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The **European Pharmacopeia (EP)** Chapter 2.2.46 contains information that is similar to the USP Chapter 621.

This includes general information about all chromatographic separations techniques, system suitability definitions and requirements, and chromatographic condition adjustments, also known as, allowable or allowed adjustments.

The extent to which the various parameters of a chromatographic test may be adjusted to satisfy the system suitability criteria without fundamentally modifying the methods are separately listed by Thin layer-, Liquid-, Gas- and Supercritical chromatography. These allowed adjustments may be necessary since the stationary phases are described in a general way, and there are a variety of phases available commercially that meet these general descriptions, which can result in chromatographic behavior differences.

The last liquid chromatography allowed adjustments revision in 2010, stated that adjustments for gradient methods are more critical than isocratic methods. These changes can lead to shifts in peaks and to a different step of the gradient. This then leads to the incorrect assignment of peaks, peak masking, or an elution shift that occurs beyond the prescribed elution time. As a result, the allowed adjustments were class-divided for isocratic and gradient methods, with minor allowed adjustments for the latter. Effective August 2014 (USP37-NF32, 1st supplement) the USP split the allowed adjustments into isocratic and gradient sections. In addition, the USP introduced a substantial change in the column related to allowable adjustments for isocratic methods to improve user flexibility.

The primary focus is keeping the column plate number, and thus resolution, fairly constant. Since the plate number is a function of the length of the column divided by the particle diameter, the L/dp ratio is the key factor here. The column length and particle diameter can be changed as long as L/dp is constant or in an allowed variation from -25% to +50%. Just recently in Pharmeuropa 29.3 (July 2017) a new draft of the **European Pharmacopeia** Chapter 2.2.46 was published, which corresponds within the **Pharmacopoeial** harmonization process (Ph. Eur., JP, USP). Major changes for the allowed adjustments for liquid chromatography have since been proposed. Similarly, to the last revision of the USP 621 chapter, the L/dp ratio was introduced for maintaining nearly constant efficiency and therefore resolution. But this change is not only valid for isocratic elution (like in the USP), it's also customized to gradient methods.

The tables below are showing the differences of the allowed adjustments for isocratic and gradient liquid chromatography methods for the new Ph. Eur. Draft, the current Ph. Eur. Supplement 9 and the current USP 40-NF35.

Allowed Adjustments: **Liquid Chromatography** – Isocratic Elution

Method Parameters	Pharmeuropa 29.3 draft	Ph.Eur. 9.0 - current	USP 40–NF 35) (1) - current
Composition of the Mobile Phase	The amount of the minor solvent component may be adjusted by ± 30 % relative or ± 2 % absolute, whichever is the larger. A minor component comprises less than (100/n) %, n being the total number of components of the mobile phase.	The amount of the minor solvent component may be adjusted by ± 30 % relative or ± 2 % absolute, whichever is the larger. However a change in any component cannot exceed ± 10 % absolute	The amount(s) of the minor component(s) (e.g. ≤ 50 %) can be modified by ± 30 % (relative). However a change in any component cannot exceed ± 10 % absolute. In ternary mixtures only one minor component can be adjusted
Mobile Phase pH	± 0.2 pH units	± 0.2 units (or ± 1.0 units for non-ionisable substances)	± 0.2 units
Concentration of salts in Buffer	± 10 %	± 10 %	± 10 %
Stationary Phase	No change of the physico-chemical characteristics of the stationary phase is permitted, i.e. chromatographic support, surface modification and extent of chemical modification must be the same; a change from Totally Porous Particle (TPP) columns to Superficially Porous Particle (SPP) columns is allowed provided these requirements are met.	No change of the identity of the substituent permitted (e.g. no replacement of C8 by C18)	No change of the identity of the substituent permitted (e.g. no replacement of C8 by C18)
Column Temperature	± 10 °C	± 10 °C	± 10 °C
Wavelength of Detector	No adjustment permitted	No adjustment permitted	no deviations permitted. The maximum error in the detector wavelength is ± 3 nm.
Column Length	see particle size	± 70 %	see particle size
Column Internal Diameter	can be adjusted if the linear velocity is kept constant	± 25 %	can be adjusted if the linear velocity is kept constant
Particle Size	be modified provided that length (L) to the particle size (dp) remains constant or in the range – 25 % to + 50 % of the prescribed L/dp ratio. For the application of particle-size adjustment to superficially porous particles, other combinations of L and dp can be used provided that the number of theoretical plates (N) is within – 25 % to + 50 % relative to the prescribed column. These changes are acceptable provided system suitability requirements are fulfilled, andselectivity and elution order of the specified impurities to be controlled are demonstrated to be equivalent. Caution is necessary when the adjustment results in a higher number of theoretical platesgenerating smaller peak volumes, a situation which may require adjustments to minimise extra-column band broadening by factors such as instrument connections, detector cell volume and sampling rate, and injection volume.	-50 %; no increase permitted	the particle size and/or length of the column may be modified provided that the ratio of the column length (L) to the particle size (dp) remains constant or in the range – 25 % to + 50 % of the prescribed L/dp ratio. For the application of particle-size adjustment to superficially porous particles, other combinations of L and dp can be used provided that the number of theoretical plates (N) is within – 25 % to + 50 % relative to the prescribed column. Caution should be taken when the adjustments lead to higher of theoretical plates which generates smaller peak volumes, which may require adjustments to minimize extra-column band broadening by factors as instrument plumbing, detector cell volume and sampling rate, and injection volume. When the monograph does not specify a particle size, the largest particle size mentioned in the L classification must be used for calculations
Flow Rate $F_2 = F_1 \frac{dc_2^2 dp_1}{dc_1^2 dp_2}$ 1: initial column 2: new column F: flow dc: inner diameter dp: particle size	After an adjustment, due to a change in column dimensions, an additional change in flow rate of ± 50 % is permitted. When the particle size is changed, the flow rate may require adjustment, because smaller-particle columns will require higher linear velocities for the same performance (as measured by reduced plate height In isocratic separations, when a change is made in particle size from ≥ 3 µm to < 3 µm, an additional increase in linear velocity (by adjusting the flow rate) may be justified, provided that the column efficiency does not drop by more than 20 %. Similarly, a change in particle size from < 3 µm to ≥ 3 µm may require additional reduction of linear velocity (by adjusting the flow rate) to avoid reduction in column efficiency by more than 20 %.	± 50 %; a larger adjustment is acceptable when changing column dimensions $F_2 = F_1 \frac{L_2 d_2^2}{L_1 d_1^2}$ 1: initial column 2: new column F: flow L: length d: inner diameter	± 50 % A lager adjustment is acceptable when column inner diameter or particle size are changed. $F_2 = F_1 \frac{dc_2^2 dp_1}{dc_1^2 dp_2}$ 1: initial column 2: new column F: flow dc: inner diameter dp: particle size
Injection Volume $Vinj_2 = Vinj_1 \frac{L_2 dc_2^2}{L_1 dc_1^2}$ 1: initial column 2: new column Vinj: Injection Volume dc: inner diameter L: length	Except for changes from TPP columns to SPP columns when the column dimensions are changed, injection volume adjustment may be guided by the equation to the left. Even in the absence of any column dimension change, the injection volume may be varied provided system suitability criteria remain within their established acceptability limits. When the injection volume is decreased, special attention is given to (limit of) detection and repeatability of the peak response(s) to be determined. An increase is permitted provided that, in particular, linearity and resolution of the peak(s) to be determined remain satisfactory.	May be decreased, provided detection and repeatability of the peak(s) are satisfactory; no increase permitted	Can be adjusted, if precision, linearity and detection limits are achieved. A too big volume can lead to band broadening and loss in resolution

Allowed Adjustments: **Liquid Chromatography** – Gradient Elution

Method Parameters	Pharmeuropa 29.3 draft	Ph.Eur. 9.0 - current	USP 40–NF 35) (1) - current
Composition of the Mobile Phase + gradient	N/A	Minor adjustments of the composition of the mobile phase and the gradient are acceptable, if the system suitability requirements are met, the principle peak(s) elute(s) within ± 15 % of the indicated retention time(s) and the final elution power of the mobile phase is not weaker	Adjustments to the composition of the mobile phase in gradient elution may cause changes in selectivity and are not recommended. If adjustments are necessary: Change in column packing (maintaining the same chemistry), The duration of an initial isocratic hold (when prescribed), and/or dwell volume adjustments are allowed.
Mobile Phase pH	± 0.2 pH units, unless otherwise prescribed	no adjustment permitted	± 0.2 units
Concentration of salts in Buffer	± 10 %	No adjustment permitted	± 10 %
Stationary Phase	No change of the physico-chemical characteristics of the stationary phase is permitted, i.e. chromatographic support, surface modification and extent of chemical modification must be the same; a change from Totally Porous Particle (TPP) columns to Superficially Porous Particle (SPP) columns is allowed provided these requirements are met.	No change of the identity of the substituent permitted (e.g. no replacement of C8 by C18)	No change of the identity of the substituent permitted (e.g. no replacement of C8 by C18)
Column Temperature	± 5 °C	± 5 °C	± 10 °C
Wavelength of Detector	No adjustment permitted	No adjustment permitted	no deviations permitted. The maximum error in the detector wavelength is ± 3 nm.
Column Length	see particle size	± 70 %	no adjustment permitted
Column Internal Diameter	See particle size and Flow Rate	± 25 %	no adjustment permitted
Particle Size	the particle size and/or length of the column may be modified provided that the ratio of the column length (L) to the particle size (dp) remains constant or in the range − 25 % to + 50 % of the prescribed L/dp ratio. For the application of particle-size adjustment to superficially porous particles, other combinations of L and dp can be used provided that the number of theoretical plates (N) is within − 25 % to + 50 % relative to the prescribed column. These changes are acceptable provided system suitability requirements are fulfilled, and selectivity and elution order of the specified impurities to be controlled are demonstrated to be equivalent. Caution is necessary when the adjustment results in a higher number of theoretical plates generating smaller peak volumes, a situation which may require adjustments to minimise extra-column band broadening by factors such as instrument connections, detector cell volume and sampling rate, and injection volume.	no adjustment permitted no adjustment permitted	no adjustment permitted no adjustment permitted
Flow Rate	After an adjustment, due to a change in column dimensions, an additional change in flow rate of ± 50 % is permitted. When the particle size is changed, the flow rate may require adjustment, because smaller-particle columns will require higher linear velocities for the same performance (as measured by reduced plate height) $F_2 = F_1 \frac{dc_2^2 dp_1}{dc_1^2 dp_2}$ 1: initial column 2: new column F: flow dc: inner diameter dp: particle size In gradient separations, when a change is made in particle size from ≥ 3 µm to < 3 µm, an additional increase in linear velocity (by adjusting the flow rate) may be justified, provided that the column efficiency does not drop by more than 20 %. Similarly, a change in particle size from < 3 µm to ≥ 3 µm may require additional reduction of linear velocity (by adjusting the flow rate) to avoid reduction in column efficiency by more than 20%. *	N/A	no adjustment permitted
Injection Volume	$Vinj_2 = Vinj_1 \frac{L_2 dc_2^2}{L_1 dc_1^2}$ 1: initial column 2: new column Vinj: Injection Volume dc: inner diameter L: length Except for changes from TPP columns to SPP columns when the column dimensions are changed, injection volume adjustment may be guided by the equation to the left. Even in the absence of any column dimension change, the injection volume may be varied provided system suitability criteria remain within their established acceptability limits. When the injection volume is decreased, special attention is given to (limit of) detection and repeatability of the peak response(s) to be determined. An increase is permitted provided that, in particular, linearity and resolution of the peak(s) to be determined remain satisfactory.	May be decreased, provided detection and repeatability of the peak(s) are satisfactory; no increase permitted	Can be adjusted, if precision, linearity and detection limits are achieved. A too big volume can lead to band broadening and loss in resolution

*A change in column dimensions, and thus in column volume, impacts the gradient volume which controls selectivity. Gradients are adjusted to the column volume by changing the gradient volume in proportion to the column volume. This applies to every gradient segment volume. Since the gradient volume is the gradient time (tG), multiplied by the flow rate (F), the gradient time for each gradient segment must be adjusted to maintain a constant ratio of the gradient volume to the column volume (expressed as L × dc2). Thus, the new gradient time (tG2) can be calculated from the original gradient time (tG1), the flow rate(s), and the column dimensions as follows:

$$tG_2 = tG_1 \frac{F_1}{F_2} x \frac{L_2 dc_2^2}{L_1 dc_1^2}$$

Thus, the change in conditions for gradient elution requires 3 steps:

(1) adjust the column length and particle size according to L/dp,

(2) adjust the flow rate for changes in particle size and column diameter, and

(3) adjust the gradient time of each segment for changes in column length, diameter and flow rate.

The example below illustrates this process.

Variable	Original conditions	Adjusted conditions	Comment
Column length (L) in mm	150	100	User's choice
Column diameter (dc) in mm	4.6	2.1	User's choice
Particle size (dp) in µm	5	3	User's choice
L/dp	30	33.3	-1
Flow rate (F) in mL/min	2	0.7	-2
Gradient adjustment factor		0.4	-3
Gradient conditions			
B (per cent)	Time (min)	Time (min)	
30	0	0	
30	3	(3 × 0.4) = 1.2	
70	13	[1.2 + (10 × 0.4)] = 5.2	
30	16	[5.2 + (3 × 0.4)] = 6.4	
	1) 11 % increase within allowed L/dp change of − 25 per cent to + 50 per cent;	**2)** calculated using F2 = F1 [(dc22 × dp1) / (dc12 × dp2)];	**3)** calculated using tG2 = tG1 × (F1 / F2) [(L2 × dc22) / (L1 × dc12)]

References

- European Pharmacopoeia 9.0, Volume 1, Strasbourg Cedex, France, 2016; General chapter <2.2.46>
- Pharmeuropa 29.3, European Directorate for the Quality of Medicines & HealthCare, Strasbourg Cedex, France, 2016; Reference: PA/PH/Exp. CST/T (17) 3 ANP

- United States Pharmacopeia 40 National Formulary 35 (USP 40-NF 35, United States Pharmacopeial Convention, Rockville, Maryland, 2017); General Chapter <621>

- W. Dolan, LCGC North Am. 35(6), 368-373 (2017); “Method Adjustment the USP Way”

Summary

Article Name

Revision of European Pharmacopeia (EP) Chapter 2.2.46

Description

The Ph. Eur. Chapter 2.2.46 is similar to the USP Chapter 621. Separations techniques, system suitability requirements, and allowed adjustments.

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