Transfer of HPLC Procedures to Suitable Columns of Reduced Dimensions and Particle Sizes

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ABSTRACT This *Stimuli* article contains proposals to help the analyst adjust HPLC column length and particle size to achieve separation power at least equivalent to that used in the original procedure, markedly increasing the range of options currently allowed in *Chromatography* ⟨621⟩. The article presents the scientific rationale for application of these proposals to isocratic procedures and follows with gradient procedures.

INTRODUCTION

Users of compendial chromatographic procedures increasingly need to develop analytically equivalent procedures that decrease analysis time and solvent consumption. In this process they face limitations because USP does not provide the necessary flexibility to change the chromatographic column without revalidation of the method. The *United States Pharmacopeia* (*USP*) General Chapter *Chromatography* ⟨621⟩ describes in detail the range of adjustments allowed in the system when the suitability test failed. These adjustments in the operating conditions, when needed, are the maximum variations that can be made without the need for validation rather than verification of method performance under the new conditions. Included, among others, are changes in column length (± 70%), changes in column diameter (± 25%), particle size (can be reduced by as much as 50%), and flow rate (± 50%). Additional changes are being implemented (*1*): The column diameter can be changed freely provided that the linear velocity is kept constant, following the formula: where *F*, *l*, and *d* are the flow rates, the column lengths, and the column diameters, respectively, before the change (subscript 1) and after the change (subscript 2). An adjustment of the flow rate by ± 50% is also allowed.

$$F_2 = F_1 \cdot \frac{l_2 \cdot d_2^2}{l_1 \cdot d_1^2} \qquad (1)$$

Except for this flexibility, ⟨621⟩ is silent on changes allowed to the column specified in the monograph. In some cases *USP* chromatographic procedures prescribe the use of a column that is no longer available and needs to be replaced with another of the same stationary phase but different dimensions. In others cases, switching to a column with different particle size and dimensions may provide a more rapid separation with equivalent chromatographic performance. Both these situations currently require revalidation. This article proposes allowing the flexibility to change column dimensions or particle size as long as equivalent or better column performance is maintained, and it provides guidance to ensure that this is achieved in a scientifically rigorous manner.

PROPOSED CHANGES TO THE *SYSTEM SUITABILITY* SECTION OF ⟨621⟩ WITH RESPECT TO PARTICLE SIZE AND COLUMN LENGTH

This *Stimuli* article proposes a new approach that will both preserve the quality of the separation as well as expand the changes in particle size beyond the current twofold decrease. The intent of this proposal is to allow the chromatographer a reduction in analysis time without sacrificing column performance or impairing the separation capability for a procedure.

Chromatography defines the relationships by which particle size, column length, and flow rate can be changed without affecting the quality of the separation (*2–8*). The column plate count *N* is determined as follows:

$$N = (l / H) = l / (d_p \cdot h) \qquad (2)$$

where *l* is the column length, *H* is the theoretical plate height, d_p is the particle diameter, and *h* is the reduced plate height. The quality of the separation is determined primarily by the plate count, which is why most *USP* chromatographic procedures require a minimum plate count. The plate count remains constant if the ratio of column length to particle diameter remains constant, provided that the reduced plate height remains the same (see equation 2).

The reduced plate height *h* depends exclusively on the reduced velocity υ, which in turn is a function of the particle diameter and the flow rate.

$$h = H / d_p \qquad (3)$$

$$\upsilon = u d_p / D_M \qquad (4)$$

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where u is the average linear velocity and D_M is the solute diffusion coefficient. To the practicing chromatographer, this means that the flow rate needs to increase in inverse proportion to the change in particle size in order to maintain the same reduced plate height h:

$$h_2 = h_1 \text{ if } \upsilon_2 = \upsilon_1 \qquad (5a)$$

and therefore

$$F_2 \cdot d_{p,2} = F_1 \cdot d_{p,1} \qquad (5b)$$

and

$$F_2 = F_1 \, (d_{p,1} / d_{p,2}) \qquad (5c)$$

As an example, if the particle size is reduced from 5 μm to 3.5 μm, the flow rate will need to be increased by slightly more than 40%. The combination of the shorter column (equation 2) and the increased flow rate (equation 5c) results in a reduction in analysis time while preserving the performance of the separation.

Analysis time decreases with both the shorter column length l and the higher flow rate F, as the square of the reduction in particle diameter: In addition, because the column length is reduced at the same time as the particle size is decreased, the quantity of solvent per analysis is reduced with the shorter column.

$$t_{a,2} = t_{a,1} \cdot \frac{l_2 \cdot F_1}{l_1 \cdot F_2} = \frac{d_{p,2}^2}{d_{p,1}^2} \qquad (6)$$

Table 1 contains sets of conditions required to maintain the quality of the separation (the same plate count), when users change the particle size.

Table 1. Change in Conditions for Achieving the Same Plate Count

4-mm columns:				
Particle Size (μm)	Column Length (cm)	Flow Rate (mL/min)	Reduction in Analysis Time	Reduction in Solvent Use
5	15	1	1×	1×
3.5	10	1.5	2×	1.5×
2.5	7.5	2	4×	2×
1.7	5	3	9×	3×
2.1-mm columns:				
Particle Size (μm)	Column Length (cm)	Flow Rate (mL/min)	Reduction in Analysis Time	Reduction in Solvent Use
5	15	0.2	1×	1×
3.5	10	0.3	2×	1.5×
2.5	7.5	0.5	4×	2×
1.7	5	0.6	9×	3×

As noted, an increase in flow rate is associated with a reduction in analysis time, and a reduction in the column length is associated with a reduction in solvent consumption. *Figure 1* provides examples from the implementation of this procedure.

From *Figure 1* it can be seen that the separation is quantitatively maintained with each change, in excellent agreement with the theoretical predictions. The good agreement between theory and practice supports allowing this degree of flexibility in the pharmacopeia.

The following rule for conversion between different particle sizes may be used to ensure the plate count remains approximately the same:

> The column length and the particle diameter should be changed approximately in proportion to each other. The flow rate should be increased or decreased in inverse proportion to the change in particle size.

This last change will be beneficial to analysts because current rules for changing the particle diameter do not provide any guidance about how to maintain the quality of the separation.

In some cases, a perfect match of column length and particle size may not be possible. For example, the change in particle size from a 10-μm packing in a 25-cm column to a 5-μm packing would require a 12.5-cm column, which is not commonly available. For such a case, some flexibility can be provided by the following rule:

> If an exact match is not readily available, the ratio of column length to particle size can be changed by ± 25%.

Additional changes in flow rate of ± 50% are already permitted by ⟨621⟩.

As the column volume is reduced, the injection volume should be reduced proportionally. This is consistent with the existing ⟨621⟩ statement that ''the injection volume can be reduced as far as is consistent with accepted precision and detection limits.'' Because scaling to a large diameter and a larger column volume is also possible, we propose changing the statement in ⟨621⟩ as follows:

> *Injection Volume (GC, HPLC)*: Can be adjusted as far as is consistent with accepted precision and detection limits.

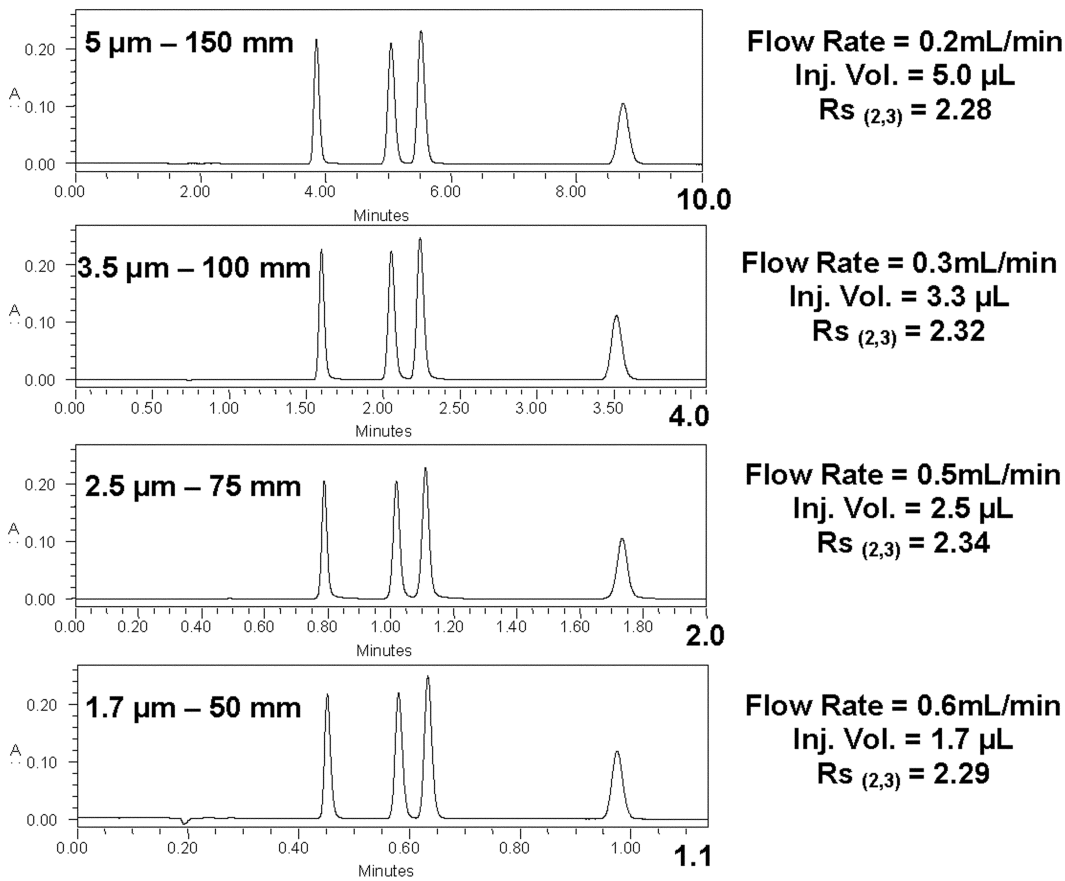

Figure 1. Scaling of a separation at a fixed ratio of column length and particle size using columns with a 2.1-mm internal diameter (reproduced with permission from Reference 7).

As a guide for accomplishing this change, chromatographers may scale the injection volume V_i relative to the column volume V_c, as shown below: The requirements to this point cover the scaling of isocratic methods, which encompass most *USP* methods. However, modern methods are increasingly gradient methods, so we propose the extension of these concepts to this area. In general, all rules written to this point for isocratic methods also apply to gradient methods. In addition, a gradient method is scaled properly if all segments of the gradient $V_{g,s}$ are scaled in proportion to the column volume V_c: Flow rates and injection volumes are scaled as in the isocratic method (see equation 5c—a faster flow rate is used and the particle diameter is reduced). The gradient segment volume $V_{g,s}$ is defined as the time programmed for the gradient segment $t_{g,s}$ multiplied by the flow rate F. This leads to the following formula for the scaling of the time for each gradient segment:

$$t_{g,s,2} = t_{g,s,1}\,[(V_{g,s,2} / F_2) / (V_{g,s,1} / F_1)] \qquad (9)$$

As was true for the isocratic method, this results in a 4-fold reduction in every gradient segment, and thus in the analysis time, for every 2-fold change in particle diameter (and column length).

$$V_{i,2} = V_{i,1} \cdot \frac{V_{c,2}}{V_{c,1}} = V_{i,1} \cdot \frac{l_2 \cdot d_2^2}{l_1 \cdot d_1^2} \qquad (7)$$

$$V_{g,s,2} = V_{g,s,1} \cdot \frac{V_{c,2}}{V_{c,1}} = V_{g,s,1} \cdot \frac{l_2 \cdot d_2^2}{l_1 \cdot d_1^2} \qquad (8)$$

Thus, we propose the following requirement for changing a gradient:

> Gradients are adjusted to the column volume by changing the gradient volume in proportion to the column volume. This applies also to every gradient segment volume.

This simple procedure may be complicated by instrument-dependent gradient delay volumes. The gradient delay volume results in an isocratic section before the beginning of the gradient proper. For a well-functioning instrument and a well-designed gradient, the gradient delay volume is small relative to the column volume, and the analysis is not affected. Alternatively, modern instruments allow for delayed injection to adjust the gradient delay volume to the column volume. An example of a well-scaled gradient method is shown in *Figure 2*. In this case, the method was scaled from a 4.6-mm × 150-mm column packed with 5-μm particles to a 2.1-mm × 50-mm column packed with 1.7-μm particles. Of course, the separation chemistry of both packings was held constant. Following the rules outlined above, the user adjusted the flow rate for both the column diameter and the particle diameter. The gradient times were adjusted to maintain a constant ratio of the gradient segment volumes to the column volumes. As a consequence, the elution pattern and the resolution remain constant, and the analysis time decreased by a factor of 8 with the shorter column.

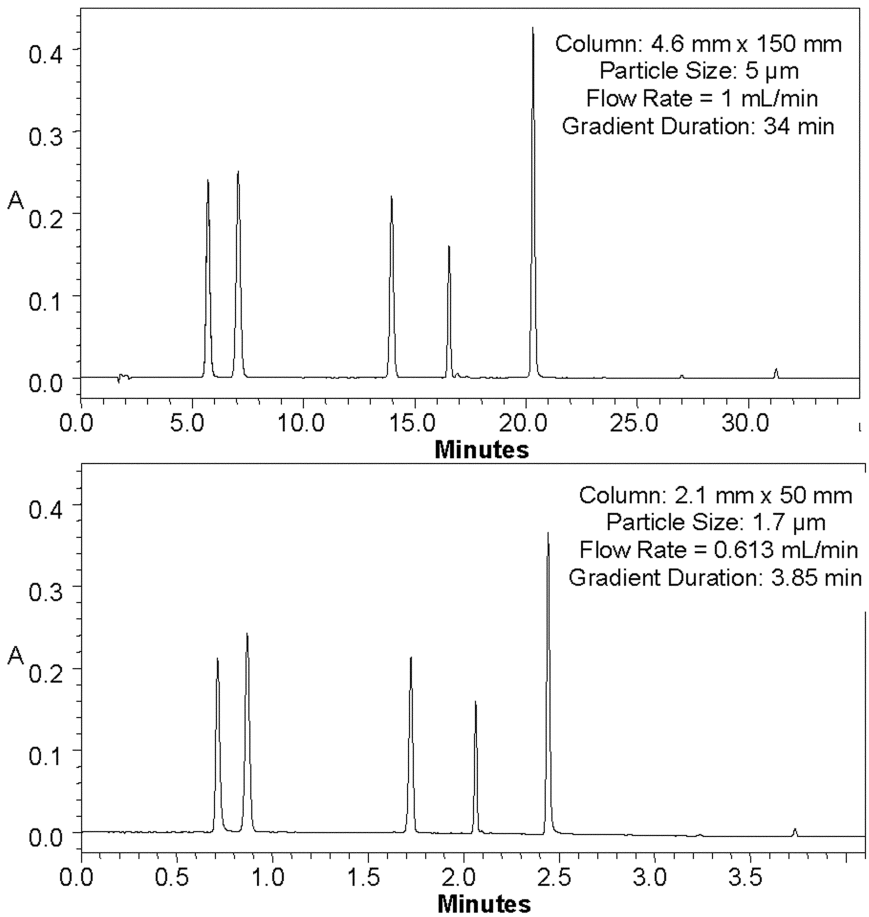

Figure 2. Comparison of two gradient chromatograms scaled by column dimension and particle diameter according to the rules described herein (reproduced with permission from Reference 9).

SUMMARY

The proposed changes to ⟨621⟩ will expand the analyst's ability to reduce solvent consumption and decrease analysis time while maintaining the quality of a chromatographic separation. By adhering to the proposed requirements, analysts can maintain the quality of the separation for the entire range of possible particle diameters, extending and improving on the rule that limits the particle diameter change to – 50% and the change in column length to ± 70%.

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