

Allowable changes to USP and Ph. Eur. monograph LC methods

INTRODUCTION

Pharmacopoeias, such as the United States (USP), Chinese (ChP), Indian (IP), British (BP), Japanese (JP) and European (Ph Eur.) pharmacopoeias, prescribe 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 analytical parameters required to run the method and have been validated and are therefore 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. Pharmacopoeias therefore include general chapters which specify allowable changes that the analyst can make to the method if necessary. This Knowledge Note summarises the allowable changes permitted for LC methods published in the USP and Ph. Eur. and additionally, demonstrates how these can be utilised to speed up methods and improve laboratory throughput.

USP AND PH. EUR. ALLOWABLE CHANGES

Monograph methods for LC separations contain details of all the materials, reagents and analytical parameters required to perform the specified analysis. This includes sample and mobile phase preparation, the LC column specifications, along with instrument parameters such as flow rate, injection volume and column temperature. When replicating analytical methods in different laboratories, often using different analytical instruments, variations in analytical data compared to the original method are not uncommon. This can arise due a number of factors, such as variation in measurement calibration

and accuracy (e.g. volumetric, weighing and pH measurements, etc.), differences in column oven temperature set points and the use of different vendor materials and reagents amongst others. One particular factor that can majorly affect the separation obtained for LC separations is the column stationary phase. For example, if a monograph method specifies the use of a

“C18 or octadecyl” column (or L1 column for USP methods), the analyst is faced with potentially hundreds of different options from different column manufacturers. The column selected may therefore demonstrate different selectivity to that used for the original method, potentially leading to loss of resolution or peak co-elution.

Table 1: Allowable method adjustments for USP and Ph. Eur. isocratic methods [3, 4].

ISOCRATIC METHODS		
	USP 40 / NF 35 <621>	Ph. Eur. 8.0
Mobile phase		
Composition	Minor mobile phase components can be changed by ±30% relative. The change in any component cannot exceed ±10% absolute. Adjustments can be made to one minor component of a ternary mixture.	The amount of the minor solvent component may be adjusted by ±30% relative or ±2% absolute, whichever is larger. No other component is altered by more than ±10% absolute
pH	pH of aqueous buffer used in mobile phase preparation can be adjusted by ±0.2 units	±0.2 units, unless otherwise stated or ±1.0 units for non-ionisable substances
Buffer salt concentration	±10% if the permitted pH variation is met	Concentration of salts in the buffer component ±10%
Column		
Stationary phase chemistry	No change to the chemical characteristics (“L” designation) of the stationary phase permitted	No change of the identity of the substituent permitted (e.g. no replacement of C18 with C8)
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%	Maximum reduction of 50% in particle size, no increase permitted. Change in column length: ±70%
Internal diameter	Any changes if linear velocity is kept constant	±25%
Method		
Flow rate	<p>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:</p> $F_2 = F_1 \times \left[\frac{(dc_2^2 \times dp_1^2)}{(dc_1^2 \times dp_2^2)} \right]$ <p>When changing from ≥3 µm to ≤3 µm, an additional increase may be justified as long as N doesn't decrease by >20%</p> <p>Additionally, flow rate can be adjusted by ±50%</p>	<p>A change in flow rate of ±50% is permitted.</p> <p>A larger adjustment is acceptable when changing column dimensions using the following equation:</p> $F_2 = F_1 \times \left[\frac{(l_2 \times dc_2^2)}{(l_1 \times dc_1^2)} \right]$ <p>F = flow, l = length, dc = internal diameter, 1 = specified column, 2 = new column</p>
Injection volume	Any changes as long as precision, linearity and detection limits are acceptable	May be decreased, provided detection and repeatability of peaks are satisfactory. No increase permitted
Temperature	±10 °C	±10 °C where the temperature is specified
Detection wavelength	No change permitted, error in detector wavelength is at most ±3 nm	No Adjustment permitted

For these reasons, pharmacopeia's generally specify allowable changes that can be made to monograph methods without requiring revalidation, to enable the analyst to meet system suitability requirements.

Importantly, whilst isocratic methods are relatively flexible, changes to gradient methods are typically much more restrictive. Although efforts to harmonise allowable changes have been made, it is important to note that different pharmacopoeias guidelines still vary to some

degree and it is therefore essential to refer to the appropriate regulatory texts (e.g. USP, Ph. Eur, ChP, IP, BP, JP, etc.) before making changes to methods. It should also be noted, that adjustments should not be made to compensate for instrument malfunction or a failure of the analytical column.

Tables 1 and 2 outline the allowable changes specified in the USP and Ph. Eur [3, 4]. The changes specified should always be verified against the latest guidance from the

Table 2: Allowable method adjustments for USP and Ph. Eur. gradient methods [3, 4].

GRADIENT METHOD ALLOWABLE ADJUSTMENTS		
USP 40 / NF 35 <621>		Ph. Eur. 8.0
Mobile phase		
Composition	not recommended	Minor adjustments of composition and gradient permitted provided system suitability requirements are fulfilled, principal peak(s) elute at $\pm 15\%$ of the specified retention time. Final concentration of mobile phase is not weaker in elution power than that prescribed
pH	pH of aqueous buffer used in mobile phase preparation can be adjusted by ± 0.2 units	No adjustments permitted
Buffer salt concentration	$\pm 10\%$ if the permitted pH variation is met	No adjustments permitted
Column		
Stationary phase chemistry	No change to the chemical characteristics ("L" designation) of the stationary phase permitted	No change of the identity of the substituent permitted (e.g. no replacement of C18 with C8)
Length & particle size	No changes	Particle size: no adjustments permitted Column length: $\pm 70\%$
Internal diameter	No changes	$\pm 25\%$
Method		
Flow rate	No changes	When changing column dimensions flow rate may be adjusted as necessary using the following equation: $F_2 = F_1 \times \left[\frac{(l_2 \times dc_2^2)}{(l_1 \times dc_1^2)} \right]$
Injection volume	Any changes as long as precision, linearity and detection limits are acceptable	May be decreased, provided detection and repeatability of peaks are satisfactory. No increase permitted
Temperature	$\pm 10^\circ \text{C}$	$\pm 5^\circ \text{C}$ where the temperature is specified
Detection wavelength	No change permitted, error in detector wavelength is at most $\pm 3 \text{ nm}$	No Adjustment permitted
Dwell volume	Adjustments to dwell volume and/or the duration of any initial isocratic hold in the gradient table	Gradient table time points may be adjusted to compensate for differences in dwell volume

relevant authority before use to ensure up-to-date compliance.

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 the 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, pharmacopoeia 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 enhance 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 relevant Pharmacopoeia for guidance on the appropriate changes that are permitted for a specific method.

Using the USP as an example, an update to the <621> general chapter for chromatography in 2014 introduced the concept of using the ratio of column length (L) to particle diameter (dp) to guide allowable changes to the

particle size for isocratic methods. 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 smaller 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 an L/dp combination outside this range, provided that the number of theoretical plates (N) is between -25% and +50% of the original prescribed column. This provides further flexibility to use superficially porous, or solid core particles, such as the Avantor® ACE® UltraCore [5].

The USP guidance also permits changing the column internal diameter as long as the flow rate is scaled to maintain the same linear velocity, which can be utilised to achieve reductions in solvent usage. This scaling can be achieved using the equation [6]:

$$F_2 = F_1 \times \frac{d_{c2}^2}{d_{c1}^2}$$

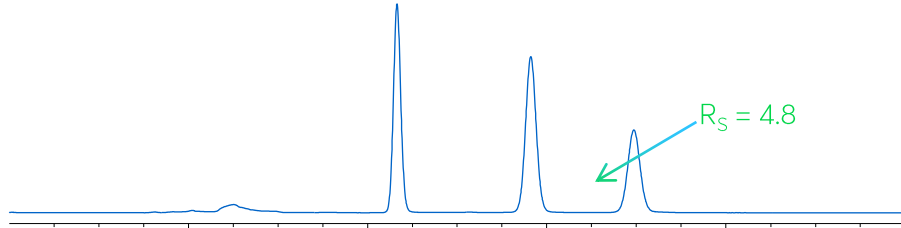
F₁ and F₂ are the original monograph flow rate and the new flow rate, whilst d_{c1} and d_{c2} are the respective column internal diameters.

Figure 1 demonstrates how this guidance can be used to speed up a USP monograph LC method for estradiol using both HPLC (B) and UHPLC (C) compatible column formats, whilst still meeting all system suitability criteria

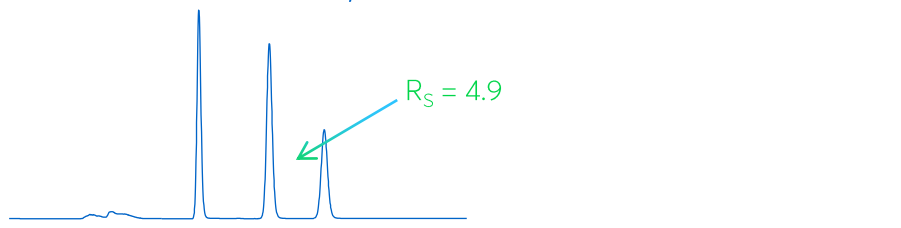
Table 3: L/dp ratios for a range of common column length and particle size combinations. Formats that provide an L/dp value of 50,000, and therefore equivalent theoretical efficiency are highlighted green. Formats within the range -25% to +50% (i.e. L/dp = 37500–75,000) are highlighted in light green.

		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

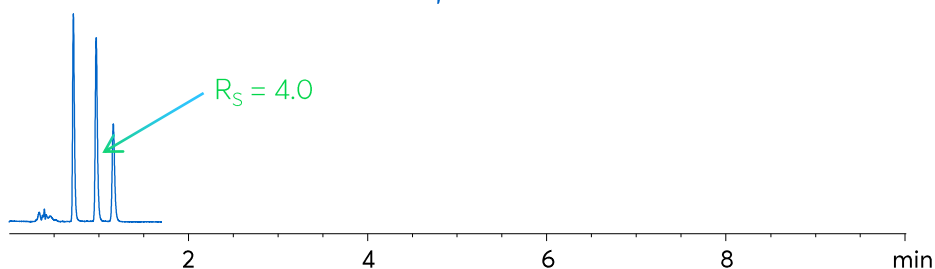
A Avantor® ACE® 10 C18, 300 x 3.9 mm



B Avantor® ACE® 5 C18, 150 x 4.6 mm



C Avantor® ACE® Excel 2 C18, 50 x 3.0 mm



	Original monograph method (A)	Translated method 1 (B)	Translated method 2 (C)
Column dimensions (mm)	300 x 3.9	150 x 4.6	50 x 3.0
d_p (μm)	10	5	2
L/d_p	30,000	30,000	25,000
Column volume (mL)	2.258	1.571	0.223
Flow rate (mL/min)	1.00	1.39	0.59
Injection volume (μL)	25	17.4	2.5
System suitability 1: Resolution between estradiol and estrone >2.0	4.8	4.9	4.0
System suitability 2: %RSD <2.0	0.1	0.1	0.2
Run time (min)	10.0	5.0 (-50%)	1.7 (-83%)
Solvent consumption (mL)	10.0	7.0 (-30%)	1.0 (-90%)

Figure 1: Method translation of the USP Estradiol assay system suitability test according to official guidelines in the USP <621> general chapter. Method conditions: Isocratic MeCN/H₂O 45:55 v/v; Column temperature: 22 °C; Detector: UV, 205 nm. Sample: 1. ethyl paraben, 2. estrone, 3. estradiol.

and therefore requiring no formal method re-validation.

In the first example (A to B), the L/dp value is maintained, therefore the resolution is essentially unaffected. In the second example (A to C), a column with a lower L/dp ratio was selected to trade some of the excess resolution and achieve a greater reduction in run time. In both cases, the column internal diameter has been changed and flow rate adjusted to maintain a constant linear velocity. In each case, the injection volume has been scaled volumetrically to the dead volume of the new column, as described in reference 4. These calculations can be easily performed using the free to download Avantor® ACE® LC Translator Tool [7]. Both solutions provide substantial savings in terms of both analytical run time and solvent consumption, whilst readily meeting the system suitability criteria specified in the monograph. The USP guidance also permits the user to further increase flow rate when the particle size is changed (see Table 1), providing further potential to speed up these methods if desired.

At the time of writing, the Ph. Eur. follows a different approach, allowing a maximum reduction of particle size of 50% and a change in column length of $\pm 70\%$. In 2017 Ph. Eur. published a revised 2.2.46 chapter for comment, that seeks future harmonisation with USP and incorporates the L/dp guidance [8]. It is therefore important for users to refer to the latest pharmacopoeia edition to verify which guidelines are applicable.

CONCLUSION

Pharmacopoeia's 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 and Ph. Eur. methods and has demonstrated how the USP 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. European Pharmacopoeia, Chapter 2.2.46 "Chromatographic separation techniques"
5. Avantor® ACE® Knowledge Note #0019 "Avantor® ACE® UltraCore and Solid Core Technology"
6. P. Petersson, M.R. Euerby, M.A. James, LCGC Europe **28** (2015) 310-320
7. Avantor® ACE® LC Translator (download at https://uk.vwr.com/cms/ace_knowledge_zone)
8. Pharmedropa 29.3 (July 2017) (https://pharmedropa.edqm.eu/app/Archives/content/Archives-37188/Pharmedropa_29.03E.pdf)