Part 1: Changing Column Inner Diameter While Adjusting Flow Rate

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Welcome everyone!

Jeff Layne here, back with another article series, and this time we are returning to our method development roots. In this new series, we are going to address the topic of column inner diameter within the context of analytical separations, not preparative chromatography (which would make another great series on its own). We receive many questions on how changing column inner diameters affects chromatography, most often in relation to LC-MS. So, to address these questions, we are going to use a series of LC-MS applications to illustrate our discussion.

To illustrate the basic principles, we did a simple LC-MS study using a well-behaved test probe (17-alpha-OH-progesterone), and attempted to verify the results using a more "realistic" sample (a pesticide panel). Of course, this is just one example and may not necessarily be representative of every application, but I think it does illustrate the basic effects of changing column inner diameter. This work was performed using an API 4000™ MS system (SCIEX) with an Agilent® 1200 series HPLC system on the front end. There is quite a bit of material to cover, so I will be spreading the results out over 3-4 separate articles, so make sure to keep an eye out for the ones to come.

Let's get started!

4.6 mm, 3.0 mm, 2.1 mm – so many choices. Why do we care about column inner diameter?

The simplest answer to this question is that, as column ID decreases, there is an increase in

peak height response. The practical upshot of this is that you should be able to improve your limits of detection and quantitation. But, the answer is actually complex due to the considerable number of other things going on. Changing your column inner diameter will have direct effects on a number of variables, including (but not limited to) peak height, signal-to-noise ratio, average column efficiency, sample loading capacity, sensitivity to strong injection solvents, pressure, analyte retention times, and solvent usage and/or liquid waste generation. The topic becomes more complex because you have to consider whether or not you are going to adjust your flow rate as you change column inner diameter, or hold flow rate constant.

Effect of changing column inner diameter with flow rate scaling

If I have not succeeded in convincing you that I am a complete nerd, the following sentence should pretty much remove any lingering doubts on the subject. In HPLC, when delivering mobile phase as a constant flow rate (e.g. 1 mL/min), the linear velocity of the mobile phase passing through the column will increase or decrease in proportion to the change in cross-sectional surface area of the column, and since analyte retention will be a function of the linear velocity of the mobile phase, analyte retention times will also change as the column inner diameter changes. For a better explaination of this principle, see chromatographyonline.com.

We can use a garden hose as an effortless way of illustrating this complex topic. Imagine you have your garden hose out on a sweltering summer day. Now, you turn the water knob and the water begins flowing out of the end of the hose in a lazy arc onto your lawn. The flow rate of water is in gallons per minute and is set by the amount that you turn the knob. The arc of water lands 3 feet in front of you and it takes one droplet of water 2 seconds to reach that point. That is the linear velocity of the water – distance per unit time – 3 feet in 2 seconds. Now, without touching the knob, you place your thumb over the end of the hose to partially block the opening. That constant flow of water (gallons per minute) is now being forced through a smaller opening, and comes out in a narrower stream and shoots out farther away for now. Now, with your finger partially blocking the end of the hose, the narrower arc of water lands 6 feet away from you, but it still only takes 2 seconds to reach that point. The linear velocity of the water has increased from 3 feet/2 seconds to 6 feet/2 seconds due to the diameter of the hose being made smaller by your thumb. Voila! Liquid chromatography

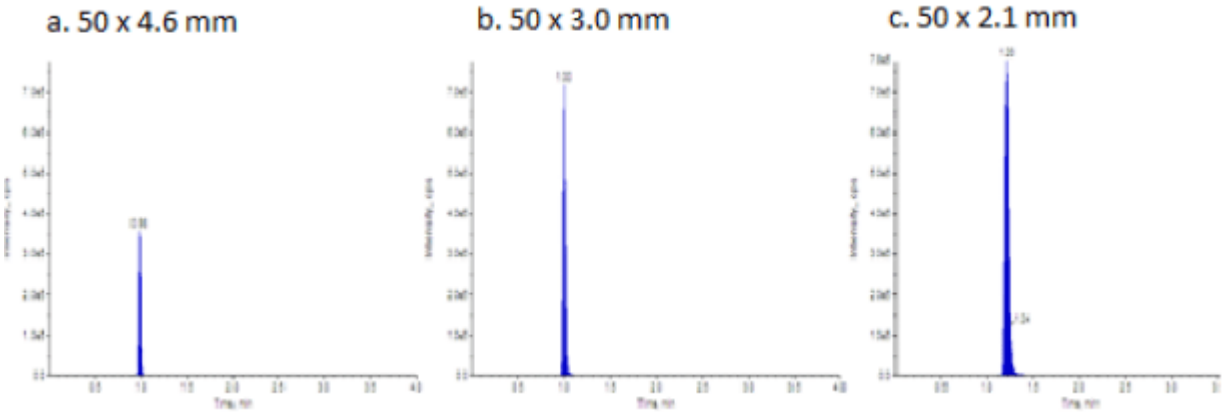
explained by a garden hose.

The same is happening in your LC column when you change the column inner diameter. If you hold the pump flow rate constant (e.g. 1 mL/min) and move to a smaller column ID (e.g. going from 4.6 mm to 2.1 mm), that mobile phase flow is being forced through a smaller ID tube and its velocity through the column increases. Thus, any analytes that are being carried by the mobile phase will also move faster through the column and will elute quicker.

For example, on a 4.6 mm ID column at a flow rate of 1 mL/min, analyte X may elute at 5 minutes. If we hold the flow rate constant and move to a 2.1 mm ID column (of identical length), that analyte X will now elute earlier, at about 1 minute (all other factors being constant). The peak height response will also increase as the column ID decreases, which is our main benefit. In fact, moving from a 4.6 mm column to a 2.1 mm ID column, we should expect to see an increase in peak height in the range of ~5-fold. To compensate for the change in retention, we can adjust our flow rate to maintain the same linear velocity as the column ID changes, resulting in a constant (or nearly constant) retention time for their analytes, while getting the advantage of an increase in signal response.

This is illustrated in **Figure 1**, which shows the ion chromatogram for 17-alpha-OH progesterone run on three columns of differing ID (2.1 mm, 3.0 mm, & 4.6 mm) and with flow rate scaling (0.2 mL/min for the 2.1 mm, 0.43 mL/min for the 3.0 mm, and 1 mL/min for the 4.6 mm). The same linear gradient was used for each column (5-95% B over 5 minutes). As the column ID is decreased from the initial 50 x 4.6 mm column (**Figure 1.a**) to the 3.0 mm (**Figure 1.b**), the peak height response is almost doubled, which is close to what we would predict based upon the change in column inner diameter and is a fantastic benefit to the analyst looking to increase sensitivity. However, as the column ID is moved from 3.0 mm to the 2.1 mm (**Figure 1.c**), there is an increase in peak height response, but you can see it is more modest at only about 7% greater than the 3.0 mm column.

Figure 1



The increase from 4.6 mm to 3.0 mm is consistent with predicted values, meaning that we are not seeing the full benefit of moving from the 3.0 mm to the 2.1 mm ID column, where we would have predicted peak height to double. Whether this deviation from expected performance is due to system dwell volume effects leading to band broadening or the nature of the interface with the MS detector itself is not clear from this study. We could tease this information out by coupling the MS to an HPLC system with a much lower system dwell volume, but we'll reserve that for another study.

The take-home message is that we do see an increase in sensitivity as we move to a smaller column ID. However, the increase is most apparent going from 4.6 mm to 3.0 mm and only a minimal benefit in sensitivity moving from the 3.0 mm to the 2.1 mm inner diameter column. This was all achieved using our HPLC system (a well-maintained Agilent 1200) and our MS detector (API 4000), connected with a minimal amount of 0.005" ID PEEK tubing (red) and by-passing the thermal heating block to minimize extra-column dead volume and scaling the flow rate to maintain a constant linear velocity. Data for peak height and pressure are in **Table 1**.

Table 1. Peak height response and system pressure for the three different columns evaluated with scaling flow rates.

Gradient with Scale Flow	H	Pressure
50 x 2.1mm @ 0.2 mL/min	7.80E+05	84
50 x 3.0mm @ 0.43 mL/min	7.30E+05	123
50 x 4.6mm @1 mL/min	3.78E+05	156

That was a lot to digest, but at least we were able to show that by decreasing our column ID it lead to an increase in peak height response for our test probe. In the next article, we will perform a similar experiment but will hold the flow rate constant as we change column inner diameter.

Looking to improve your HPLC analysis. Check out the HPLC troubleshooting guide for the latest tips and tricks for LC analysis.

Back to the lab!