

December 2022 revision to USP <621> allowable changes to monograph LC methods

INTRODUCTION

The United States pharmacopoeia (USP) prescribes analytical methods for the testing of pharmaceutical active ingredients and final product to ensure quality and safety for the consumer. Liquid chromatography (LC) is commonly employed in monographs for active substance assay and impurity testing. Monograph methods contain all the analytical parameters required to run the method. They have been validated and are ready to use by the analyst (although the analyst is required to verify that the method can be used for its intended purpose, for example, selected analytical performance characteristics of USP methods require verification according to general chapter <1226> when run for the first time, but do not require full validation according to chapter <1225>).[1, 2]

Although replicating monograph methods is, in principle, relatively straightforward, a number of factors can lead to differences in the results obtained from different laboratories running the same monograph method. The USP therefore includes a general chapter on

chromatography (chapter <621>),[3] which specifies allowable changes that the analyst can make to the method if necessary.

In a 2014 update, the guidance was amended to allow the analyst more flexibility to translate methods to more modern LC practices (i.e. narrower bore columns packed with smaller particles). However the guidance applied primarily to isocratic methods, with changes to gradient methods remaining restricted.

In December 2022, a further revision of this chapter became official, which is a result of harmonisation with the European and Japanese pharmacopoeias through the Pharmacopoeial Discussion Group (PDG) in 2021.[4,5] In the latest update, further revisions have been included around isocratic methods, whilst the guidance on allowable changes to gradient methods has been overhauled. The permitted changes for gradient methods now allow analysts to translate monograph methods to alternative column formats to take advantage of modern approaches, i.e. smaller format, narrow bore columns / UHPLC. This will allow for improvements in laboratory

efficiency and throughput, along with potential for achieving significant reductions in waste generated.

This Knowledge Note summarises the allowable changes permitted for LC methods previously published by the USP and compares them to the changes now permitted under the 2022 update. The information presented is for reference, please refer the full text for clarity to ensure full compliance.^[5]

ISOCRATIC LC METHODS

For isocratic LC methods, the allowable changes to the column format (length, ID and particle size) remain similar to the previous guidance (Table 1). Most of the changes that have been made are aimed at providing more guidance and flexibility for translating isocratic

methods to more modern column formats (i.e. smaller format columns packed with smaller particle sizes and/or superficially porous particles).

Further guidance to cover changes from totally porous particles (TPP) to superficially porous particles (SPP) has been added. Additionally, a statement advising extra-column dead volume may need to be minimised due to the small peak volumes produced when using narrow bore, small particle size column is now included.

The guidance on allowable flow rate adjustment has been expanded, whilst new guidance on adjusting injection volume when moving to shorter and/or narrower bore columns has been incorporated. Other changes include a new definition of minor mobile phase constituents.

Table 1: Comparison of allowable method adjustments for USP isocratic methods in the previous and updated text.^[5,6]

ISOCRATIC METHODS		
	USP 40 / NF 35 <621>	USP <621> 2022 revision
Mobile phase		
Composition	Minor mobile phase components can be changed by $\pm 30\%$ relative. The change in any component cannot exceed $\pm 10\%$ absolute. Adjustments can be made to one minor component of a ternary mixture.	Minor mobile phase components can be changed by $\pm 30\%$ relative. The change in any component cannot exceed $\pm 10\%$ absolute. A minor component is defined as $\leq (100/n)\%$, where n is the total number of mobile phase components.
pH	pH of aqueous buffer used in mobile phase preparation can be adjusted by ± 0.2 units.	pH of the aqueous buffer component can be adjusted by ± 0.2 units.
Buffer salt concentration	$\pm 10\%$ if the permitted pH variation is met.	$\pm 10\%$ salt concentration in aqueous buffer component.
Column		
Stationary phase chemistry	No change to the chemical characteristics ("L" designation) of the stationary phase permitted.	No change to the identity of the substituent (e.g. no replacement of C18 by C8) permitted. Other physicochemical stationary phase characteristics (i.e., chromatographic support, surface modification and extent of chemical modification) must be similar. A change from totally porous particle (TPP) columns to superficially porous particle (SPP) columns is allowed provided the above are met.
Length & particle size	Particle size (dp) and length (L) may be changed if a) L/dp is constant or varies -25% to $+50\%$ OR b) number of plates (N) is -25% to $+50\%$.	Particle size (dp) and length (L) may be changed if L/dp is constant or varies -25% to $+50\%$. Refer to flow rate adjustment section. For particle-size adjustment from totally porous to superficially porous particles, other combinations of L and dp are permitted provided N is within -25% to $+50\%$, relative to the prescribed column.

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ISOCRATIC METHODS (CONTINUED)

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Column

Internal diameter

Any changes if linear velocity is kept constant.

In the absence of a change in dp and/or L , the column internal diameter may be adjusted. For adjustment of flow rate, refer to flow rate section.

Minimization of extra column band broadening may be required (e.g. instrument connections, flow cell volume and sampling rate and injection volume) due to smaller peak volumes.

Method parameters

Flow rate

When the particle size is changed, the flow rate may require adjustment. Flow rate changes for both a change in column diameter (dc) and particle size (dp) can be made by:

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

When changing from $\geq 3 \mu\text{m}$ to $< 3 \mu\text{m}$, an additional increase may be justified as long as N doesn't decrease by $>20\%$.

Additionally, flow rate can be adjusted by $\pm 50\%$

When the particle size is changed, the flow rate (F) requires adjustment.

Flow rate changes are adjusted for both a change in column diameter (dc) and particle size (dp) by:

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

When changing from $\geq 3 \mu\text{m}$ to $< 3 \mu\text{m}$, an additional increase may be justified as long as N doesn't decrease by $>20\%$. Similarly, when changing from $< 3 \mu\text{m}$ to $\geq 3 \mu\text{m}$, an additional reduction may be justified to avoid reduction in column performance decrease by more than 20% .

After adjustment due to a change in column dimensions, an additional change in flow of $\pm 50\%$ is permitted.

In absence of a change in column dimensions, an adjustment in flow of $\pm 50\%$ is permitted.

Injection volume

Any changes as long as precision, linearity and detection limits are acceptable

When column dimensions are changed, injection volume (V_{inj}) can be adjusted by:

$$V_{inj2} = V_{inj1} \times \left[\frac{(L_2 \times dc_2^2)}{(L_1 \times dc_1^2)} \right]$$

This equation may not be applicable to changes from TPP to SPP columns.

Even in the absence of column dimension changes, V_{inj} may be varied provided system suitability criteria are within accepted limits. When V_{inj} is decreased, special attention should be given to (limit of) detection and repeatability. An increase is permitted provided linearity and resolution remain satisfactory.

Temperature

$\pm 10^\circ\text{C}$

$\pm 10^\circ\text{C}$ where temperature is specified, unless otherwise prescribed.

Detection wavelength

No change permitted, error in detector wavelength is at most $\pm 3 \text{ nm}$.

No change permitted.

GRADIENT LC METHODS

Previously, minimal changes were permitted to gradient monograph methods. No changes to the column dimensions or particle size were allowed. As part of the harmonisation process however, substantial changes have been implemented which permit the analyst to run gradient monograph methods on alternative column dimensions and particle sizes. This change allows methods developed on older format columns (i.e. 150 and 250 mm lengths with 5-10 µm particles), which necessarily involved long gradient times, to be translated to smaller format columns, thus providing considerable scope for modernising monograph methods by increasing sample throughput and reducing solvent waste.

Adjustment of gradient methods does however require considerably more caution compared to adjusting isocratic methods. A key consideration is that the selectivity in gradient separations is determined by the volume of each gradient segment (i.e. $t_G \times F$). When changing the column dimensions and flow rate of a

gradient separation, it is imperative that the ratio of the column volume to gradient volume remains constant to avoid any changes in selectivity. Additionally, changes in system dwell volume can result in selectivity changes and the new USP guidance provides information on this.

When changing a gradient method to utilise a different format column, 3 key steps should be performed:

- 1. Adjust column length and particle size keeping L/d_p constant.
- 2. Adjust flow rate for changes in column internal diameter and particle size.
- 3. Adjust the gradient time of each segment for changes to flow rate and column dimensions.

In addition the injection volume should also be volumetrically adjusted to the new column volume. Table 2 summarises the key equations that should be used for these steps and describes allowable changes according to the 2022 USP <621> updates and compares these to the previous guidance.

Table 2: Comparison of allowable method adjustments for USP gradient methods in the previous and updated text.^[5,6]

GRADIENT METHOD ALLOWABLE ADJUSTMENTS		
USP 40 / NF 35 <621>		USP <621> 2022 revision
Mobile phase		
Composition	not recommended.	Adjustments of composition of the mobile phase are acceptable provided that: • System suitability criteria are fulfilled • The principle peak elutes within ±15% of the retention time obtained with the original conditions. This does not apply for changes in column dimensions. • The first peaks are sufficiently retained and the last peaks are eluted.
pH	pH of aqueous buffer used in mobile phase preparation can be adjusted by ±0.2 units	pH of the aqueous component of the mobile phase: ±0.2 units
Buffer salt concentration	±10% if the permitted pH variation is met	±10%

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GRADIENT METHOD ALLOWABLE ADJUSTMENTS (CONTINUED)

USP 40 / NF 35 <621>

USP <621> 2022 revision

Column

Stationary phase chemistry

No change to the chemical characteristics ("L" designation) of the stationary phase permitted.

No change to the identity of the substituent (e.g. no replacement of C18 by C8) permitted. Other physicochemical stationary phase characteristics (i.e., chromatographic support, surface modification and extent of chemical modification) must be similar.

A change from totally porous particle (TPP) columns to superficially porous particle (SPP) columns is allowed provided the above are met.

Length & particle size

No changes.

Particle size (dp) and length (L) may be changed if L/dp is constant or varies -25% to +50%. Refer to flow rate adjustment section.

For particle-size adjustment from totally porous to superficially porous particles, other combinations of L and dp are permitted provided $(t_R/W_R)^2$ is within -25% to +50%, relative to the prescribed column for all peaks used for system suitability parameters. These changes are permitted provided system suitability criteria, selectivity and elution order are equivalent.

Internal diameter

No changes.

In absence of a change in dp and/or L , the column internal diameter may be adjusted. For adjustment of flow rate, refer to flow rate section.

Minimization of extra column band broadening may be required (e.g. instrument connections, flow cell volume and sampling rate and injection volume) due to smaller peak volumes.

Method Parameters

Flow rate

No changes.

When the particle size is changed, the flow rate requires adjustment.

Flow rate is adjusted for both a change in column diameter (dc) and particle size (dp) by:

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

Gradient

No changes.

A change in column dimensions (and thus column volume) requires adjustment of every gradient segment to maintain a constant ratio of the gradient volume to the column volume ($L \times dc^2$). The new gradient time (t_{G2}) can be calculated from the original gradient time (t_{G1}), the flow rate (F) and column dimensions (L and dc) as follows:

$$t_{G2} = t_{G1} \times \left(\frac{F_1}{F_2} \right) \times \left(\frac{L_2 \times dc_2^2}{L_1 \times dc_1^2} \right)$$

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GRADIENT METHOD ALLOWABLE ADJUSTMENTS (CONTINUED)

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Method Parameters

Gradient (continued)	No changes.	Please refer to reference 5 for a detailed example of how to apply these changes using the gradient conversion factor (t_{G2}/t_{G1}).
Injection volume	Any changes as long as precision, linearity and detection limits are acceptable.	When column dimensions are changed, injection volume (V_{inj}) can be adjusted by: $V_{inj2} = V_{inj1} \times \left[\frac{(L_2 \times dc_2^2)}{(L_1 \times dc_1^2)} \right]$ This equation may not be applicable to changes from TPP to SPP columns. Even in the absence of column dimension changes, V_{inj} may be varied provided system suitability criteria are within accepted limits. When V_{inj} is decreased, special attention should be given to (limit of) detection and repeatability. An increase is permitted provided linearity and resolution remain satisfactory.
Temperature	± 10 °C	± 5 °C where temperature is specified, unless otherwise prescribed.
Detection wavelength	No change permitted, error in detector wavelength is at most ± 3 nm.	No change permitted.
Dwell volume	Adjustments to dwell volume and/or the duration of any initial isocratic hold in the gradient table.	Monograph methods preferably include an isocratic step before the gradient so that gradient time points can be adapted to account for dwell volume between the system used for development of the method and the actual equipment used. It is the user's responsibility to adapt the length of the isocratic step to the analytical equipment used. If the dwell volume used is specified in the monograph, the gradient time points (t, min) stated can be replaced by adapted time points (t_c, min) using the following equation: $t_c = t - \frac{(D - D_0)}{F}$ D = dwell volume (mL) D_0 = dwell volume used for method development (mL) F = flow rate (mL/min) The isocratic step may be omitted if validation data without the step included is available.

INCREASING SAMPLE THROUGHPUT

Many monograph LC methods were developed using traditional HPLC technology, often utilising large format columns (e.g. 250 x 4.6 mm columns, packed with 5–10 µm particles). Over the last couple of decades, the increased availability of smaller stationary phase particles, along with optimisation and higher pressure capabilities of LC instrumentation (particularly UHPLC), mean that many monograph methods have great potential for drastic improvements in sample throughput.

With this in mind, USP <621> allowable changes permit the analyst to scale the method to more modern, smaller column formats and utilise smaller particle sizes without needing to formally revalidate the method. This allows laboratories to achieve considerable reductions in method run times and increase sample throughput. However, after implementing these changes, it is important that the analyst should verify the suitability of the method under the new conditions, by assessing the analytical performance characteristics potentially affected by the change, thereby ensuring system suitability compliance. It is also essential to refer to the USP directly for guidance on the appropriate changes that are permitted for a specific method.

Guidance for both isocratic and gradient monograph methods in the USP <621> general chapter for chromatography is based on using the concept of the ratio of column length (L) to particle diameter (dp) to guide allowable changes to the particle size and column length. When adjusting the length and particle size of a column, if the L/dp ratio remains constant, then the same column efficiency is achievable. Table 3 shows L/dp ratios for some common format columns. From this table,

it can be seen that a 250 x 4.6 mm column packed with 5 µm particles has an L/dp value of 50,000. This method could therefore be migrated to 3 alternative L/dp combinations to maintain the same theoretical efficiency. The guidance also states that a change in L/dp of -25% to +50% is acceptable, which clearly presents a large number of additional possibilities. Alternatively, the analyst may instead use superficially porous, or solid-core particles, such as the Avantor® ACE® UltraCore,^[7] in which case L/dp combinations outside this range may be used, as described in Tables 1 and 2.

The guidance also allows for the columns internal diameter to be reduced (flow rate should also be scaled, see flow rate section in Tables 1 and 2). It is important to note that the small peak volumes generated by smaller internal diameter columns and small particle sizes may require optimisation of system extra-column band broadening, which can be achieved through optimisation of system connections and tubing, smaller internal volume detector cells and faster data acquisition rates.

For gradient monograph methods, sample throughput can also be increased by using smaller column dimensions. Three key steps are highlighted in the USP guidance to translate a gradient method in this way:

1. Adjust column length and particle size according to L/dp .
2. Adjust flow rate for changes in column internal diameter and particle size.
3. Adjust the gradient time of each segment for changes to flow rate and column dimensions.

Table 4 demonstrates this approach for moving a

Table 3: L/dp ratios for a range of common column length and particle size combinations. Formats that provide an L/dp value of 50,000, and therefore equivalent theoretical efficiency are highlighted green. Formats within the range -25% to +50% (i.e. L/dp = 37,500–75,000) are highlighted in light green.

		Column Length (mm)							
Particle Size (µm)		30	50	75	100	125	150	250	300
	1.7	17,647	29,412	44,118	58,824				
	2	15,000	25,000	37,500	50,000	62,500	75,000		
	2.5	12,000	20,000	30,000	40,000	50,000	60,000		
	3	10,000	16,667	25,000	33,333	41,667	50,000	83,333	
	5	6,000	10,000	15,000	20,000	25,000	30,000	50,000	
	10	3,000	5,000	7,500	10,000	12,500	15,000	25,000	30,000

gradient method to a smaller format column. In addition, the injection volume should be volumetrically adjusted to the new column volume. All the key equations that should be used for these steps are summarised in Table 2.

These calculations can be easily performed using the free to download Avantor® ACE® LC Translator Tool.^[8]

Whilst this document provides a summary of the changes that have been implemented as of December 2022, it is important for users to refer to the full text and specific monograph methods to ensure that changes are correctly implemented and system suitability criteria met. Additionally, the latest pharmacopoeia edition should be checked to ensure the most up to date guidance is followed.

Table 4: Example translation of a gradient method from a 100 x 4.6 mm, 5 µm column to a 50 x 2.1 mm, 2 µm column using the guidance outlined in Table 2.

Parameter	Original method	Adjusted method
Column length (L):	100 mm	50 mm
Column ID (dc):	4.6 mm	2.1 mm
Particle size:	5 µm	2 µm
L/dp:	20,000	25,000 (+25%)
Flow rate:	1 mL/min	0.52 mL/min
Injection Volume:	5 µL	0.5 µL
Gradient:		
%B	Time (min)	Time (min)
5	0	0
5	2	0.4
60	22	4.4
60	24	4.8
5	25	5

CONCLUSION

Pharmacopoeias typically contain guidance on how monograph LC methods can be adjusted without requiring formal validation. These allowable changes can be used by the analyst to ensure an analysis meets system suitability criteria and can also allow the method to be run using alternative column lengths and particle sizes. It is important for analysts to refer to the latest guidelines to ensure regulatory compliance when adjusting monograph methods. This article has summarised current guidance on allowable changes to USP methods as of December 2022 and has demonstrated how the guidance can be used to utilise more modern format columns and achieve substantial reductions in method run times.

REFERENCES

1. United States Pharmacopoeia, Chapter <1226> "Verification of compendial procedures"
2. United States Pharmacopoeia, Chapter <1225> "Validation of compendial procedures"
3. United States Pharmacopoeia, Chapter <621> "Chromatography"
4. Press release: "The Harmonised General Chapter Chromatography (G-20) Was Signed-Off By The Pharmacopoeial Discussion Group (PDG)" (<https://www.usp.org/sites/default/files/usp/document/harmonization/gen-chapter/press-release-chromatography-pdg-sign-off-110821.pdf>).
5. United States Pharmacopoeial Forum, Chapter <621> "Chromatography", (downloaded from <https://www.uspnf.com/pharmacopoeial-forum>, on 5th December 2022).
6. United States Pharmacopoeia, Chapter <621> "Chromatography", First Supplement to USP 40-NF 35.
7. Avantor® ACE® Knowledge Note #0019 "Avantor® ACE® UltraCore and Solid Core Technology" (https://uk.vwr.com/cms/ace_knowledge_notes)
8. Avantor® ACE® LC Translator (download at https://uk.vwr.com/cms/ace_knowledge_zone)