Harnessing the Power of Core-Shell Technology to Improve Older, Validated HPLC Methods Within Acceptable USP Guidelines

Part One: Quality Control Methods

Guest Author: Dr. Jeff Layne

Hello everyone, I hope you enjoyed my last two series, **"Jeff Tries Cannabis"** and **"The Effects of Changing Column Inner Diameter on LC-MS Applications"**. I now want to start a new conversation about an often-overlooked aspect of HPLC—Quality Control methods. Specifically, methods that have been validated using USP criteria. Now let's be honest, when people think about exciting and fun activities, running quality control methods is probably low on everyone's list. And not without reason–it can be routine and repetitive and not particularly intellectually challenging. Confession … I ran the same ODS steroid method every day for almost two years, so when it comes to boring repetitive activities, I can speak from personal experience).

But I am here to tell you that it does not have to be boring. Running QC methods can be super fun and, if you consider the allowable adjustments that can be made within acceptable USP guidelines, it can also be very rewarding. And the key reason for that is, with the guidelines that the **USP outlines in General Chapter <621>**, you can now make certain reasonable adjustments to validated USP methods to improve their performance or productivity WITHOUT having to go through an entire re-validation process. This means that you can finally take that old method on a 10 µm irregular silica C18 particle and bring it into the 21st century without having to go through the tedious, time-consuming, and expensive process of revalidation.

"Why bother? What's in it for me?"

Well, other than breaking up tedious routine analyses, if you apply these guidelines outlined in Chapter <621>, you can take older methods developed on outdated LC column technology and bring them up-to-date with the most recent improvements in LC column technology. Practically, this can translate into dramatically improved chromatography and increase productivity through shorter analysis times.

"Chapter <621>? I am not familiar with that."

The allowable adjustments to validated methods was first proposed in **USP General Chapter <621>** back in 1999, and has undergone a few revisions since that time. Basically,

Chapter <621> defines a series of adjustments that could be made to a method, such as column length and particle size, or even mobile phase composition, WITHOUT having to undergo a complete revalidation. Further revisions in Chapter <621> in 2007 and 2014 further refined that allowable changes more in harmony with the allowable adjustments laid out in the European Pharmacopeia. It is important to note that these allowable adjustments only apply to USP monograph methods which are ISOCRATIC, and any changes to column dimensions for gradient methods will currently require a complete revalidation. For an in-depth review of Chapter <621> and its practical implications, please visit this link http://www.phenomenex.com/usp. (will also link to our blog article)

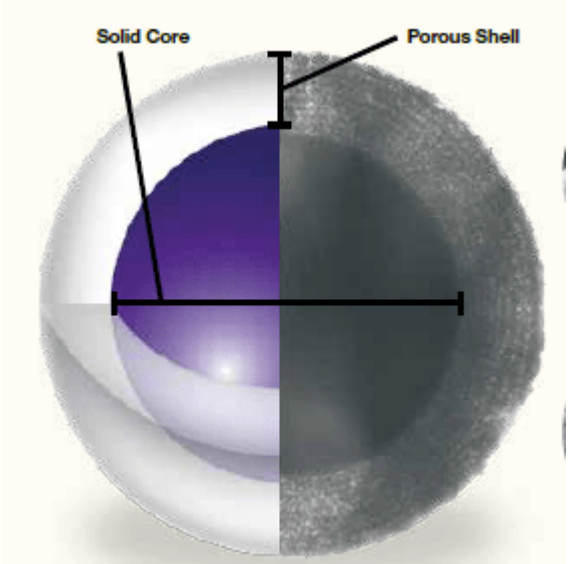
"But I don't have a UHPLC/UPLC® system, Jeff".

You don't need one. That's the best part. By taking advantage of **core-shell HPLC particles**, you can get the performance benefit of sub-2 µm UHPLC media on your conventional HPLC systems!

"What is this core-shell technology of which you speak?"

I'm glad you asked that. In contrast to the typical silica-based LC particles, core-shell particles consist of a solid, impermeable inner silica core that is surrounded by an outer shell of conventional fully porous silica (**Figure 1**). Without going into a thorough, technical explanation of the basis of core-shell performance (which can be found at http://www.phenomenex.com/kinetex), the practical take-home message is that, due to the unique core-shell morphology, columns packed with core-shell particles will deliver significantly greater efficiency than columns packed with fully porous media of equivalent particle size. Greater efficiency will translate into improved resolution and increased peak height (sensitivity). With that improved resolution, you can afford to go to shorter column lengths and/or increased flow rates (within allowable adjustment ranges) to dramatically reduce analysis times compared to your original method while still meeting your initial system suitability requirements. Tell me that doesn't sound like fun?

Figure 1. Core-shell particles consist of a layer of traditional fully porous silica surrounding an impermeable inner core.



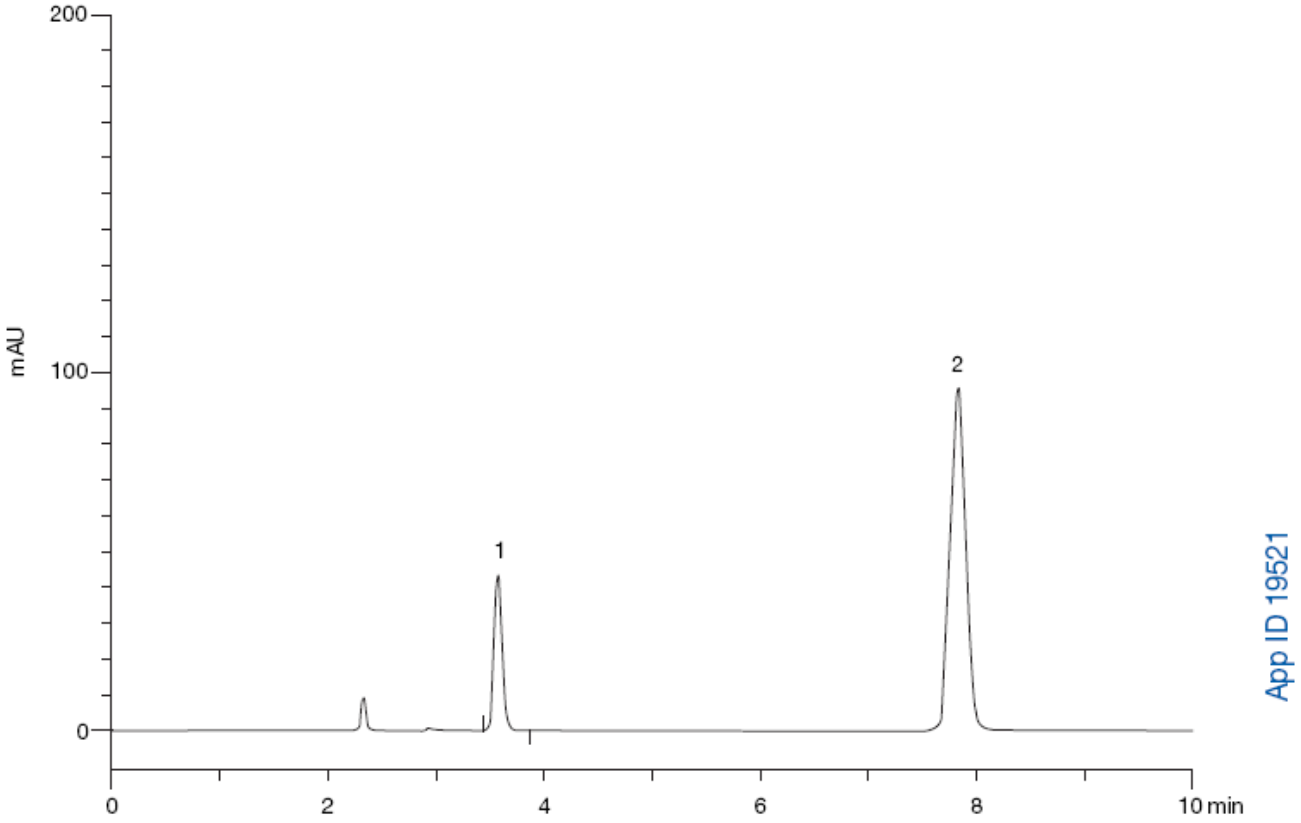
"Hmmm. It sounds good to me, but my boss will want to see some data before we commit to something like that."

They would be crazy not to. So, let's walk-through a few examples, beginning with the USP Assay method for propranolol.

The USP Assay method for propranolol calls for an L7 column (C8), with a 5 µm particle diameter, and a 250 x 4.6mm column dimension (**Figure 2**). The system suitability requirements call for a resolution of not less than 2.0 between propranolol and procainamide, and a tailing factor of no more than 3.0. Mobile phase conditions are detailed in the **Figure 2** caption. A representative chromatogram obtained by following exactly the USP conditions is shown in **Figure 2** using a **Luna 5 µm C8(2) column**. The method passes all system suitability requirements and the elution time of the last peak is about 8 minutes.

Figure 2. USP Assay for Propranolol using an L7 250 x 4.6mm column.

Column: Luna 5 µm C8(2)
Dimensions: 250 x 4.6 mm
Part No.: 00G-4249-E0
Mobile Phase: 0.5 g SDS in 18 mL of 150 mM Phosphoric acid, 90 mL Acetonitrile, 90 mL Methanol diluted to 250 mL with water
Flow Rate: 1.5 mL/min
Temperature: 25 °C
Detection: UV @ 290 nm
Backpressure: 186 bar
Sample: 1. Procainamide
2. Propranolol



So how can we utilize core-shell technology to improve this method within the acceptable USP Chapter <621> guidelines>?

Well, for isocratic methods, if we want to reduce our analysis times, one way is to simply use

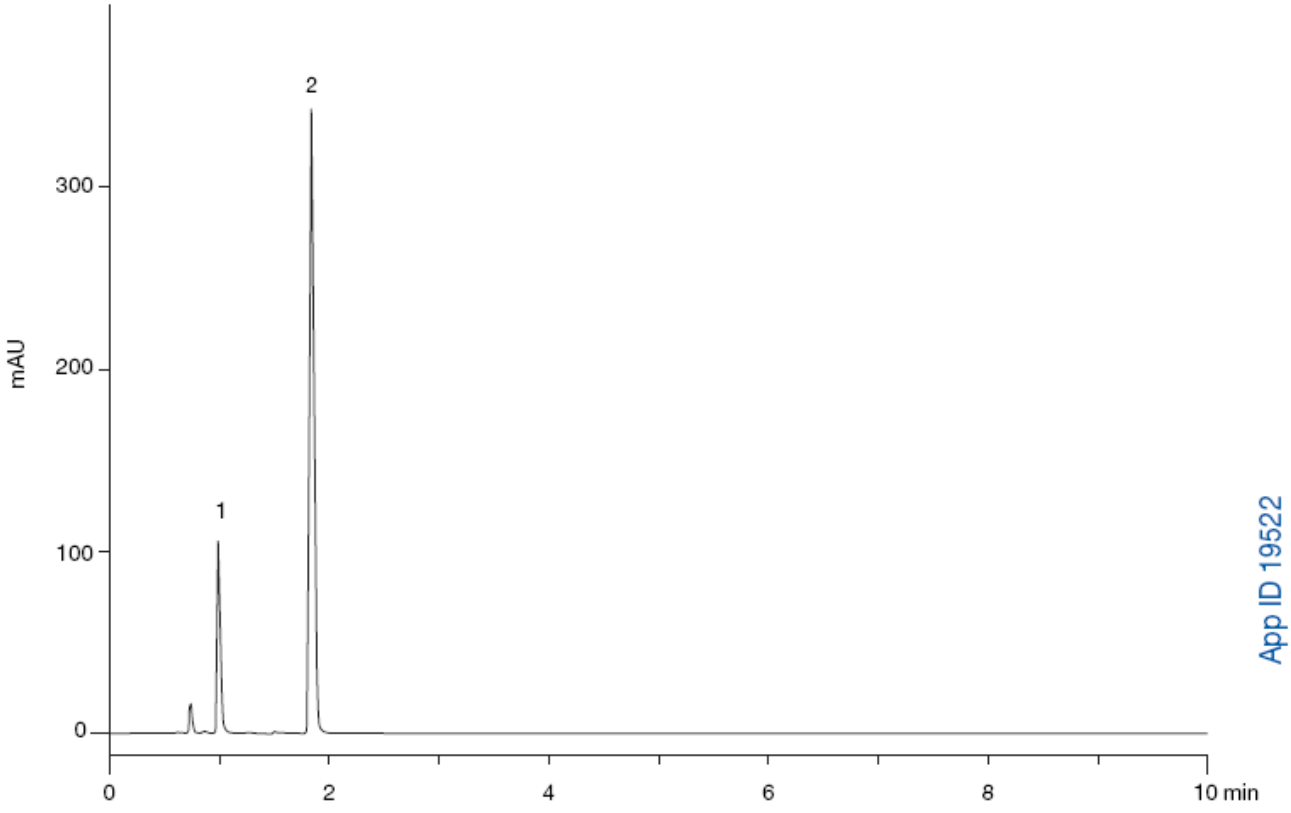
a shorter column. Under isocratic conditions, retention time is directly proportional to column length, if we hold all other factors equal. Thus, if we reduce our column length in half, we should reduce run time by about half. The problem is that, if we reduce column length, we also reduce our efficiency, since that is also directly proportional to column length as well. And, when we reduce efficiency, we lose resolution between peaks. So, if we want to decrease our analysis time without sacrificing too much resolution, we can achieve that by using a shorter column packed with more highly efficient media. And how do we get more efficient media? By going to smaller particle sizes and/or using core-shell media.

But how much are we ALLOWED to change column length and particle size?
Chapter <621> states that changes to column length and particle size are based upon a RATIO of length (L) to particle diameter (dp). So, for instance, a 250 mm long column packed with 5 µm media would give an L/dp ratio of 50,000 (250,000 um/5um = 50,000). Chapter <621> states that the L/dp ratio for isocratic method can be adjusted anywhere between -25% and +50% of the starting ratio value. For our 5 µm 250 x 4.6mm column, the allowable range of L/dp ratio would be 37,500 – 75,000. We can make any adjustments to length and particle size so long as they L/dp ratio falls within that allowable range.

So, we are going to use a much shorter column (100 x 4.6mm) packed with **2.6 µm Kinetex® C8** core-shell media. The resulting L/dp for this column is 38,462, which is still within our adjustable range of L/dp values. When we run that column using the exact same running conditions as the original method, the resulting chromatogram is shown in **Figure 3**. Using this new column, the retention time of our last peak has been cut from 8 minutes down to about 2 minutes, a four-fold decrease in run time. Because of the efficiency of the Kinetex core-shell media, we still can maintain excellent resolution (Rs = 14.5) even with that shorter column, and we are easily able to meet our system suitability requirements. Best of all, we achieve this four-fold increase in sample throughput by simply changing our column from the original 250 x 4.6mm 5 µm fully porous column to the 100 x 4.6mm Kinetex core-shell column and do not even have to perform a revalidation.

Figure 3. Modified USP Assays for Propranolol using a Kinetex 2.6 µm C8 100 x 4.6mm column.

Column: Kinetex 2.6 µm C8
Dimensions: 100 x 4.6 mm
Part No.: 00D-4497-E0
Mobile Phase: 0.5 g SDS in 18 mL of 150 mM Phosphoric acid,
90 mL Acetonitrile, 90 mL Methanol diluted to 250 mL with water
Flow Rate: 1.5 mL/min
Temperature: 25 °C
Detection: UV @ 290 nm
Backpressure: 348 bar
Sample: 1. Procainamide
2. Propranolol



And there we have one example of how using core-shell technology can make QC method fun and productive. Now this was a very basic example in which we simply changed the column length and particle diameter for a routing drug assay method. In my next article, we will look

at applying these same principles to a more complex impurity profiling method.

Back to the lab!

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Summary

Article Name
Using Core-Shell Technology to Improve HPLC Methods within USP: Part 1
Description
Harness the power of core-shell technology to improve older, validated HPLC methods within acceptable USP Guidelines, starting with an often-overlooked aspect of HPLC—Quality Control methods.
Author
Dr. Jeff Layne