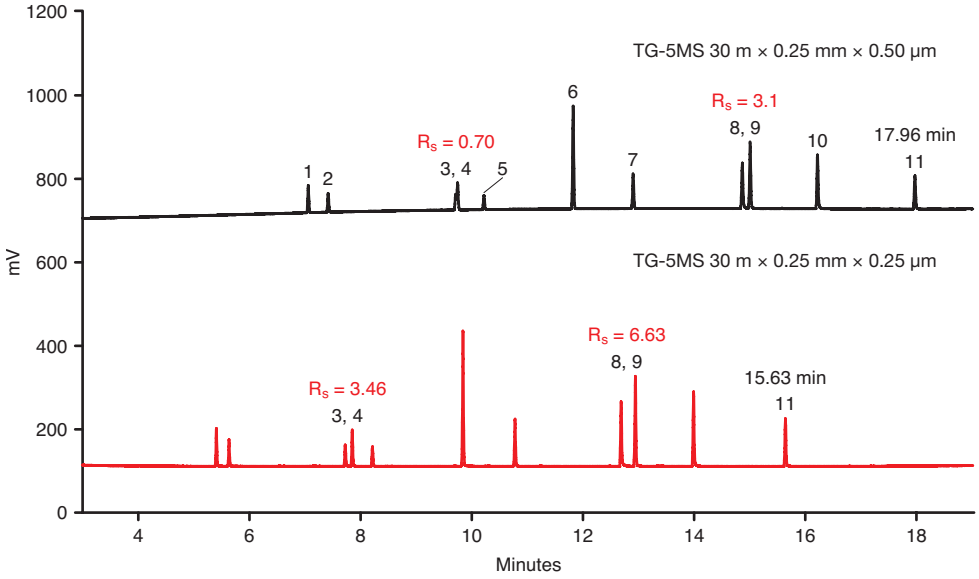

Figure 4: Film thickness effect on analysis time and resolution for the separation of an eleven phenol standard mix

Experimental conditions: Inlet – SSL at 250 °C; Carrier gas – 1.2 mL/min helium, constant flow; Split injection – 80:1; Injection volume – 1.0 µL; Oven – 60 °C (1 min), 10 °C/min, 240 °C; Detector – FID at 280 °C

Analytes: 1. Phenol, 2. 2-Chlorophenol, 3. 2-Nitrophenol, 4. 2,4-Dimethylphenol, 5. 2,4-Dichlorophenol, 6. 4-Chloro-3-methylphenol, 7. 2,4,6-Trichlorophenol, 8. 2,4-Dinitrophenol, 9. 4-Nitrophenol, 10. 2-Methyl-4,6-dinitrophenol, 11. Pentachlorophenol.

If the phase ratio (Equation 2) is kept consistent, then the elution order of compounds is anticipated to be the same. As Table 2 shows, replacing a 0.25 mm × 0.25 µm GC column with a 0.15 mm × 0.15 µm column and thus maintaining the same phase ratio, the same elution order of compounds will be seen provided the column stationary phase is kept the same. However, the efficiency on the 0.15 mm column diameter will be greater, allowing for a similar separation to be performed with a shorter column length.

Equation 2.

$$\beta = \frac{d_c}{4d_f}$$

β – Phase ratio of the column
d_c – Column diameter (µm)
d_f – Film thickness (µm)

Column diameter, d_c (mm)	Film thickness, d_f (µm)										
	0.15	0.18	0.25	0.5	1	1.4	1.5	1.8	2.65	3	5
0.15	250	208	150	75	38	27	25	21	14	13	8
0.18	300	250	180	90	45	32	30	25	17	15	9
0.25	417	347	250	125	63	45	42	35	24	21	13
0.32	533	444	320	160	80	57	53	44	30	27	16
0.53	883	736	530	265	133	95	88	74	50	44	27

Table 2: Phase ratio values to ensure correct dimensions are selected for optimizing methods

Temperature Ramp Rate

For the example shown in Figure 5, the separation of a phenol mix, the speed of analysis increases with increasing GC oven temperature ramp rate. Each 16 °C/min increase in temperature ramp rate reduces the retention factor by approximately 50%, but at the expense of resolution. However, if resolution is sufficient then high temperature ramp rate can be used.

The performance of the column also reduces with increasing ramp rate. In this case, efficiency cannot be used as a measure of column performance, instead peak width or peak capacity are generally used. The peak capacity (P_c) decreases by 22% as temperature ramp rate increases from 10 °C/min to 20 °C/min. The practical implication is that the temperature of late-eluting analytes is also increased. The operating temperature of the column may not be high enough to elute compounds. In addition, at a rate above 40 °C/min, there will be a greater variation in retention time of the late eluting compounds. This is due to inaccurate reading of the GC oven temperature >40 °C/min.

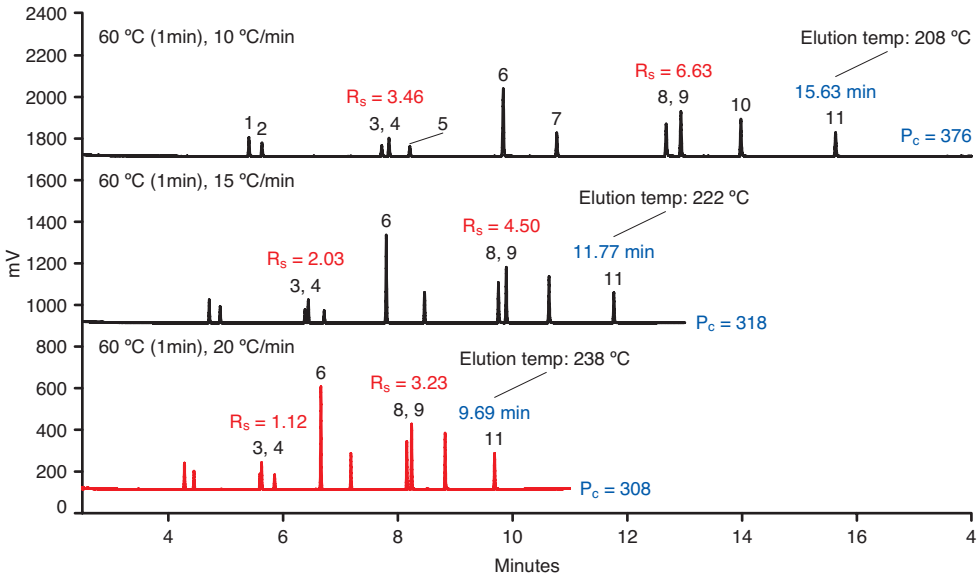

Figure 5: The effect of oven temperature ramp rate on analysis time, resolution and elution temperature

Experimental conditions: Column – TG-5MS 30 m × 0.25 mm × 0.25 µm; Inlet – SSL at 250 °C; Carrier gas – 1.2 mL/min helium, constant flow; Split injection – 80:1; Injection volume – 1.0 µL; Detector - FID at 280 °C

Analytes: 1. Phenol, 2. 2-Chlorophenol, 3. 2-Nitrophenol, 4. 2,4-Dimethylphenol, 5. 2,4-Dichlorophenol, 6. 4-Chloro-3-methylphenol, 7. 2,4,6-Trichlorophenol, 8. 2,4-Dinitrophenol, 9. 4-Nitrophenol, 10. 2-Methyl-4, 6-dinitrophenol, 11. Pentachlorophenol. Peak capacities are calculated over 11 peaks and an average is taken.

Linear Velocity of Carrier Gas

Using the same phenol mixture, the effect of varying the linear velocity (u, cm/s) of the carrier gas can be shown in Figure 6. It can be seen that the speed of analysis and the resolution increases with increasing linear velocity.

Under the isothermal conditions, if the linear velocity deviates from the optimum linear velocity (U_{opt}), relative peak broadening and loss of resolution is observed. At linear velocities above the optimum flow, chromatographic efficiency decreases due to a non-equilibrium of the solute between the stationary and mobile phases. Although, the efficiency drops, the absolute peak width reduces because of the increase of the linear velocity driving the peak off the column in a reduced time.

The effect of the increased carrier gas flow on the temperature gradient is to decrease the temperature gradient relative to the time the compounds stay on the column. This is seen in Figure 6, where peak capacity increases. The increased flow rate results in an increase in the peak capacity, due to the peak widths getting narrower and the temperature gradient effectively being decreased.

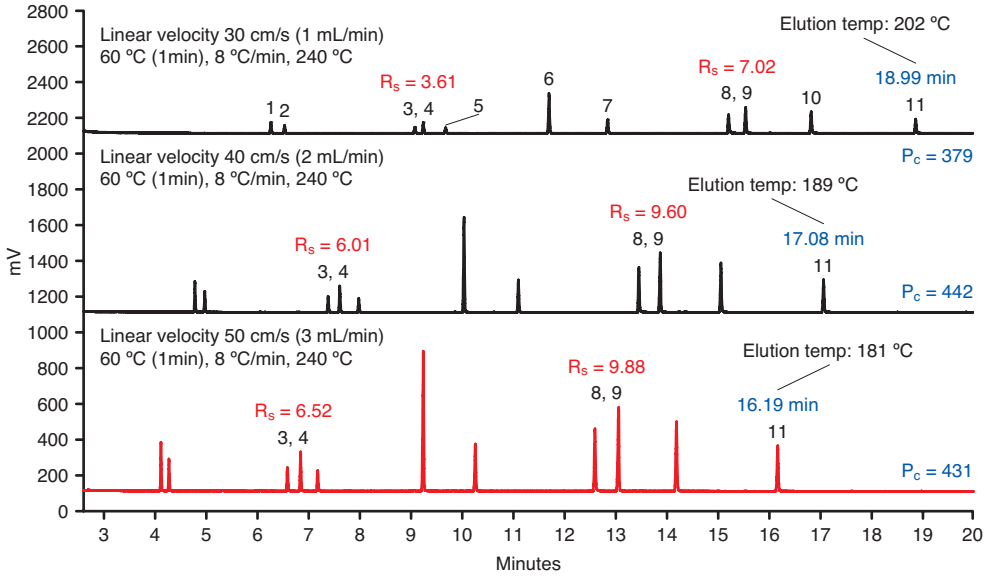

Figure 6: The effect of linear velocity on analysis time, resolution and elution temperature

Experimental conditions: Column – TG-5MS 30 m × 0.25 mm × 0.25 µm; Inlet – SSL at 250 °C; Carrier gas – helium, constant flow; Split injection- 80:1; Injection volume – 1.0 µL; Detector – FID at 280 °C.

Analytes: 1. Phenol, 2. 2-Chlorophenol, 3. 2-Nitrophenol, 4. 2,4-Dimethylphenol, 5. 2,4-Dichlorophenol, 6. 4-Chloro-3-methylphenol, 7. 2,4,6-Trichlorophenol, 8. 2,4-Dinitrophenol, 9. 4-Nitrophenol, 10. 2-Methyl-4,6-dinitrophenol, 11. Pentachlorophenol. Peak capacities are calculated over 11 peaks and an average is taken.

Temperature Ramp Rate vs. Linear Velocity of Carrier Gas

Figure 7 illustrates the separation of a phenol mix on a TG-5MS GC column, where the resolution of peaks is decreased but the baseline is still resolved, as the linear velocity is increased. The ramp rate and isothermal times were adjusted using the simple equations displayed in Equation 3 and this reduced the analysis time by up to 50%.

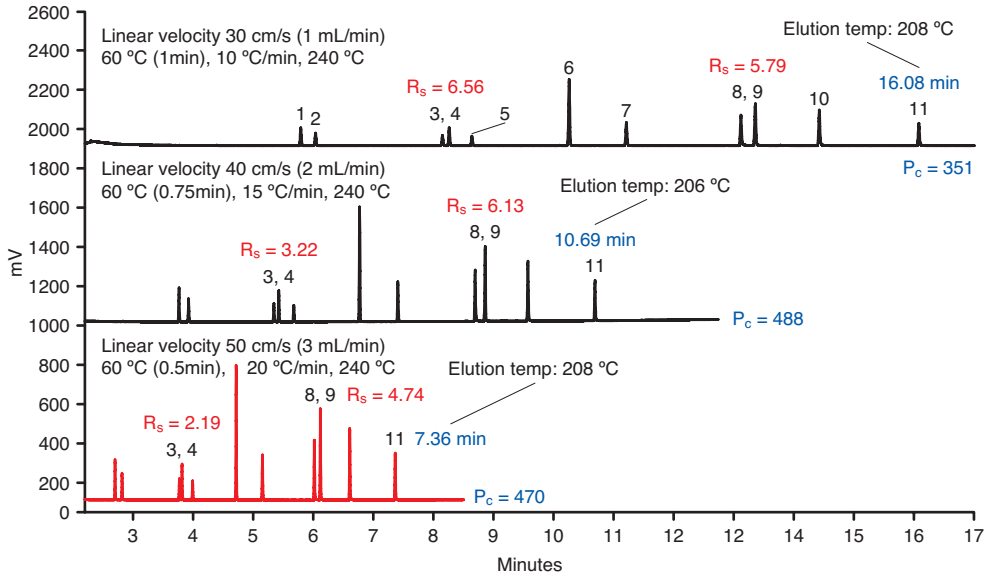

Figure 7: Effect of adjusting the linear velocity combined with varying the oven temperature ramp rate

Experimental conditions: Column – TG-5MS 30 m × 0.25 mm × 0.25 µm; Inlet – SSL at 250 °C; Carrier gas – helium, constant flow; Split injection – 80:1; Injection volume – 1.0 µL; Detector – FID at 280 °C.

Analytes: 1. Phenol, 2. 2-Chlorophenol, 3. 2-Nitrophenol, 4. 2,4-Dimethylphenol, 5. 2,4-Dichlorophenol, 6. 4-Chloro-3-methylphenol, 7. 2,4,6-Trichlorophenol, 8. 2,4-Dinitrophenol, 9. 4-Nitrophenol, 10. 2-Methyl-4,6-dinitrophenol, 11. Pentachlorophenol.

Method Transfer to a Fast GC Column

To demonstrate the applicability of this approach, a fatty acid methyl ester (FAME) separation was transferred from a conventional column to a fast GC column.

Table 3 displays the equivalent conventional and fast GC column configurations, which allow method analysis times to be readily reduced with only minor modifications to the method. The ratio of column length to I.D. and phase ratio are kept approximately the same, with the carrier gas flow rate and oven ramp rate variable to ensure that a similar chromatographic performance is obtained, while reducing the overall analysis time.

Original column	Replaced by
15 m × 0.25 mm × 0.25 µm	10 m × 0.15 mm × 0.15 µm
30 m × 0.25 mm × 0.25 µm	20 m × 0.15 mm × 0.15 µm
60 m × 0.25 mm × 0.25 µm	40 m × 0.15 mm × 0.15 µm
15 m × 0.32 mm × 0.25 µm	10 m × 0.15 mm × 0.15 µm
30 m × 0.32 mm × 0.25 µm	15 m × 0.15 mm × 0.15 µm
60 m × 0.32 mm × 0.25 µm	30 m × 0.15 mm × 0.15 µm

Table 3: Faster analysis with same separation achieved using shorter, narrower bore columns

The following equations (Equation 3) were used to determine the system parameters required to optimize performance using a fast GC column. Separation of FAMEs standard mix C8-C24 is used as an application illustrated in Figure 8.

Equation 3.

$$t_{g2} = t_{g1} \frac{v_2}{v_1} \frac{\beta_2}{\beta_1} \frac{l_1}{l_2} \qquad T_2 = T_1 \frac{v_1}{v_2} \frac{\beta_1}{\beta_2} \frac{l_2}{l_1}$$

Where
t_{g1}, t_{g2} - temperature gradient for original and new conditions
v_1, v_2 - linear velocity of gas for original and new conditions
T_1, T_2 - hold time for isothermal part of separation for original and new conditions
β_1, β_2 - phase ratio for original and new conditions
l_1, l_2 - length of column for original and new conditions

Standard method (I): TG-WaxMS 30 m × 0.25 mm × 0.25 µm, β = 250
Carrier gas: 1.2 mL/min helium flow rate, Linear velocity 30 cm/s, constant flow
Split injection: 50:1, 1.0 µL
Oven: 100°C (0.5 min), 15 °C/min, 220 °C, 5 °C/min, 250 °C (5 min), total run time 19.50 min

Fast method (II): TG-WaxMS 20 m × 0.15 mm × 0.15 µm, β = 250
Carrier gas: 0.6 mL/min helium flow rate, Linear velocity 30 cm/s, constant flow
Split injection: 50:1, 0.5 µL
Oven: 100 °C (0.3 min), 22.5 °C/min, 220 °C, 7.5 °C/min, 250 °C (3.5 min), total run time 13.13 min

Faster method (III): TG-WaxMS 20 m × 0.15 mm × 0.15 µm, β = 250
Carrier gas: 1.0 mL/min Helium flow rate, linear velocity 43 cm/s, constant flow
Split injection: 50:1, 0.5 µL
Oven: 100 °C (0.25 min), 30 °C/min, 220 °C, 10 °C/min, 250 °C (3 min), total run time 10.25 min

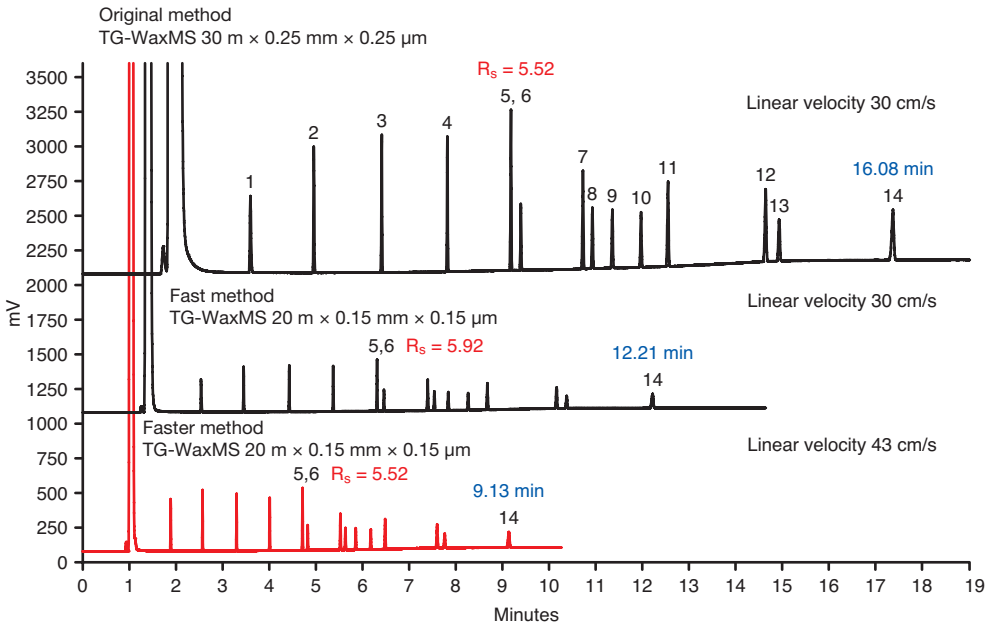

Figure 8: Chromatogram for FAME standard mix C8–C24 illustrating method transfer for faster analysis

Experimental conditions: Inlet – SSL at 220 °C; Carrier gas – helium, constant flow; Detector – FID at 240 °C.

Analytes: 1. C8:0, 2. C10:0, 3. C12:0, 4. C14:0, 5. C16:0, 6. C16:1 [cis-9], 7. C18:0, 8. C18:1 [cis-9], 9. C18:2 [cis-9,12], 10. C18:3 [cis-6,9,12], 11. C20:0, 12. C22:0 FAME, 13. C22:1 [cis-13], 14. C24:0.

Figure 8 shows the analysis time was decreased by 30% by using a modified method incorporating a fast GC column compared to a standard geometry column. The reduction in analysis time does not compromise the resolution. The speed of separation was further increased by increasing the linear velocity by approximately 40%–50%. Overall, the analysis time was reduced by approximately 50% of the original method, with no loss of resolution.

Conclusion

GC analysis time can be significantly reduced by transferring a method to fast GC columns. Performance not need be compromised by careful consideration of:

- Column length
- Column I.D.
- Column film thickness
- Carrier gas linear velocity
- Temperature ramp rate

This approach has been used to transfer a FAMEs C8–C24 mix from a standard 30 m × 0.25 mm × 0.25 µm GC column to a fast GC column, giving up to 50% faster analysis time, with no compromise in resolution and with no changes to system configuration.

References

1. Modern Practice of Gas Chromatography; Grob, R.L. and Barry, E.F., Eds.; Wiley: Hoboken, 2004.

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