Acceptable Modifications to an HPLC Validated Method

Even if you are using an FDA validated or a USP recommanded method, some operating conditions can be adjusted if the modifications respect the acceptable specifications proposed by Pharmacopeias[1-3] and the FDA[4]. A side-by-side comparison of both the original and the adjusted method needs to be performed to demonstrate that the method's accuracy and precision is not affected by these modifications.

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Parameters	Allowable modification	Examples of possible modifications
Mobile phase pH	± 0.2 units	Validated pH: 7.0 Allowed pH range: 6.8 – 7.2
Concentration of salts in buffer	± 10%	Validated concentration: 20 mM Allowed concentration range: 18 - 22 mM
Ratio of components in mobile phase	Only the minor components can be adjusted by ± 30% or ± 2% absolute (*i.e.: in regards to the total mobile phase*), whichever is the larger but should never exceed ± 10% absolute or removed totally.	**Binary mixtures:** Validated ratio: 50/50 Allowed ratio: 40/60 to 60/40 Validated ratio: 95/5 Allowed ratio: 93.5/6.5 to 96.5/3.5 **Ternary mixtures:** Validated ratio: 60/35/5 Allowed % of the 1st component: 60% Allowed % of the 2nd component: 25 – 45% Allowed % of the 3rd component: 3.5 – 6.5% The total of the three components together need to be 100%.
Wavelength of UV detector	No modification allowed.	n/a
Column length	± 70%	Validated length: 150 mm Allowed length range: 45 - 255 mm
Column inner diameter	± 50%	Validated inner diameter: 4.6 mm Allowed inner diameter range: 2.3 – 10.6 mm
Flow rate	± 50%	Validated flow rate: 1.00 mL/min Allowed flow rate range: 0.5 – 1.5 mL/min
Injection volume	May be increased to as much as 2 times if no adverse effects on LOD and repeatability.	n/a
Particle size	No increase permitted. May be decreased by as much as 50%.	Validated particle size: 5 µm Allowed particle size range: 2.5 - 5 µm
Column temperature	± 20%	Validated temperature: 23°C Allowed length range: 18.4 – 27.6°C

1. USP. USP 32–NF 27, Chromatography <621>. Rockville, MD: USP; 2009:227.
2. USP. Second Supplement to USP 32–NF 27. Rockville, MD: USP; 2009:4147.
3. USP. USP 32–NF 27, Verification of Compendial Procedures <1226>. Rockville, MD: USP; 2009:736.
4. ORA Laboratory Procedure, Food and Drug Administration, modification criteria.