LC Troubleshooting Series
Retention Time Shifts

Introduction

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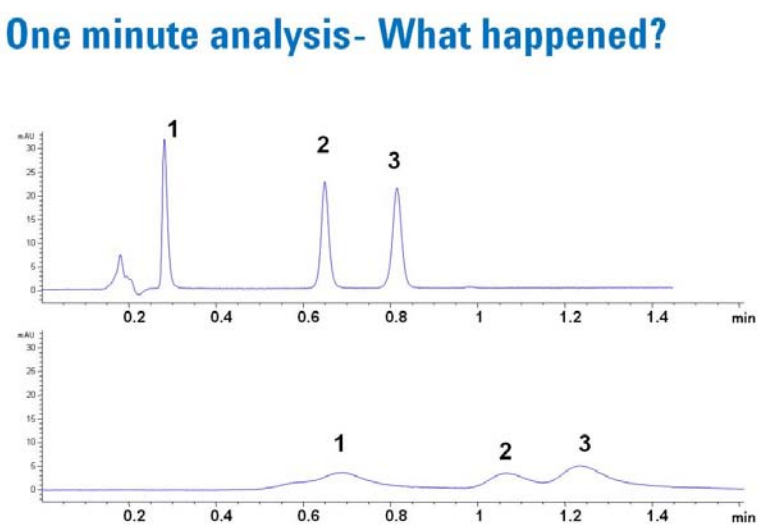
Some possible causes to consider for retention time changes are:

- Column aging
- Column contamination
- A poor column/mobile phase combination for your analytes
- Insufficient column re-equilibration
- Changes in flow rate or column temperature

Figure 1 below shows both changing retention times and poor peak shape. In this case, the capillaries were connected to the autosampler valve improperly.

Figure 1. Changing retention times and poor peak shape.



Change in Column Dimensions or Flow Rate

It's important to remember to adjust system and method parameters – flow rate, injection volume, gradient time-- when you change your column dimensions. Try the **Agilent LC Method Translator**. Search for "LC Method Translator" at www.agilent.com.

Figure 2 shows a gradient separation of pesticides and flame retardants in drinking water, first on a 100mm column and then on a 50mm column, but hardly any time savings are recognized with the shorter column.

Figure 2. A gradient separation of pesticides and flame retardants in drinking water on a 100mm column and a 50mm column.

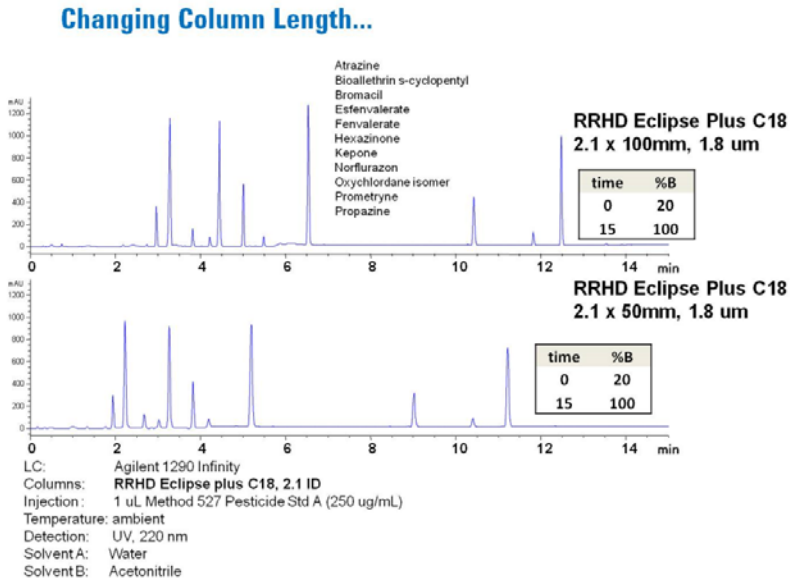
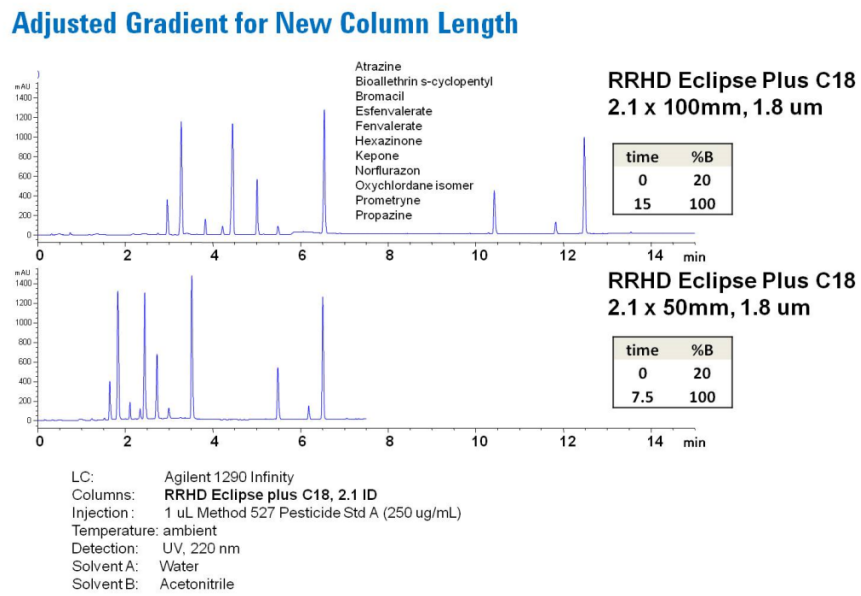


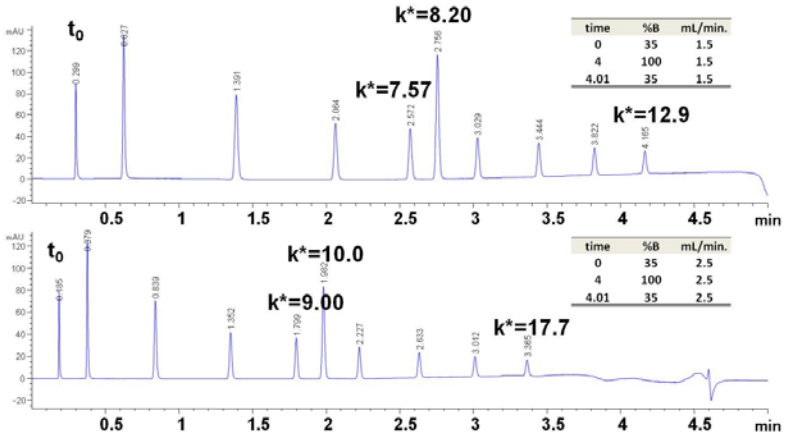
Figure 3. Adjusting the gradient to accommodate a new column length.



Because the column length was cut by 50%, the gradient time must also be cut in half to realize the time savings and keep the separation the same. When the gradient time is cut in half to match the column length and change in volume, **Figure 3** shows that the analysis time is also cut in half.

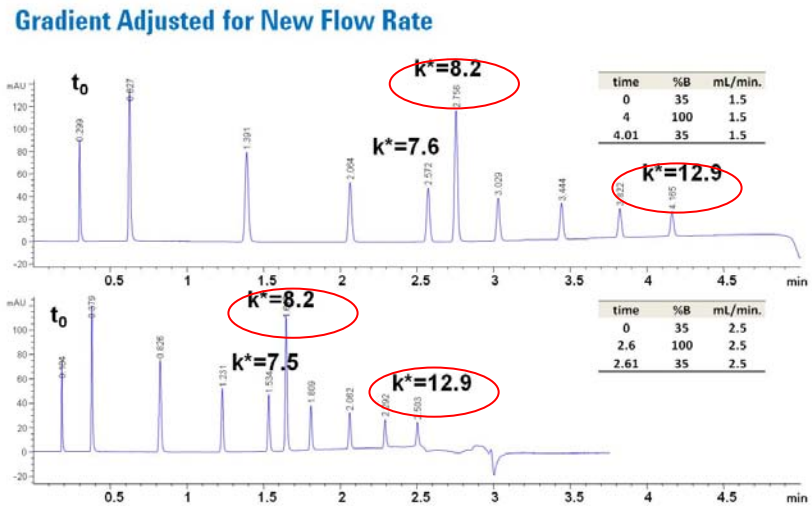
Figure 4 demonstrates the same principle as **Figure 3**, except the **flow rate**, instead of the column length has been changed to decrease analysis time.

Figure 4. Altering the flow rate to decrease analysis time.



When we increased the flow rate 60% to speed up our analysis, as we would in an isocratic method, we find that analysis time did not decrease 60%, and retention factors have changed, in fact they've increased. We need to adjust the gradient to match the flow rate change. **Figure 5** displays the new chromatogram:

Figure 5. Adjusting the gradient to correspond with a new flow rate.



As you can see, our retention factors have adjusted correctly, and we've cut our analysis time. In **Figure 5**, many people might suspect there is a problem with the LC pump delivering the right flow rate— in this case, it was just a method issue. This is the first thing you should check.

The Gradient Equation

$$t_{G1}F_1/Vm_1 = t_{G2}F_2/Vm_2$$

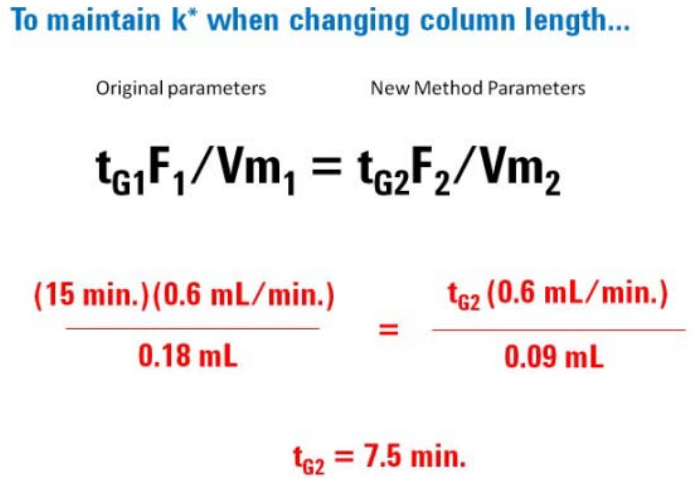
The retention factor in gradients, k*, depends on several parameters:

- Flow rate (F)
- Gradient time (t)
- Column void volume (proportional to column size) (Vm)

Remember:

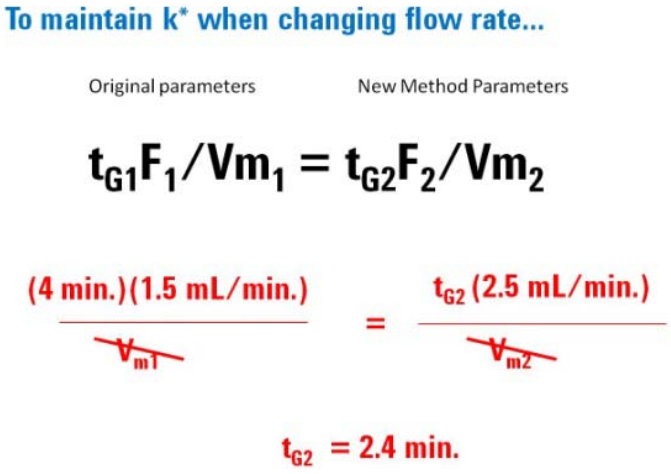
- Any change in flow rate, gradient time or void volume will change k* or retention factor.
- Vm is proportional to column length, so any change in column length will also change k*.
- To keep k* for method 1 the same when changing to method 2, these factors must balance.

Figure 6. Maintaining retention factor (k*) when changing column length.



In **Figure 6**, the column length was halved and the column length is directly proportional to the void volume, so since the void volume was halved, the gradient or flow rate must be doubled to keep the equation balanced; not doing so resulted in barely any time savings at all with the shorter column.

Figure 7. Alkylphenone: Maintaining retention factor (k*) when changing flow rate.



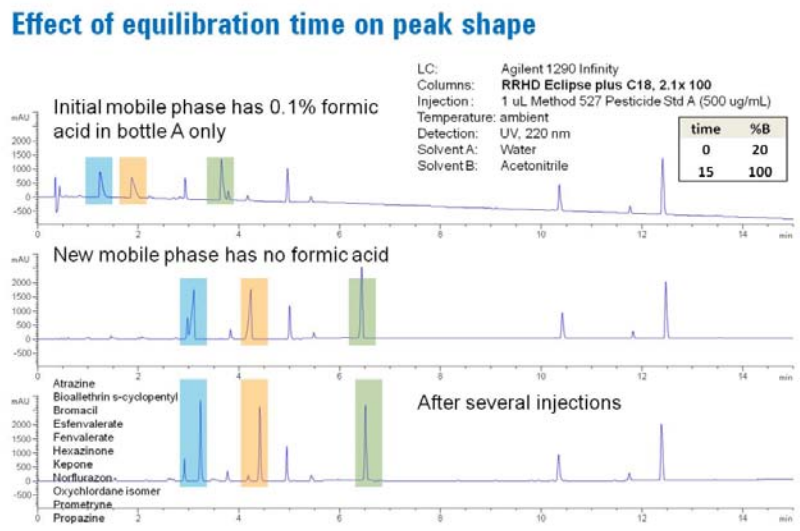
In **Figure 7**, flow rate (F2) in method 2 was increased, but the gradient time (tG2) was not adjusted, resulting in the surprising increase in retention factors. When we decreased the gradient time by 60% to balance the equation and keep k* equivalent, the retention factors matched.

Buffers and Mobile Phase

- Buffers and mobile phases impact a column's retentiveness over time. Keep a chromatogram of your column's performance right out of the box, using a standard or your common method, so you can run periodic comparisons to evaluate its performance.
- Guard columns are a good way to extend the life of your analytical column.
- A change in mobile phase may change your retention. Sometimes mobile phases can differ because of the way they are mixed in the lab.
- Mobile phases can become contaminated over time.
- It's a good practice to degas your mobile phase, to prevent air bubbles from entering your flowpath and causing retention shifts.

Figure 8 shows an analysis with a ZORBAX Rapid Resolution HD column on the 1290 Infinity. Note how the peak shapes and retention differ in this analysis from run to run, as the mobile phase is changed.

Figure 8. The effect of equilibration time on peak shape with a ZORBAX RRHD Eclipse Plus C18 2.1 x 100mm column.



Column Re-equilibration

In gradients, column re-equilibration is necessary to keep your method reproducible.

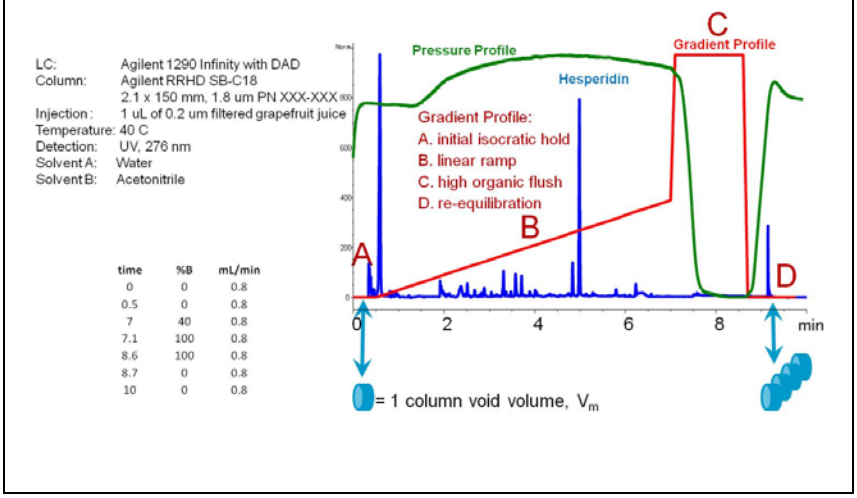
Measure your column void volume by:

- Measuring the time from the start of the chromatogram to the first disturbance on the baseline.
- Dividing this time in minutes by the flow rate in ml/min to find mL of void volume.

A general rule is to use ten column volumes for method development analyses, but for established methods, the equilibration time can be evaluated and shortened. The optimal equilibration time needs to be determined by trial and error, running the gradient repeatedly and looking for changes in retention.

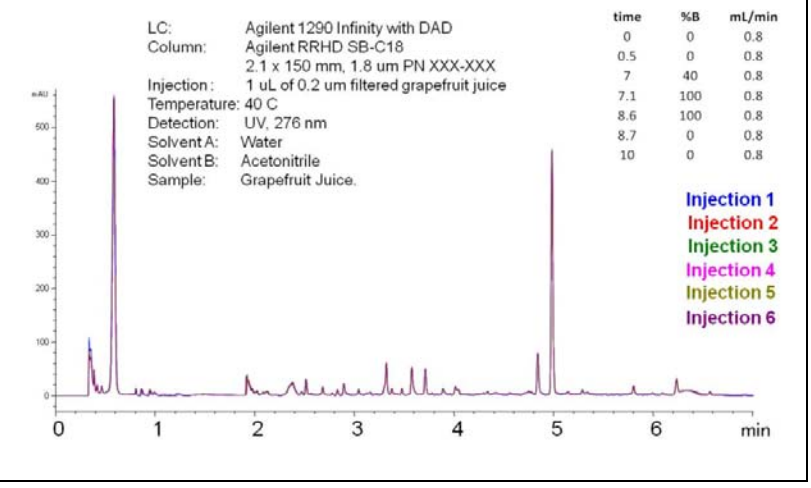
In **Figure 9,** an example of a gradient separation for grapefruit juice analysis, we can see the column void volume and amount of time dedicated to column re-equilibration. In this case, equilibration time was 1.4 min., equivalent to 4 column void volumes.

Figure 9. Gradient Separation in Grapefruit Juice.



The rule of thumb is to use ten column volumes for method development analyses, but for established methods the equilibration time can be evaluated and shortened.

Figure 10. Identical repeated injections.



The minimum equilibration time has to be determined by trial and error, by running the gradient repeatedly and looking for changes in retention. If several repeated injections are identical, as in this **Figure 10**, equilibration time is sufficient.

Summary

Tips for keeping retention times consistent:

- Know your column, and keep a sample chromatogram of its performance in new condition, to compare over time.
- Make sure you adjust your method if you change your column size – use the LC Method Translator Tool for help.
- Be sure you use a consistent method for mixing your mobile phase.
- If you're using gradients, test and confirm the number of column volumes you need to re-equilibrate during method development.

If you require additional assistance, you can always contact Agilent technical support by logging on to www.agilent.com/chem/contactus.