Part III. Chromatographic Impact of Making Adjustments to Existing Isocratic Methods

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Welcome back to the third article of my series ***"Using Core-Shell Technology to Improve HPLC Methods Within USP"***, which focuses on harnessing the power of core-shell technology to improve the quality and productivity of existing compendia (**European Pharmacopeia (Ph. Eur.)** or **United States Pharmacopeia (USP)) methods**. In the first two articles, I explored two methods showing how to make simple changes to existing compendial methods that can greatly decrease analysis times, and most importantly, fall within the allowable adjustments as specified by the USP and Ph. Eur. Those changes did NOT require method re-validation.

It is important to explore the various allowable changes and how they might affect chromatographic performance because there are a lot of changes that can be made to a method. Knowing these changes beforehand, with a fundamental understanding of how they might affect your subsequent chromatography, is significant so that you are not going into the method blind. In the next two articles, I am going to go through, step-by-step, the allowable changes stated in the current version of the USP and European Pharmacopeia and give a brief explanation of how they may affect your isocratic methods.

Table 1 lists out the current allowable changes to USP and Ph. Eur. methods, respectively. You can see that for many of the method parameters, both the Ph. Eur. and USP, have identical allowable adjustment specifications. However, there are slight differences due to column format. Again, as we go through these adjustments they only pertain to ***ISOCRATIC*** methods.

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

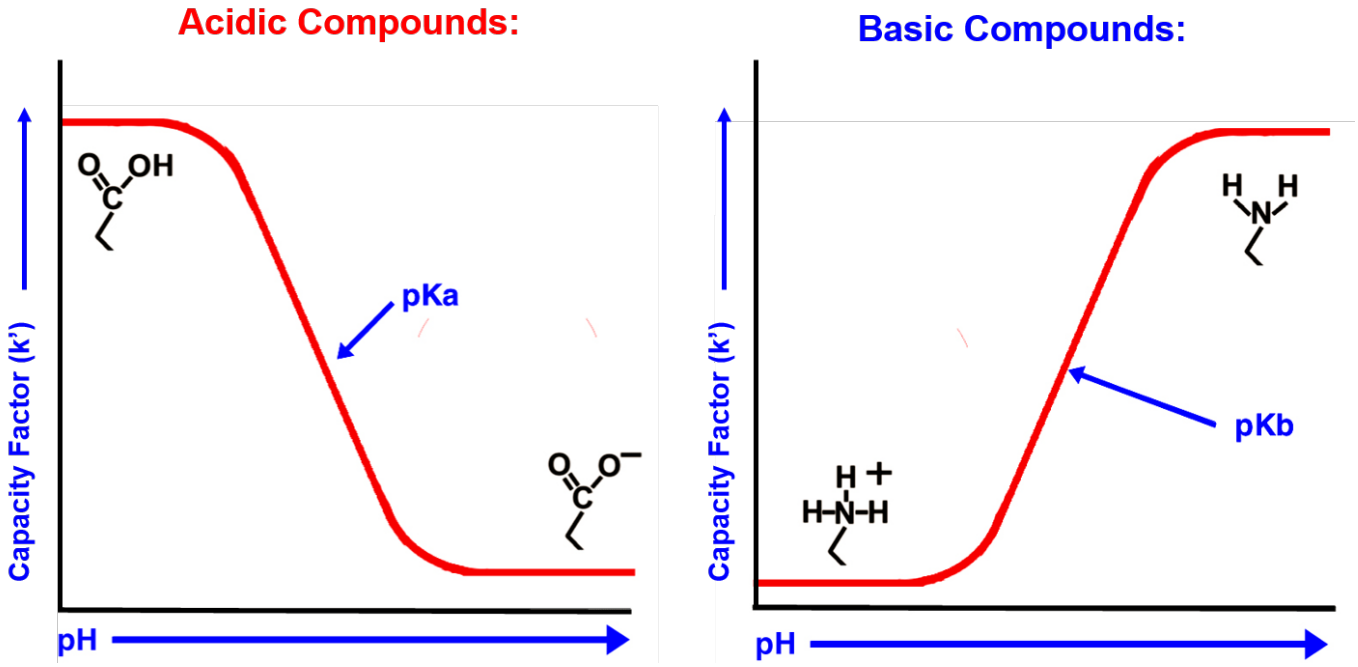
Method Parameter	USP	Ph. Eur.
Mobile phase pH	±0.2 Units	±0.2 units
Concentration of Salts in Buffer	**±10%**	**±10%**
Composition of the mobile phase	±30% relative; Cannot exceed ±10% absolute change in any component; Cannot be reduced to zero	±30% of the minor solvent component relative or 2% absolute, 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%

Mobile phase pH.

Both the Ph. Eur. and USP allow for a slight (± 0.2 unit) adjustment in mobile phase pH. **Mobile phase** pH affects the ionization state of analytes in sample mixtures and it can affect the protonation state of residual silanols on the surface of your silica. Under typical reversed-phase conditions, these changes can lead to noticeable changes in analyte retention, primarily for analytes that contain ionizable functional groups (i.e. acids and bases primarily, but hydroxyls to a lesser extent) and are unlikely to significantly alter the retention behavior of neutral species.

The retention behavior of acidic and basic molecules as a function of mobile phase pH shown in **Figure 1**. You can see that as the mobile phase pH is changed, you will get a decrease or increase in retention depending upon whether the analyte in question is an acid or a base. Given the relatively small amount of pH adjustment that is within the acceptable range, I would be hesitant to rely on this small adjustment as a method development "tool". It would likely have more value as a possible way to troubleshoot or fine-tune a given method when in mobile phase, and a small change in mobile phase pH might be able to push a method into a more acceptable performance region. **Overall, though, I would not rely on a small change in pH (± 0.2 unit) as a way of "improving" a method or increasing productivity through decreasing analysis time.**

Figure 1. Retention behavior (capacity factor) of acids and bases as a function of mobile phase pH.



Concentration of buffer salts.

According to USP and Ph. Eur., the **concentration of buffer** salts can be adjusted ± 10%. The effect of changing the concentration of buffer salts will be most apparent when analyzing ionizable compounds, as the chromatographic behavior of neutral species is much less sensitive to buffer concentration. Basic molecules are most likely to display noticeable changes in chromatography as a direct consequence of changing the salt concentration. This is because there will usually be a degree of secondary interactions (ionic or polar) between positively charged analytes and the negatively charged residual silanols on the silica surface. These secondary interactions can lead to peak tailing and contribute to the overall retention of basic analytes. As the concentration of buffer salts increase, there will be a reduction of secondary ionic and polar interactions between charged amines and the underlying silica. In many cases this will reduce peak tailing and may also reduce retention. However, given the relatively modest amount of change permitted (± 10%), you are unlikely to see a dramatic benefit (or negative impact) from such a relatively subtle change in buffer concentration (e.g. for a method that states 20mM buffer, the allowable range would be 18-22mM). **As with adjustments to mobile phase pH, changing the buffer**

concentration within this narrow acceptable range may be useful as a tool to "fine-tune" a method that was on the cusp of acceptable performance. I would not look to it as a tool for significantly improving chromatography or productivity for an established method.

Detector wavelength.
No deviations permitted under Ph. Eur. or USP guidelines.

Injection volume.
There is some difference between the allowable adjustments for sample injection, as shown in **Table 1**. The USP states that that the injection volume may be "adjusted as much as needed…", whereas the Ph. Eur. guidelines only mention a "decrease" is allowable. From a practical perspective, both guidelines are important to consider when you are trying to convert an older method using a longer column to a newer method using a much shorter column, and particularly if that shorter column uses core-shell media. The reason for this is that, all things being equal, the amount of sample you can load onto a column will be relatively proportional to the amount of media within the column. If we exceed the loading capacity of a given column, you will begin to see peak distortion effects (fronting, tailing, and/or broadening). So, if you had been injecting 20 µL onto a 300 x 7.8mm column with your old method and you are trying to move to a 150 x 4.6mm format, you might find that if you inject that same 20 µL sample load, you get an unacceptable amount of peak tailing due to sample overload. This can be remedied by simply decreasing the injection in a step-wise manner until you achieve a suitable peak shape while maintaining your required LOD/LOQ values.

Reducing the sample load is particularly important if you are trying to move to a core-shell format since the core-shell media has a much lower surface area, and therefore, lower sample loading capacity than fully porous media. This is illustrated in **Figure 2** below, where you can see the improvement in peak shape as we decrease the sample loading on the core-shell column. If you are concerned that reducing your sample load might compromise your ability to hit LOD/LOQ values, don't forget that the increase in efficiency with core-shell media, combined with the shorter elution times, should result in a dramatic increase in peak height for the same sample load on-column. Therefore, even when you reduce your injection volume and sample load, the performance gains of using core-shell

media should offset the reduced load on-column and allow you to maintain or even improve your LOD/LOQ with a smaller injection volume.

Figure 2. Improving peak shape for a strongly basic analyte as the sample load on-column is decreased.

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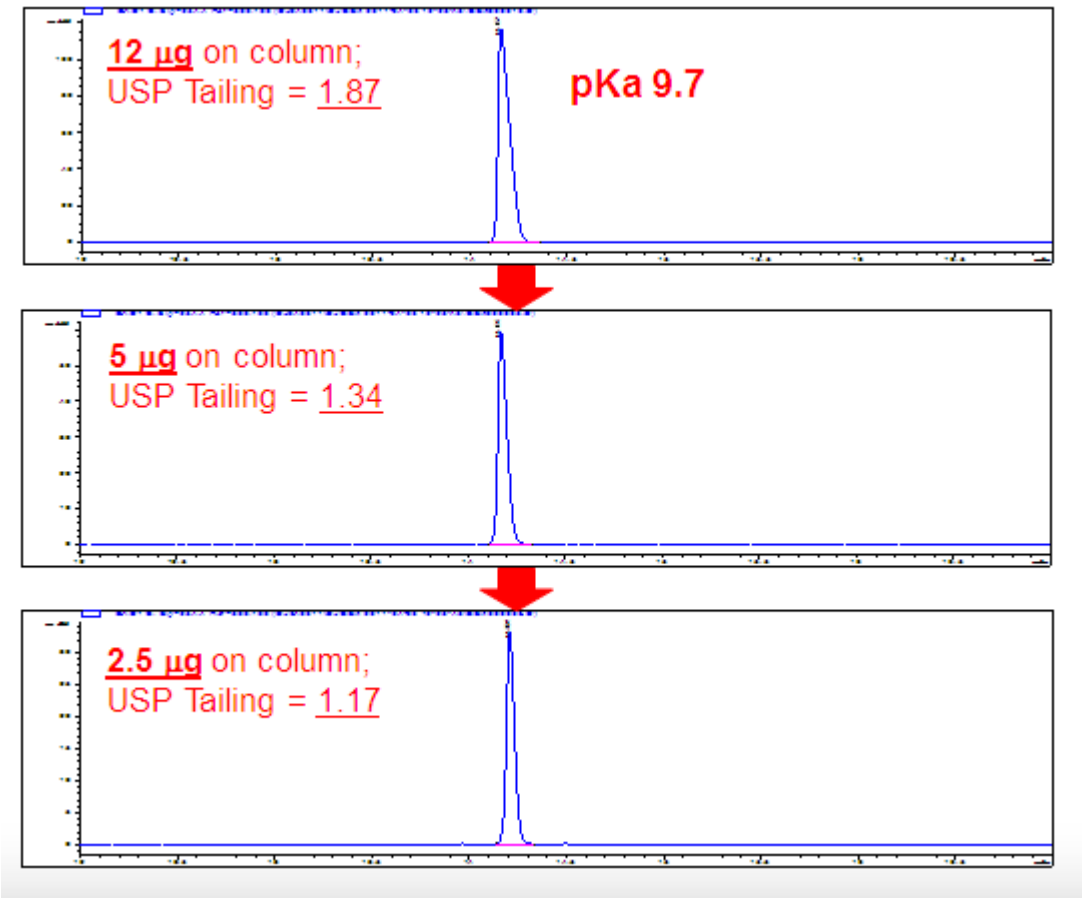

In the next article, we will continue our exploration of the chromatographic effects of making allowable changes to USP and Ph. Eur. Methods.

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Summary

Article Name
Using Core-Shell Technology to Improve HPLC Methods Within USP: Part 3
Description
A step-by-step of allowable changes stated in the USP and European Pharmacopeia and a brief explanation of how they may affect your isocratic methods.