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

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

Isocratic Elution

Column length	**± 70 %**
Column inner diameter	**± 25 %**
Particle size	**- 50 %**
Flow rate	**± 50 %** *(at given ID of column)*
Injection volume	*Can be reduced as long as precision and detection limits are achieved*
Composition of mobile phase	**± 30 %** *(relative for excess component)* **or ± 2% absolute** (*whichever is larger)*
Mobile phase pH	**± 0.2 pH units** *(± 1.0 pH units for non-ionizable analytes)*
Buffer concentration	**± 10 %**
Column temperature	**± 10 °C**
Detector wavelength	**± 3 nm**

Gradient Elution

Column length	**± 70 %**
Column inner diameter	**± 25 %**
Particle size	*no adjustment*
Flow rate	*only adjustment to maintain linear velocity at new column ID*
Injection volume	*Can be reduced as long as precision and detection limits are achieved*
Composition of mobile phase	*Minor adjustments allowed, if system suitability parameters are met, retention times of compounds are in a range of* **± 15 %** *indicated in the method and the final elution strenghts of mobile phase is not weaker.*
Mobile phase pH	*no adjustment*
Buffer concentration	*no adjustment*
Column temperature	**± 5 °C**
Detector wavelength	*no adjustment*
Dwell volume	*Gradient time points may be adjusted to compensate differences between two systems*

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