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Part V: Some Final Points on the Chromatographic Impact of Making Adjustments to Existing Methods

We are finally coming to the end of our series, "Using Core-shell Technology to Improve HPLC Methods Within USP", which examines the effects of modifying existing European Pharmacopeia (Ph. Eur.) or United States Pharmacopeia (USP) methods. In this series, we have reviewed some of the various adjustments that could be made to USP and Ph. Eur. methods without having to go through a complete re-validation, and finished with the role of adjusting the column temperature and column length. Now, let's complete our review with the last three allowable adjustments.

Table 1 below lists out the current allowable changes to USP and Ph. Eur. methods, respectively. For many of the method parameters, both the Ph. Eur. and USP have identical allowable adjustment specifications, but for some, particularly when it comes to column format, there are slight differences. Again, as we go through these, remember these adjustments pertain only to isocratic methods.

Table 1. Allowable adjustments to isocratic USP and European Pharmacopeia methods.

Method Parameter	USP	Ph. Eur.
Mobile phase pH	±0.2 Units	±0.2 units
Concentration of Salts in Buffer	**±10%**	**±10%**
Composition of the mobile phase	±30% relative; Cannot exceed ±10% absolute change in any component; Cannot be reduced to zero	±30% of the minor solvent component relative or 2% absolute, whichever is the larger. No other component is altered by more than 10% absolute.
Detector wavelength	**No deviations permitted**	**No deviations permitted**
Injection volume	Can be adjusted as much as needed; must be consistent with linearity, precision, and detection requirements	May be decreased, provided detection and repeatability of the peak(s) to be determined are satisfactory.
Column temperature	**±10° C**	**±10°C**
Stationary phase	No change of the identity of the substituent permitted (e.g. no replacement of C18 by C8)	No change of the identity of the substituent permitted (e.g. no replacement of C8 by C18)
Column length	**Column length (L) to particle size diameter (dp) ratio can be adjusted between -25% and +50%**	**±70%**
Column inner diameter	Can be adjusted so long as linear velocity if maintained	±25%
Particle size	**Column length (L) to particle size diameter (dp) ratio can be adjusted between -25% and +50%***	**-50%**
Flow rate	±50% (at given ID)	±50%

Column Inner Diameter.

Adjustments to column inner diameter will have dramatic effects on chromatography and should not be made without a thorough understanding of the consequences and how flow rates may be changed to maintain the same linear velocities. For this article, I will limit our explanation to a reasonable scenario: an analyst would like to move from a conventional 4.6mm ID column to a narrower inner diameter column. In such a scenario of decreasing inner diameter, you will need to decrease the flow rate to maintain the same linear velocity and analyte retention times. The USP is very lenient in this aspect, and **you can adjust the column ID if you maintain a constant linear velocity**.

Probably the most obvious application of this would be to move from a standard 4.6mm ID column to a 2.1mm ID column. We would want to adjust our flow rate by a factor of about 5, so from 1 mL/min on the 4.6mm column to 0.2 mL/min on the 2.1mm column. This would allow you to maintain the linear velocity, retain the same approximate analyte retention times, and take advantage of the increased sensitivity and decreased solvent consumption that comes with narrow ID columns.

For the Ph. Eur., the limitations are more stringent and you can only adjust column ID by a

factor of **±25%.** So, you could NOT move from a 4.6mm ID column to a 2.1mm column because that would be more than a 50% change. Thus, your options are much more limited within the guidelines of the Ph. Eur. There is probably not much benefit in exploring this aspect of method adjustment within that framework.

Particle Size.

For the USP guidelines, we discussed particle size adjustments in the last article in the context of column length. To recap, within USP guidelines, you can adjust the L/dp ratio between **-25% to +50%.** The most logical use of this adjustment would be to move from a long column packed with large diameter particles (e.g. 150 x 4.6mm, 5µm) to a shorter column packed with smaller ID particles (e.g. 100 x 4.6mm, 2.6µm) to take advantage of the increase in efficiency of smaller particles and the decrease in analysis time with shorter columns. In this case, the L/dp ratios would be 150,000/5 = 30,000 and 100,000/2.6 = 38,461, or a 28% change, which would be acceptable. So, you could go even shorter (e.g. 75 mm) if you were still able to meet your method system suitability requirements.

For Ph. Eur., the guidelines are much simpler—you can decrease particle size by up to 50%, independent of column length. This would allow you to move from a 5µm to a 2.6µm, or maybe from an older method using a 10µm particle size media to a more modern column packed with 5µm particles. There is obviously a lot more flexibility in method development since it is not necessary to make changes to column length as the particle size is adjusted.

Flow Rate.

For both the USP and Ph. Eur., the guidelines are the same—you can adjust your flow rate by a factor of **±50%** for a given column ID. This can be a very useful tool in increasing

productivity as, under isocratic conditions, analyte retention is directly proportional to flow rate. If we have a method running at 1mL/min, we can increase the flow rate to 1.5mL/min and have **our run time decreased by about 50%.** Of course, as you increase flow rate, there will also be a proportional increase in system pressure.

Chromatographic media has an optimal flow rate (for a given column ID) that depends upon its diameter and morphology. In general, if we operate above or below that optimal flow rate, you will see a decrease in efficiency, which can also lead to a decrease in resolution. As this optimal flow varies depending upon the method, you will need to experiment to see if the increase in flow rate has an unacceptable impact on your method system suitability. However, as resolution under isocratic conditions is proportional to the square root of the change in efficiency, the modest drop in efficiency (let's assume 20%) that comes with a 50% increase in flow rate would result in a much smaller change in resolution. In this example, a 20% change in efficiency (0.2) only causes a about a 4.4% decrease in resolution. It is not a lot, but you need to consider it as a factor in your decision.

With that, we end the overview of allowable adjustments that can be made to compendial methods according to the USP and Ph. Eur, guidelines.

However, the conversation doesn't have to end here! For a more thorough explanation, **please contact your local Phenomenex representative or chat nearly 24/7 with our Technical Experts.**

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Summary

Article Name

Using Core-Shell Technology to Improve HPLC Methods within USP: Part 5

Description

Dr. Layne finishes examining the effects of modifying existing European Pharmacopeia (Ph. Eur.) or United States Pharmacopeia (USP)) methods in HPLC methods

Using Core-Shell Technology to Improve HPLC Methods within USP: Part 5

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