Developing Fast GC Methods – Avoiding Changes in Elution Order or Separation Efficiency

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Recently I spoke with a customer who was trying to speed up an analysis. He had decided to use a shorter column with a smaller diameter, to maintain separation efficiency and decrease run times by a factor of 2. However, using this approach the customer did not get the same separation as peaks moved, even swapping direction. What was happening and how could the risk of peak swapping be reduced? In this article we discuss the phenomena of relative peak-shifting which can occur because of a possible change in the elution temperature.

Reducing analysis time

In GC analysis time can be reduced in several ways. It is important is to set a clear objective regarding resolution by asking:

1. Do I have ENOUGH resolution between the peaks of interest, allowing me to give up some efficiency for the sake of speed?
2. Do I need the SAME efficiency (plates) as before?

Depending on our choice, different approaches are required:

For sufficient separation the options are:

- Using a shorter column length (15 m instead of 30 m, or 30 m instead of 60 m)
- If the injection technique allows, starting at a higher temperature or using a faster oven program rate to elute the components
- Operating the column at a higher flow rate or use flow programming for late eluting compounds

If resolution (efficiency) must be maintained, our options are:

- Using a faster carrier gas: use hydrogen instead of helium. This is the easiest way to reduce analysis time and implementation can be quite fast. In our labs, all columns and applications are run using hydrogen. Hydrogen has about twice the optimum velocity of helium, meaning analysis times are 2x faster. Of course we need to consider safety procedures and potential risks; for some labs, hydrogen is not an option. One also needs to be aware that helium will become more expensive as the supply will not last forever. Last year there was an issue with helium availability and many labs experienced problems with their supply. With hydrogen the supply is guaranteed.
- Using a shorter capillary with a smaller bore. This produces similar efficiency, but run times will be shorter.

There are other ways to reduce run time: for instance, using selective detection devices (only detect the component of interest). Here we can use shorter columns, operated with high velocities as co-elutions are not a problem, providing quenching effects can be negated. Investments are usually significant and method development will take time. Another approach is to use a different, more selective stationary phase. If only a

few compounds have to be resolved, one can take this route, but with increasing complexity, the method development time will also increase.

One can also choose to use the same column dimensions but with a thinner film. As retention is lower, the components will elute faster. This may present challenges for early eluting components and we may need to start at a lower temperature to compensate, but then we lose the speed advantage. For components with higher retention factors, this approach may work fine, but we must be aware of changes in peak elution order as elution temperatures will be lower if the oven program is not changed.

Peak elution order

If we want a faster method we would also like to see the same separation and the same peak elution order. Here we have to be careful. In GC, the relative peak position depends directly on the elution temperature. (Retention index values of stationary phases are always listed at a certain temperature because they change with temperature). The more polar the phase is, the bigger the impact elution temperature will have. Figure 1 shows the impact of temperature programming on a simple test mixture. Here we analysed at 5 and 15 °C/min. The separation is similar, but twice as fast using the 15 °C ramp rate. Looking

Figure 1

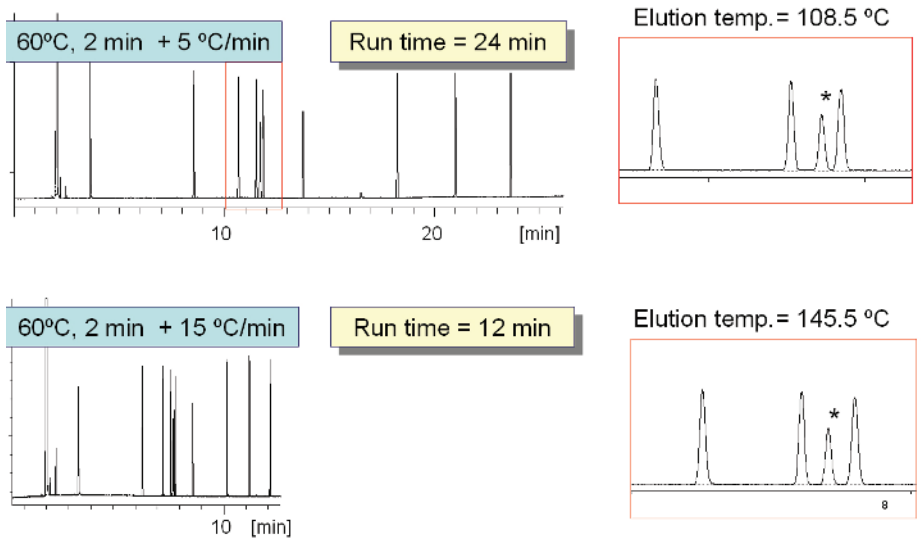

Figure 1: Impact of different program rates on analysis time, using the same column and gas velocity. shorter runtimes are obtained, but relative peak-position begins to shift because of the difference in elution temperature.

Figure 2

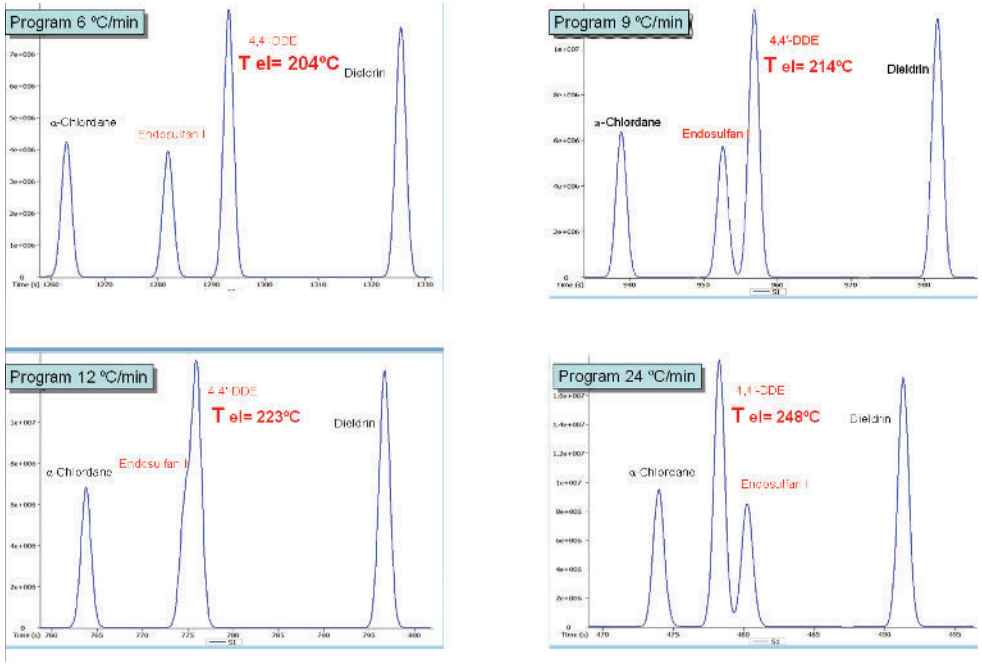

Figure 2: Practical example of peak switching based on elution temperature. The sequence of pesticide elution depends directly on their elution temperatures. Using faster ramp rate, the 4,4'-DDE moves towards to the front of the chromatogram , relative to the position of the endosulfan I.

Figure 3

$$\text{New Temp Program (2)} = \text{Old Temp. program(1)} \times \frac{\text{Gas velocity (2)}}{\text{Gas velocity (1)}}$$

$$\text{New Iso Times (2)} = \text{Old Iso Times (1)} \times \frac{\text{Gas velocity (1)}}{\text{Gas velocity (2)}}$$

(1) : original program and gas velocity

(2) : new program and gas velocity for faster method

Times in seconds, Program in ºC/min, Gas Velocity in cm/s

Figure 3: Formula for calculating temperature program and iso-times using higher linear gas velocity with the same column.

Figure 4

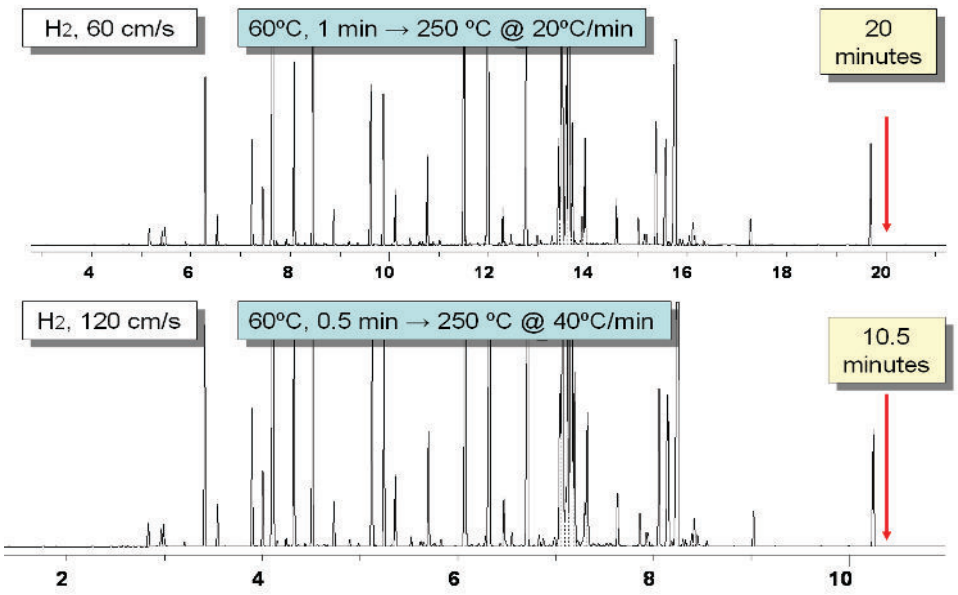

Figure 4: Perfume analysis on Rxi-5Sil MS, 30/0.25/0.25 at 60 and 120 cm/s; carrier gas: Hydrogen; Injection: Split.

more closely at the marked peak, we see that its relative position has changed. The reason for this is that this separation takes place under a different temperature profile. One can take the elution temperature of a certain component as a good indicator for possible peak position changes. In this case the difference in elution temperature is 37 °C. Depending on the type of compounds and phase interaction this effect can be very large. Figure 2 for instance, shows a pesticides separation. The column was operated under constant flow, but different oven programming rates were utilized, resulting in different elution temperatures.

With increasing program rates (resulting in higher elution temperatures), we see the 4,4'-DDE moving towards the Endosulfan I, using 6 and 9 °C/min, co-eluting at 12 °C/min and passing the Endosulfan I at 24 °C/min. In this case a very fast temperature program resulted in good separation and a very short analysis time.

Elution temperatures change with column flow and/or column dimensions

The peak elution temperature (and elution order) directly depend on the linear gas velocity (column flow) and the column length. If the column flow is increased, the components will elute at a lower temperature and we can expect peaks to move. Similarly, if we use a shorter column with similar temperature program, the components will elute at lower temperatures compared to the same conditions on a longer column. This is something we need to be aware of in three situations:

1. If we want to perform separations using a higher column flow rate
2. If we are using a different (faster) carrier gas, say Hydrogen instead of Helium.
3. If we use a shorter column or a shorter column with a smaller internal diameter.

Situation 3 was similar to the challenge the customer referred to but without loss in efficiency.

All peak elution order change issues, are related to change of elution temperature when setting up a new method.

Our goal in all these cases is to keep the elution temperature the same. This means when we change flows, type of carrier gas and/or columns we need to change the temperature program rate also. In setting a new program, we always keep the start-, the end- and

Figure 5

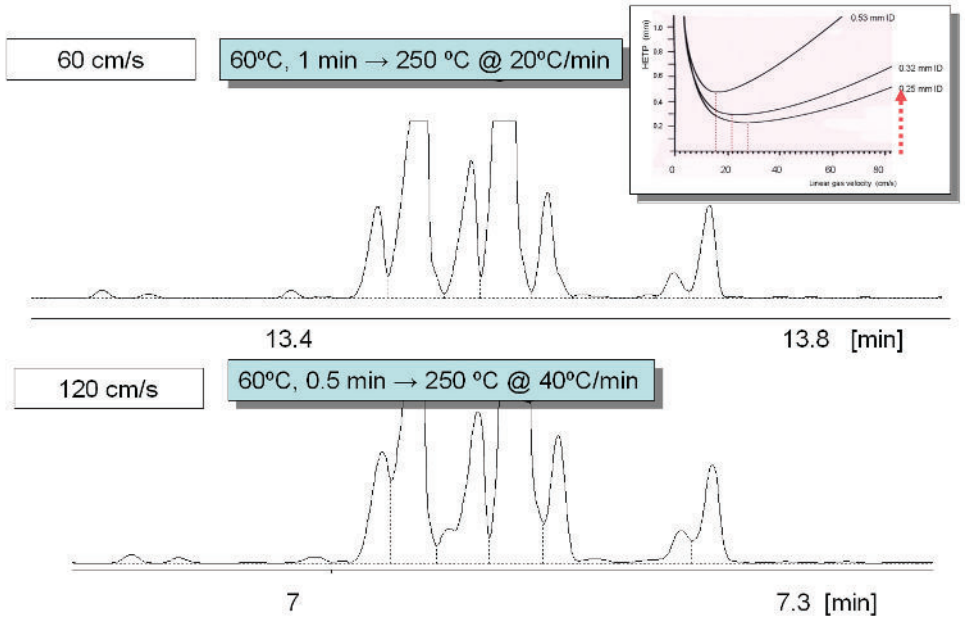

Figure 5: Expanded detail of perfume analysis of Figure 4. The elution order is similar, but we lose some resolution because we do not operate at the optimum velocity.

Figure 6

$$\text{New Temp. Program (2)} = \text{Old Temp. program(1)} \times \frac{\text{Length column 1}}{\text{Length column 2}} \times \frac{\text{Gas velocity column 2}}{\text{Gas velocity column 1}}$$

$$\text{New Iso Times (2)} = \text{Old Iso Times (1)} \times \frac{\text{Length column 2}}{\text{Length column 1}} \times \frac{\text{Gas velocity column 1}}{\text{Gas velocity column 2}}$$

(1) : Original column and velocity used

(2) : New column for faster method

Times in seconds, Program in °C/min, Gas Velocity in cm/s

Figure 6: Formula for calculating temperature program and iso times when a different column dimension is used Columns must have the same phase ratio.

eventually intermediate platform temperatures the same.

Using existing column with a higher gas velocity

This is one of the easiest changes we can implement to reduce the analysis time. However it is rarely used to its full power. Most GC systems have the capability of setting flow programming, or pressure programming. Beside reducing run time by increasing the flow for eluting the most heavy elutors, one can apply this very easily to reduce the analysis time.

The only downside is, that at a higher linear velocity, the column will not produce its maximum efficiency. That means that we cannot do this if we have "critical" separations in the present method. If we use higher linear gas velocity, the components will elute faster and therefore also at a lower elution temperature.

In order to get a similar elution order we need similar elution temperatures and for that we need to use the equations shown in figure 3. As we double the linear gas velocity we have to double the program rate and half the isothermal times. For simple samples, this is a very easy way to reduce run times. We will lose some efficiency though as the column is not being operated optimally. To show the impact on efficiency, we ran a complex perfume sample at 60 and 120 cm/s using the listed programs. Figure 4 shows the resulting chromatograms which demonstrates that exactly the same peak elution order was obtained. Because we chose to increase speed, we gave up some resolution power, shown in Figure 5. If we need to change the column dimension while maintaining the same efficiency, as as for the 30 m x 0.25 mm we cannot use this approach and we must look for a different solution.

Also note that a program temperature rate of 40 °C/min was used here. The maximum program value depends on instrument type. Some instruments cannot accommodate fast programming and operation with faster programs may result in a variation in retention times. In order to accommodate faster programming, one can reduce the oven size by using an "oven-insert". Reducing the oven size will allow much faster programming. Especially for instruments that use 110 V, that may be of interest as fast programming requires energy. In this case 220 V, instruments have a clear advantage.

Using a shorter column

If there is enough resolution, we can chose a shorter column, using the same capillary diameter and stationary phase film. If the column is 2x shorter, the analysis time can be 2x faster. For temperature programmed analysis we must adjust the program rate, to get the same elution temperatures. Use the equation in Figure 6 for this. Be aware that we will have lower efficiency, so the effect will be comparable to using a higher gas velocity.

Figure 7

OLD METHOD

Column	: Rxi-5Sil MS, 30m x 0.25mm, df = 0.25 µm
Carrier	: H_2, u = 36 cm/s constant flow
Spit inject	: 1:100; 1.0 µl
Oven	: 100°C, 2 min, 5°C/min → 250 °C
GC	: type 6890

NEW METHOD

Column	: Rxi-5Sil MS, 20m x 0.15mm, df = 0.15 µm
Carrier	: H_2, u = 46 cm/s constant flow
Spit inject	: 1:100; 1.0 µl
Oven	: 100°C, 1 min, 9.6°C/min → 250 °C
GC	: type 6890

Figure 7: Translation conditions for a complex perfume analysis moving from a 30 m x 0.25 mm to a 20 m x 0.15 mm ID capillary.

Figure 8

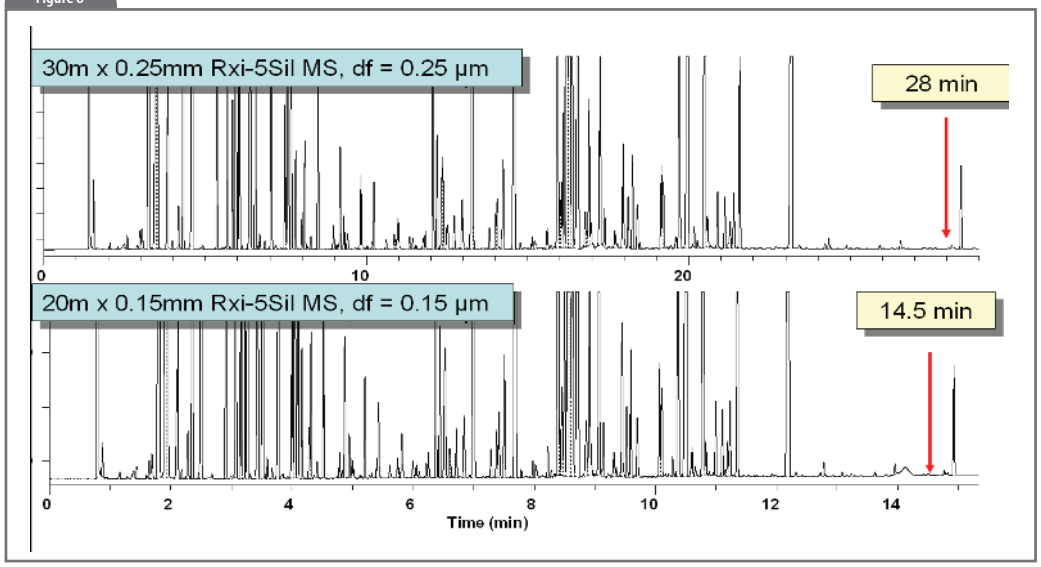

Figure 8: Comparison of perfume analysis using conditions from Figure 7. Resolution is similar while analysis time has been reduced close to a factor of 2.

Using hydrogen to speed up analysis – keeping the efficiency

Using hydrogen is one of the easiest changes to reduce analysis time. Most systems have digital flow control, meaning that its almost impossible to get large amounts of hydrogen in the oven. If safety is an issue, there are hydrogen-monitoring systems available that measure the oven for hydrogen presence.

One can also use metal type columns to reduce the risk of column breakage. If hydrogen is used instead of helium, we also have to adjust the temperature program, the same way as described before because hydrogen has an optimum velocity that is 2x higher then helium. We can reduce analysis time by a factor 2 while keeping the efficiency. So, also for critical separations, we will find similar separation quality.

If no critical separations are present we can also run hydrogen at higher velocities allowing 3-4 times shorter run times.

Using a shorter capillary with a smaller internal diameter – keeping the efficiency

Another way to reduce run time, while maintaining efficiency, is to use a column with a smaller diameter. There are several choices, varying from 0.1 mm – 0.18 mm. Though the 0.1mm ID columns have been available for a long time, the practical application is not as simple as is often is claimed. The biggest challenges are sample introduction, loadability and robustness. A good intermediate diameter that has been proven to work in nearly all existing systems, is a 0.15mm ID column. A 20 m x 0.15 mm column will provide on average 10% higher separation efficiency compared with a 30 m x 0.25 mm and is a good replacement column. Similarly a 10m x 0.15mm will replace a 15m x 0.25mm column. Practically the 0.15mm column can be operated above the optimum gas velocity, making a 2x reduction in analysis time possible.

For similar peak elution, the temperature programming rate must be adjusted. When changing to smaller internal diameter columns, the best way is to choose a column with similar phase ratio. That means that the retention factor for all components remains the same. If we do that, we can use the equation in Figure 7 to help us in our calculations. The new programmed conditions depend on the new linear gas velocity and the column length. An example of a complex mixture is shown in Figure 8. Here a perfume was analysed using a 30 m x 0.25 mm and a 20 m x 0.15 mm column, both with similar phase ratio. The chromatograms are nearly identical as we managed to generate similar elution temperatures. We maintained resolution, but we gained in speed.

Different phase ratios

If we use a column with a lower phase ratio, we will find it very difficult to get faster analysis, as relatively slow programs will be required to get similar elution temperatures. In contrast, if we use smaller bore with higher phase ratio, we need to increase the programming rate again by a factor related to the ratio of the phase ratios (and reduce isothemal times also accordingly).

The calculations as expressed here are generic as they do not account for compressibility and expansion using different outlet pressures. For many applications they work well, but for more accurate calculations, method translation software is available from the bigger instrument suppliers.

This article was written by Jaap de Zeeuw. Jaap is a GC specialist working for Restek.