Allowable Adjustments to European Pharmacopeia (EP) Methods

ISOCRATIC ELUTION

1. Ratio of components in mobile phase: ± *30 % (relative)*

The amount(s) of the minor component(s) can be adjusted by ± 30 % relative or ± 2 % absolute, whichever is the larger. However a change in any component cannot exceed ± 10 % absolute.

- 60:40 Acetonitrile/Water could be adjusted to ± 12 % water (= 30 % of 40), but this exceeds the ± 10 % maximum absolute change. Therefore the amount of water can range from 30 % to 50 % in this case.

2. Mobile phase pH: ± 0.2 units *(or ± 1.0 units for non-ionizable substances)*

- pH of 7.6 can be adjusted from 7.4 – 7.8

3. Concentration of salts in buffer: ± *10 %*

- 20 mM Potassium phosphate can be 18 – 22 mM, as long as proper pH is maintained as above

4. Stationary phase: *No change of the identity of the substituent permitted*

- No replacement of C18 by C8

5. Particle size: *Can be reduced as much as 50 %*

- 10 µm particles can be switched with 5 µm particles

6. Column length: ± *70 %*

- A 150 x 4.6 mm column can be varied from 45 – 255 mm in length

7. Column inner diameter: ± *25 %*

- A 150 x 4.6 mm column can be varied from 3.45 – 5.75 mm in diameter

8. Flow rate: ± *50 % (larger adjustment is ok when changing column dimensions)*

When column dimensions are changed (e.g. 125 x 4.0 mm at 0.8 mL/min to 100 x 4.6 mm), the flow rate may be adjusted using the following equation:

$$F_2 = F_1 \frac{l_2 d_2^2}{l_1 d_1^2} = 0.8x \frac{100 \times 4.6^2}{125 \times 4.0^2} = 0.85 \text{ mL/min}$$

- 1 mL/min can be varied from 0.5 to 1.5 mL/min

9. Column temperature: ± *10° C*

10. Wavelength of detector: *No deviations permitted*

11. Injection volume: *Can be reduced as long as precision and detection limits are achieved*

Source: European Pharmacopeia 7.0, Chapter 2.2.46. Chromatographic separation techniques, p. 70-77.

Allowable Adjustments to European Pharmacopeia (EP) Methods

GRADIENT ELUTION

1. Ratio of components in mobile phase and gradient

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.

2. Dwell volume

Gradient time points (t in min) can be adapted to compensate differences in dwell volume between the system used for method development (D_0 in mL) and that actually used (D in mL). The adapted time points (t_c) at the current flow rate (F in mL/min) can be calculated using he following equation:

$$t_c = t - \frac{(D - D_o)}{F}$$

3. Mobile phase pH: ***No adjustment permitted***

4. Concentration of salts in buffer: ***No adjustment permitted***

5. Stationary phase: ***No change of the identity of the substituent permitted***

- No replacement of C18 by C8

6. Particle size: ***No adjustment permitted***

7. Column length: ***± 70 %***

- A 150 x 4.6 mm column can be varied from 45 – 255 mm in length

8. Column inner diameter: ***± 25 %***

- A 150 x 4.6 mm column can be varied from 3.45 – 5.75 mm in diameter

9. Flow rate: ***Adjustment is acceptable when changing column dimensions***

When column dimensions are changed (e.g. 125 x 4.0 mm at 0.8 mL/min to 100 x 4.6 mm), the flow rate may be adjusted using the following equation:

$$F_2 = F_1 \frac{l_2 d_2^2}{l_1 d_1^2} = 0.8x \frac{100 \times 4.6^2}{125 \times 4.0^2} = 0.85 \text{ mL/min}$$

10. Column temperature: ***± 5° C***

11. Wavelength of detector: ***No deviations permitted***

12. Injection volume: ***Can be reduced as long as precision and detection limits are achieved***

Source: European Pharmacopeia 7.0, Chapter 2.2.46. Chromatographic separation techniques, p. 70-77.