Technical Note

Allowable Adjustments to HPLC Methods in the European Pharmacopoeia (Ph.Eur.)

11. Edition

A variety of factors can lead to differences in results produced by different laboratories using the same HPLC methods. These factors include for example the differences in dwell volumes of HPLC systems of different brands or manufacturers, which can lead to changes in separation efficiency and thus resolution. It is a widely accepted fact that different stationary phases can show vastly different chromatographic properties, due to the quality of packing, surface coverage and effective area, pore size, as well as particle shape and size uniformity, even if they are formally listed as the same class of material (e.g. C18).

Because of this, laboratories are allowed to change parameters in their isocratic and gradient separations to optimise their analyses to their specific conditions, enhance reproducibility and productivity or even make the separation possible in the first place. It's also possible to replace your column with an alternative of the same classification, for example a conventional C18 column with a more modern C18 phase from a different manufacturer.

Here we provide a helpful overview of adjustments allowed by the Ph.Eur. (European Pharmacopoeia, Chapter 2.2.46) in your HPLC methods:

Isocratic Elution

Column length (L)/particle size (d_p)	**-25%** *to* **+50%** *from original L/d_p ratio*
Column inner diameter	*May be adjusted*
Flow rate	**± 50%** *(from constant linear velocity at given ID and d_p of column)*
Injection volume	*Can be reduced as long as system suitability criteria remain in acceptable limits*
Composition of mobile phase	*Minor component* **± 30%** *relative / no more than* **10%** *absolute*
Mobile phase pH	**± 0.2 pH** *units*
Buffer concentration	**± 10%**
Column temperature	**± 10 °C**
Detector wavelength	*No adjustments permitted*

Gradient Elution

Column length (L)/particle size (d_p)	**-25%** *to* **+50%** *from original L/d_p ratio*
Column inner diameter	*May be adjusted*
Flow rate	*Only adjustment to maintain linear velocity at given column ID and particle d_p*
Gradient volume	*Adjustment of gradient time for each gradient segment volume to maintain constant ratio of gradient volume to column volume*
Injection volume	*Can be reduced as long as system suitability criteria remain in acceptable limits*
Composition of mobile phase	*Minor adjustments allowed, if system suitability parameters are met, retention times of principal peaks are in a range of* **± 15%** *compared to original conditions (requirement does not apply when column dimensions are changed), first peaks are sufficiently retained and all peaks elute.*
Mobile phase pH	**± 0.2 pH** *units*
Buffer concentration	**± 10%**
Column temperature	**± 5 °C**
Detector wavelength	*No adjustments permitted*
Dwell volume	*Gradient time points may be adjusted to compensate differences between two systems*

YMC Europe GmbH · Schöttmannshof 19 · 46539 Dinslaken · Phone +49 2064 427-0 · Fax +49 2064 427-222 · Email: info@ymc.eu · **www.ymc.eu**