Boosting LC Method Efficiency with Allowable Compendial Adjustments

Adjusting USP Methods: What’s Allowable without Re-Validation?
Adjusting USP Compendial Methods
Technical Proof: Working within Allowable Changes to Save Time and Money
In-Depth Examples of USP Method Adjustments

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As the official compendium for all pharmaceutical products marketed in the United States, drug makers heavily rely on the *US Pharmacopeia–National Formulary* (USP-NF) compendium for science-based quality standards and testing methods that ensure the purity and potency of drug products and substances. Many USP methods were developed when the drug was first developed, before faster liquid chromatography techniques were in existence. While the compendium is updated every few years, laboratories looking for time and cost efficiencies made possible through modern technology would be hindered by using USP methods right “out of the box.” A method may use an older, longer column, while a shorter, smaller-particle column could do the job in half the time or less.

Recognizing this limitation, USP revised *USP General Chapter on Liquid Chromatography* <621> in an attempt to create space for certain allowable adjustments to the methods. The latest revision (published in *USP 37–NF 32*, effective Aug. 1, 2014) makes the largest...
step forward in defining exactly what are the limits of allowable method adjustments and which changes actually modify methods to the point that they require revalidation. It is also the user’s responsibility to perform verification tests of the method under the new conditions by assessing the analytical performance characteristics potentially affected by the change.

How can your lab take advantage of the allowable changes laid out in USP <621>?

Examples of Allowable Changes

In the “System Suitability” chapter of USP <621>, USP lays out this framework for making method adjustments. In a nutshell, certain changes are allowed without revalidation if they remain within the ranges specified and the method passes system suitability tests. To stay within the ranges, the method should not be modified for the purposes of acquiring additional or different information; generating additional resolution or making the method find analytes that the other method would not be able to find, for instance, is not the point of method adjustment. Again, USP intends method adjustments to be directed at substituting in commercially similar products or taking advantage of newer technologies in an effort to increase the laboratory’s efficiency (i.e., saving time and money).

So, how much can you change a method without changing the method?

Column length and particle size. If you want to change the type of column—such as moving from the C18 column (i.e., L1) indicated in the USP method to a C8 (i.e., L7)—you cannot do so without revalidating the method. You must stay in the same column chemistry.

However, you can make changes to the column length or particle size, which is exactly the kind of adjustment that makes sense to pursue. Taking advantage of shorter columns and newer technologies make analyses faster and more efficient. If you use a column that’s one-third as long, two-thirds less solvent will be used and analysis time will be slashed. The impact of such changes on a lab is almost immediate.

There’s an important caveat to consider, though. The method cannot degrade or substantially improve in efficiency. Changes are only allowed if they keep the column length-to-particle diameter ratio \(L/d_p\) constant or if the efficiency (\(N\)) of the method is kept within the range of -25% to +50%.

Here’s an example of the first scenario. If you have a column size of 250 mm x 4.6 mm with a particle size of 5 µm, the \(L/d_p\) is 50,000. You could switch to a 150 mm x 4.6 mm, 3 µm column and still keep the \(L/d_p\) constant at 50,000. This allowable change in column length and particle size would make the method faster, shortening the runtime of this method by the change in column length (40%).

When moving to a different particle type like superficially porous particles (SPP), there is an additional aspect of particle size and
“Injection volume can also be adjusted, provided it remains consistent with the original method’s precision, linearity, and detection limits.”

column length changes to consider: the plate number (N) allowance of -25% to +50%. This is a very useful part of the ratio because SPP materials tend to be more efficient than totally porous particles of the same size.

If one were to evaluate the change from a totally porous column to one with SPP simply based on the \( L/d_p \) ratios, it would seem like it wasn’t allowed. However, the option of the SPP column remains on the table because of the N allowance range. In this case, however, it is necessary to make the assessment of efficiency based on an actual analysis of the compound on both columns.

To reduce analysis time in the above example even further, you could use a 2.7 µm column with the shorter column length (100 mm), which has an \( L/d_p \) of 37,035. This change is 26% below the -25% allowed by the \( L/d_p \) ratio rule. However, the original method generated an efficiency of 16,122 plates measured with a standard. The new method generates an efficiency of 13,000 plates. A change of 19% within the allowable N allowance of -25% to +50%. This increases the throughput of the method by 60%.

Overall, just switching from a 5 µm column to a 2.7 µm column would provide about 60% faster throughput and 60% times less solvent if you keep the diameter the same. These changes become significant and add up quickly on the very large scale that many pharmaceuticals or over-the-counter drugs are produced.

**Flow rate and column inner diameter.** With any USP method, the flow rate can be adjusted ±50%, provided N decreases ≤20%. This means the initial method has to be run first with the analyte. When moving to a smaller particle column, adjustments to flow rate are also allowed.

If a 5 µm column runs optimally at about 1.2 mL per minute, 2.7 µm column might run better at 2 mL per minute. USP allows such changes, based on this rule:

\[
F_2 = F_1 \times \left( \frac{(dc_2)^2 \times dp_1}{(dc_1)^2 \times dp_2} \right)
\]

where \( F_1 \) and \( F_2 \) are the flow rates for the original and modified conditions, respectively, \( dc_1 \) and \( dc_2 \) are the respective column diameters, and \( dp_1 \) and \( dp_2 \) are the particle sizes.

However, these adjustments are not cumulative. You can only adjust flow rate from the original method.

As a result, the throughput of the method might increase by about much as 5x when taking the increase in flow rate and shorter column length into account.

Changes in \( F \), \( dc \), and \( dp \) are not allowed for gradient separations.

USP also allows for adjustments to the method in terms of column inner diameter, per this equation. The improvement can be
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**TABLE 1: Summary of allowable adjustments per USP General Chapter <621>**

<table>
<thead>
<tr>
<th>Parameters for System Suitability</th>
<th>USP 37–NF 32 S1</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isocratic</td>
<td>Gradient</td>
</tr>
<tr>
<td><strong>Particle size</strong></td>
<td>L/dₚ: -25% to +50% or N: -25% to +50%</td>
<td>No changes allowed</td>
</tr>
<tr>
<td><strong>Column length</strong></td>
<td>Flexible, with constant linear velocity</td>
<td>No changes allowed</td>
</tr>
<tr>
<td><strong>Column inner diameter</strong></td>
<td>Flexible, with constant linear velocity</td>
<td>No changes allowed</td>
</tr>
<tr>
<td><strong>Flow rate</strong></td>
<td>Based on (d_p^2 \times F_2 = F_1 \times \frac{(dc_2)^2 \times dp_1}{(dc_1)^2 \times dp_2}) Additional adjustments: ±50%, provided N decreases ≤20%</td>
<td>No changes allowed</td>
</tr>
<tr>
<td><strong>Injection volume</strong></td>
<td>May be adjusted, as far as is consistent with precision and detection limits</td>
<td>May be adjusted, as far as is consistent with precision and detection limits</td>
</tr>
<tr>
<td><strong>Column temperature</strong></td>
<td>±10°C</td>
<td>±10°C</td>
</tr>
<tr>
<td><strong>Mobile phase pH</strong></td>
<td>±0.2 units</td>
<td>±0.2 units</td>
</tr>
<tr>
<td><strong>Salt concentration</strong></td>
<td>Within ±10% if the permitted pH variation is met</td>
<td>Within ±10% if the permitted pH variation is met</td>
</tr>
<tr>
<td><strong>Ratio of components in mobile phase</strong></td>
<td>Minor component (≤50%): ±30% relative, but cannot exceed ±10% absolute; may only adjust one minor component in ternary mixtures</td>
<td>No changes allowed*</td>
</tr>
<tr>
<td><strong>Wavelength of UV-visible detector</strong></td>
<td>No changes allowed</td>
<td>No changes allowed</td>
</tr>
</tbody>
</table>

*Not specified in <621>, assume no changes are allowed
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for solvent saving rules alone when keeping the particle size constant. 3 mm columns are an excellent choice, as they will save approximately 50% of the solvent.

**Injection volume.** Injection volume can also be adjusted, provided it remains consistent with the original method’s precision, linearity, and detection limits. Again, this is an important area to focus on when moving to a shorter, smaller particle column. If you’re using a column that has only two-thirds of the volume, then you would only inject two-thirds of the sample as well.

Generally, original and adjusted methods would be the same peak height though it might be a sharper peak with the more efficient particles than the older column’s particles.

Reducing the injection volume has another benefit. Since you’re injecting less material onto the column, smaller amounts of excipients and binder material will go through the column. These materials can clog the column and degrade the column’s performance over time, so injecting less volume could actually increase the column lifetime.

Note that larger injection volume can lead to increased dispersion resulting in decreased \( N \) (efficiency) and \( R \) (resolution).

**Mobile phase.** It is possible to make small changes in mobile phase without re-validation. These changes include ratio of mobile phase components and salt or buffer concentration. These changes allow for slight adjustment of chromatographic conditions to allow minor selectivity changes in the chromatography. These changes can be useful when multiple active materials are used together with flavors or colors. It is not possible to substitute buffers (e.g., if sodium phosphate is specified potassium phosphate is not allowed). Also, it is not possible to reduce any component to zero.

Additional adjustments are outlined in TABLE 1.

**A Note on Gradient Methods**

The adjustments described in this article apply to isocratic methods. The right-hand column in TABLE 1 shows allowable adjustments for gradient methods. In some cases, gradient method adjustments are not allowed. It is expected that USP will make some changes in this area in the next year or two.

**Conclusion**

Analytical laboratories currently analyzing generic pharmaceuticals with compendial methods can benefit from increased speed and solvent savings that can be provided by adjusting methods using superficially porous columns. The allowed adjustments described

“If you’re using a column that has only two-thirds of the volume, then you would only inject two-thirds of the sample as well.”
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in this work, using the $L/d_p$ or $N$ rules permit laboratories to increase sample throughput up to 3 times, saving a proportional amount of chromatographic solvent. Additional speed is gained by increasing linear velocity by either the allowed 50% or by the ratio of the particle size change (for example, 5 to 2.7). The combination of changing to a shorter column with smaller particle size and an increased flow rate creates a faster method. These improvements in throughput can be achieved in many cases with no changes in equipment. By applying these allowed adjustments according to *USP <621>* , companies can take advantage of proven technology to boost productivity with no need for additional validation. In this case, short 2.7 µm superficially porous columns can achieve faster results than 5 µm columns resulting in a more productive laboratory while easily meeting system suitability requirements.

William Long

William Long, PhD, is an application scientist at Agilent.
Compendial testing, such as that described in the United States Pharmacopeia-National Formulary (USP-NF), is essential for ensuring the safety and quality of finished drug products and raw materials. Many USP monographs were created for older drugs, however, and tend to be based on traditional column formats such as a 4.6 x 250 mm column packed with 5 μm material. Thus, compendial methods are often more time-consuming and less efficient to run than they would be if they took advantage of newer liquid chromatography (LC) technologies (e.g., smaller particles and superficially porous, core-shell particles) and smaller column sizes.

To address this issue, USP published a revision to USP General Chapter on Liquid Chromatography <621> in August 2014, which offers parameters for “allowable adjustments” that can be made to USP methods without the need for revalidation. Previously, the guidance on such changes was vague. Thus, adjusting compendial methods according to the allowable adjustments clearly defined in USP <621> can help bring down costs while boosting productivity.
and improving the efficiency of analytical laboratories.

Here, William Long, application scientist at Agilent Technologies, spoke with LCGC about how tactful method adjustment can lead to time and cost efficiencies.

**LCGC: How are USP methods used in the pharmaceutical industry and why is the updated USP <621> so important?**

**Long:** USP is a 200-year-old independent, not for profit, non-governmental organization that publishes methods and standards for drug products and raw materials. The USP’s collection of information (i.e., its pharmacopeia) is published digitally in a combined volume with the National Formulary, which is known as the **USP-NF**.

Since 1989, USP has worked in partnership with FDA to create standards that are enforced by the agency. If a drug ingredient or drug product has an applicable USP quality standard for identity, strength, and purity, it must conform to USP’s standard in order to use the designation USP or NF. Drugs subject to the USP standards include human drugs (prescription, over-the-counter, generics, and otherwise) as well as animal drugs. In addition, USP methods are used as starting points for many pharmaceutical methods, including those for generic pharmaceuticals. Many other countries also use the **USP-NF** instead of issuing their own pharmacopoeia or to supplement their own government’s pharmacopeia. USP methods and standards are used in more than 140 countries.

An advantage of using USP methods is that they are already validated. Pharmaceutical companies can even use them if an old material is being included as a part of a newer delivery system. For instance, when developing a new asthma inhaler, a larger pharmaceutical company might simply use the methods that have already been prepared to assay the quality of the asthma drug. As long as the compounds will be used and tested in the same conditions as the USP methods, the previously validated USP methods can still be applied.

USP <621>, updated and published on August 1, 2014, defines the types of adjustments or changes that can be made without substantially changing the method so that it does not require revalidation. Essentially, the USP methods are pre-validated and only needs to be verified at the customer site.

**LCGC: Why should a company consider adjusting USP compendial methods?**

**Long:** Many USP methods were developed with name brand pharmaceuticals several decades ago with the introduction of the drug. Since the adoption of these compounds and methods, new analytical technologies have been developed. While the original columns are useful and reliable, newer columns can increase the throughput of the method. For example, a method
that would typically take 30 minutes to complete could be done in five minutes when using shorter, smaller particle columns. Increasing throughput by five to six times can be invaluable, especially considering the pressure labs often feel to increase throughput and cut turnaround time.

By modernizing compendial techniques to use newer analytical technologies—allowable adjustments defined in USP <621>—you end up with a faster method that may even be more efficient from a cost and environmental perspective because less solvent is being used.

**LCGC: How do you know if an adjustment requires full validation versus verification?**

**Long:** If we follow the adjustments that are outlined in USP <621>, only verification is needed. Avoid generating additional resolution or making the adjusted method able to find things that the other method would not be able to find. Instead, focus the adjustment on using newer technologies and decreasing run time while staying within the same guidelines of the original method. It is most important, however, to follow the Standard Operating Procedure (SOP) for your laboratory or company. If changes to method require validation, then you must follow your own SOP.

**LCGC: Are there any areas that should not be changed when adjusting a method?**

**Long:** Changes from C18 to C8 column may change selectivity and are not allowed adjustments. Even if no apparent change occurs in selectivity some other change may take place. The analyst is free to make changes to methods, but those changes will require full validation of methods. Substitution of reagents described in methods is also not an allowed adjustment. This includes small changes, for example, sodium phosphate to potassium phosphate. Again, while this may or may not cause apparent changes, no substitutions are allowed without validation of methods.

**Long:** Some changes can be very impactful. For example, making a change to the particle size or length of column creates almost an immediate calculable impact. If a column is used that is one-third as long, that means two-thirds of the solvent will be saved. If a new particle size is used that gives a more efficient chromatography, speed will be increased.

Another impactful change is increasing flow rate. A change in flow rate of up to 50% is allowed, if efficiency is not changed by 20%.

Generally, if you switch from a 5 μm totally porous particle column to a 2.7 μm superficially porous particle column, the impact is about five times faster throughput and three times less solvent if the diameter is kept the same.

Considering how much time it takes to adjust a method and verify that any changes made are useful, you may as well use the USP
method for infrequently run methods (e.g., once a month) because you won't see much impact otherwise. Focus your changes on frequently run methods where you will see the most impact.

One important thing to consider is the impact of multiple changes. While this is allowed, the USP cautions the analyst to carefully consider and evaluate each change as it is made.

**LCGC: How can column choice accelerate timelines during method adjustment?**

**Long:** When developing a new method or implementing compendial methods, it is important to use the best technologies available. As a person involved in the development of new columns, I can attest that we continuously evaluate existing materials and new technologies as part of our process to create new products. Existing technologies are benchmarked during new column development, so the newly developed columns can offer improved performance reliably under typically used conditions.

For instance, a few years ago, Agilent released high-pH stable, superficially porous material technology (Poroshell HPH) that could be used at pH 10 in bicarbonate buffer. This material enabled us to develop a superficially porous column with enhanced durability in phosphate buffer, which is normally very destructive to columns at mid-pH. This improved material improves column lifespan and results in less downtime in the laboratory over many existing products.

Another instance is the use of short high-efficiency columns, such as those containing superficially porous particles to minimize development time. This allows fast turnaround time during method adjustment, verification and subsequent use.

Finally, it is also a good idea to consider what instruments will be used to analyze your compound with a finished method. In many cases, having a method that runs at 700 bar or 10,000 psi will limit the instruments available to run your method and leave no flexibility in scheduling testing or perhaps even outsourcing analysis.

**LCGC: If you are considering using smaller particles with shorter columns to implement an isocratic method adjustment, can you explain the best way to move to a superficially porous particle column?**

**Long:** The best way to answer this would be to talk though an example. Let’s use a method for the analysis of benzocaine cough drops.

The USP assay method uses a mobile phase consisting of acetonitrile, water and a 1 molar potassium phosphate pH 3.0 buffer, in a 250:700:50 ratio with a C8 or L7 column. This method also utilizes a 4.6 x 250 mm column packed with 5 μm material. That method takes about 15 minutes to run each sample.

Considering how inexpensive cough drops are and how common their materials are, a more efficient method would be very beneficial, but we want to speed up the analysis time without dramatically increasing the efficiency of the method.

Keeping the $L/d_p$ ratio constant is an important concept in modernizing methods.
This ratio is related to the efficiency or N of the separation. So, if you wanted to switch to a 3.5 μm particle column, you could do the same method with a 150 mm column.

You would essentially shorten the runtime of this method by 40%. Changing the particle size and length of column would result in an increase in throughput while keeping the ratio constant. Now, you could use a 2.7 μm column in this; the same column length would work just as well. So instead of having an analysis time of 15 minutes, you would have a time of only about 9 minutes to run each sample.

You could also increase the flow rate of this method to take advantage of the fact that you have a more efficient particle, because now the particles would run more efficiently at different flow rates. The combination of changing to a shorter column with a smaller particle size with an increased flow rate would give you a faster method.

Superficially porous particles can utilize the $L/d_p$ rule but since they are more efficient, they follow a more direct N rule. Using this technology, you might be able to use a 100 mm column instead of 150 mm column. While it initially does not sound like much of a difference, there are various pharmaceutical analyses being made on a large scale during the drug production process. So, in a way, every bit of time saved helps, and in this case, you are talking about an adjustment that will further shorten the analysis time by as much as 33%. This change from the initial column of 250 mm to a 100 mm column can result in a 60% saving of solvent and time, and more if the flow rate is increased.

Note that the adjustment neither changes how the samples are prepared nor how the solvent being used is made but will reduce the amounts of solvents that are used—and most importantly how time in the laboratory is utilized as well.
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Overview

This article will review some high impact changes you can make to USP compendial methods to save time and money that will not require re-validation. The focus is on how you can adjust older methods to leverage modern technology (superficially porous particle columns). Adjustments made will be to particle size, column length, flow rate, and injection volume.

Introduction

Compendial testing, such as that described in the United States Pharmacopeia-National Formulary (USP-NF), is essential for ensuring the safety and quality of finished drug products and raw materials. Many USP monographs were created for older, generic drugs, however, and tend to be based on traditional column formats such as a 4.6 x 250 mm column packed with 5 μm material. Thus, compendial methods are often more time-consuming and less efficient to run than they would be if they took advantage of newer
liquid chromatography (LC) technologies (e.g., smaller particles and superficially porous, core-shell particles) and smaller column sizes.

Taking advantage of new column technology to increase laboratory productivity while ensuring high-quality separations is key to remaining competitive. Time and solvent are often large costs associated with pharmaceutical testing. Adjusting compendial methods according to the allowable adjustments clearly defined in USP <621> can help bring down these costs while boosting productivity and improving the efficiency of analytical laboratories. A chart summarizing the allowable adjustments within USP <621> can be found here.

This paper will use the USP Naproxen Sodium tablet method to demonstrate how making allowable adjustments can increase your throughput and cut costs.

**USP Naproxen Sodium Tablet Method**

Naproxen is classified as a non-steroidal anti-inflammatory drug (NSAID) and is available as generic tablets. It was patented in 1967 and while it remains a prescription-only drug in much of the world, the Food and Drug Administration (FDA) approved it as an over-the-counter (OTC) drug in 1994 in the United States. The structure of naproxen sodium is shown in **FIGURE 1**. Its IUPAC name is (S)-6-methoxy-a-methyl-2-naphthaleneacetic acid sodium salt.

The method for the analysis of Naproxen Sodium Tablets and Naproxen Impurities was updated as additional standards became available from the USP (2). The USP tablet assay method previously used a 5 µm C18 or L1 column, but has recently been revised to utilize a 5 µm C8 or L7 column with the same mobile phase. In this Technical Proof, the method published in the USP for the tablet assay is adjusted within allowable limits to increase sample throughput using superficially porous particle columns.

While the method calls for a 5 µm column, modern technology can be utilized to achieve chromatographic efficiencies. Superficially porous particle columns, like Agilent InfinityLab Poroshell 120 columns, provide improved performance using a typical LC instrument. These columns have a 2.7 µm superficially porous particle that can provide faster analysis and high resolution in shorter columns for testing more samples in less time on existing instrument. The columns are available in many phases including L1 (C18), L7 (C8), L11 (Phenyl-Hexyl), L10 (Cyano), and many others. To
Method Adjustment Examples

Examples

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Allowable Changes

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fit the USP guidelines for the Naproxen method, this work will utilize the L7 phase (InfinityLab Poroshell 120 EC-C8) to achieve these benefits.

**Approach**

This work will show how the two rules for particle size and column length adjustment can be used to increase laboratory productivity. These two rules are referred to as the “$L/d_p$” rule and the “N” rule. A summary of instrument conditions, part numbers, and the experimental set up is included in the Appendix.

Results and Discussion

One example of an allowed change is the ratio of column length to particle size. This ratio should be kept within a range of -25% to +50%. By keeping the efficiency, a new method is not created. The intent is not to create a more efficient method, but rather a faster method. No changes can be made to the detection without revalidation. Finally, while injection volume may be adjusted as far as consistent precision and detection limit, the injection volumes are scaled geometrically (1).

Original method example: Following the USP method with the original column required, an analysis time of approximately 9 minutes can be met given a retention time of 4.55 minutes with a system suitability requirement of not less than 2 times the retention. The $L/d_p$ ratio is 30,000. In addition, a tailing factor of not more than 2.0 is easily met with a tailing factor of 1.05. This chromatogram appears as **FIGURE 2**.

Particle size adjustment using $L/d_p$ to speed up analysis: To speed up analysis using a 75 mm column with a 2.7 μm particle, we must apply the $L/d_p$ rule. The original method had an $L/d_p$ ratio of 150/5 equaling 30,000 and the new method has an $L/d_p$ of 75/2.7 equaling 27,778. This means that the new method is 92.6% of the $L/d_p$ of the original, which is within the range of -25% to +50%.
Using this rubric, we can find an allowable change that cuts analysis time in half (from 8.9 to 4.6 minutes), also reducing mobile phase consumption by 50%. Injection volume is cut geometrically proportionally to 10 μL, half of the original 20 μL injection volume. Peak height is comparable with the original 5 μm, 150 mm method and tailing factor is 1.03. This chromatogram appears in FIGURE 3A.

Adjusting your flow rate to take advantage of smaller particles: The USP recognizes that smaller particles have a higher optimal linear velocity and thus the flow rate of methods may be increased over the original compendial method.

F1 and F2 refer to the initial and final flow rate, dc refers to the internal diameter of the column, and dp is the column particle diameter. This equation can also be used to adjust to smaller diameter columns. This chromatogram appears in Figure 3b. You can see that the analysis time is reduced even further for a 64% time saving over the
original method. Additionally, solvent saving of 50% is constant when changing to the shorter column.

Particle size adjustment using N to speed up analysis: An alternative to using the L/d_p ratio method is to keep the efficiency of the method with a shorter column. This rule is generally applicable to superficially porous particle columns. In the case of a 50 mm column with a 2.7 µm particle, the efficiency of the column is measured with the analyte of interest (naproxen). This is compared with the naproxen efficiency of the original column with a 5 µm particle. If the efficiency of the new shorter column is within the range of -25% to +50% of the efficiency of the original column, the adjustment is acceptable and only method verification needs to be carried out.

FIGURE 4A shows the chromatogram of naproxen sodium run at 1.2 mL/minute using a 4.6 x 50 mm column. Since the column volume is one-third of the original specified column, the injection volume is reduced similarly. In this example, an efficiency of 11,697 is achieved, which is 90.4% of the original 5 µm, 150 mm method (12,943). This solution is acceptable. Furthermore, the pressure at 160 bar is only slightly above the 132 bar of the original method. Analysis time is approximately 3 minutes. This adjustment will save

TABLE 1: Summary of experiments

<table>
<thead>
<tr>
<th>Approach</th>
<th>Column</th>
<th>Column Length (L, mm)</th>
<th>Particle Size (d_p, µm)</th>
<th>L/d_p Ratio</th>
<th>Allowable L/d_p Range</th>
<th>Injection Volume</th>
<th>N Naproxen Standard</th>
<th>% Time Saving</th>
<th>Pressure (Bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original Method</td>
<td>Fully Porous C8 (L7)</td>
<td>150</td>
<td>5</td>
<td>30,000</td>
<td>22,500–45,000</td>
<td>20 µL</td>
<td>12,943</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Particle size adjustment</td>
<td>Superficially Porous C8</td>
<td>75</td>
<td>2.7</td>
<td>27,778</td>
<td>Yes</td>
<td>10 µL</td>
<td>17,223</td>
<td>48%</td>
<td>217 (1.2 ml/min)</td>
</tr>
<tr>
<td>Using L/d_p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusting Flow Rate</td>
<td>Superficially Porous C8</td>
<td>75</td>
<td>2.7</td>
<td>27,778</td>
<td>Yes</td>
<td>10 µL</td>
<td>15,479</td>
<td>64.7%</td>
<td>318 (1.8 ml/min)</td>
</tr>
<tr>
<td>Particle size adjustment</td>
<td>Superficially Porous C8</td>
<td>50</td>
<td>2.7</td>
<td>18,518</td>
<td>No</td>
<td>6.7 µL</td>
<td>11,679</td>
<td>66%</td>
<td>160 (1.2 ml/min)</td>
</tr>
<tr>
<td>Using N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusting Flow Rate</td>
<td>Superficially Porous C8</td>
<td>50</td>
<td>2.7</td>
<td>18,518</td>
<td>No</td>
<td>6.7 µL</td>
<td>11,113</td>
<td>76%</td>
<td>244 (1.8 ml/min)</td>
</tr>
</tbody>
</table>

Order of experiments follows order of figures: Figures 2, 3A, 3B, 4A, 4B
approximately 66% of the original solvent and 66% of the analysis time. The tailing factor is 1.03. As shown in the previous example, the flow rate may be increased up to 1.5 times the original linear velocity.

Adjusting your flow rate to take advantage of smaller particles: You can adjust the flow rate again to get an even faster analysis. FIGURE 4B shows the chromatogram of naproxen sodium run at 1.8 mL/minute using a 4.6 x 50 mm column.

In this case, the new run time is 2.1 minutes and an efficiency of 9343 is achieved. Our solvent savings is the same (66%), but the time savings is 76% of the original method at 244 bar, which is well within the capabilities of the instrumentation.

TABLE 1 summarizes the experiments approaches followed in this article to achieve significant time and solvent savings.

System suitability requirements are the acceptance criteria for adjustments. In the case of the naproxen sodium assay, we were able to reduce the analysis time from 10 minutes with the original method to 2 minutes on a 4.6 x 50 mm InfinityLab Poroshell 120 EC-C8 2.7 µm column. In addition to the efficiency change, we also earned a 66% decrease in solvent consumption. This is typical of methods adapted to 50 mm InfinityLab Poroshell 120 2.7 µm columns. System suitability requirements using for the naproxen sodium tablet method are not more than (NMT) 2.0%. This is easily met with a 0.064% area RSD and a 0.050% RSD. In addition, USP tailing factor of NMT 2.0 is met, with a tailing factor of 1.05. This is summarized in TABLE 2.

Conclusions

Laboratories performing compendial analyses with fully porous 5 µm columns can benefit from the increased speed and solvent savings that superficially porous 2.7 µm InfinityLab Poroshell 120 columns can provide without needing to replace instrumentation. Faster analysis times, leading to higher throughput, can lead to a more productive laboratory. By applying allowed adjustments with these shorter columns, no additional validation is needed.

“System suitability requirements are the acceptance criteria for adjustments.”
required while easily meeting system suitability requirements.

References
1. USP General Chapter 621, USP 37-NF32, First supplement

Appendix

Agilent 1260 Infinity LC II system was configured using 0.17 mm tubing throughout.

USP Grade or HPLC Certified materials were used in this work as is typical of laboratories that carry out these analytical methods. These include solvents such as acetonitrile, water, glacial acetic acid and naproxen standards. Mobile phase was prepared per USP method by mixing acetonitrile, water, and glacial acetic acid (500 mL:490 mL:10 mL). Samples were prepared following the procedure described in USP 42(4) 2017 (2).

The Agilent columns used in this work were:
- Zorbax Eclipse XDB-8, 4.6 x 150 mm, 5 µm (PN: 993967-906)
- InfinityLab Poroshell 120 EC-C8, 4.6 x 75 mm, 2.7 µm (PN: 699975-906)
- InfinityLab Poroshell 120 EC-C8, 4.6 x 50 mm, 2.7 µm (PN: 697975-906)

William Long

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In-Depth Examples of USP Method Adjustments

The following are examples of other method adjustments that can be made without revalidation.

**Adjusting Injection Volume**
As columns’ dimensions change within allowed adjustments outlined in USP General Chapter <621>, the injection volume should also be adjusted. The simplest way to do this is to use simple geometry. Failure to make this adjustment will lead to loss of efficiency, peak broadening, and poor resolution.

**Adjusting Temperature**
The effect of temperature on liquid chromatography is described and demonstrated with various active pharmaceutical ingredients. As temperature affects pressure, retention time, selectivity, resolution, and efficiency, its control is critical for robust measurements. Even differences of 5°C may have a significant effect on chromatographic separations. United States Pharmacopeia (USP) guidelines allow temperature changes of ±10°C without the need for revalidation.
**Adjusting Mobile Phase Ratio**

Adjusting the ratio of mobile phase minor components in liquid chromatography systems is allowed by the United States Pharmacopoeia without revalidation, provided the parameters published in USP <621> are followed. Here, the benzocaine lozenge method is used to illustrate the allowable changes and their effect on analysis speed.
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