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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
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<table>
<thead>
<tr>
<th>Method Parameters</th>
<th>Phenomenex</th>
<th>PHS-100</th>
<th>Phenomenex NW 90 (2) sensor</th>
</tr>
</thead>
</table>

- **Composition of the Mobile Phase**
  - The amount of the minor solvent component may be adjusted by ±10% relative or ±1% absolute, whichever is the larger. A minor solvent component less than 10% of the total number of components of the mobile phase.
  - The amount of the minor solvent component may be adjusted by ±2% relative or ±1% absolute, whichever is the larger. However, a change in any component cannot exceed ±1% absolute.

- **Mobile Phase pH**
  - 5.0 ± 0.2 units (±0.1 units for non-ionizable substances)

- **Stationary Phase**
  - No change of the identity of the stationary phase (e.g., no replacement of C8 by C18)

- **Column Temperature**
  - 35°C

- **Wavelength of Detector**
  - No adjustment permitted

- **Coulomb Length**
  - 1.7 m

- **Particle Size**
  - 5-10 µm

- **Flow Rate**
  - After an adjustment, due to a change in column dimensions, an additional change in flow rate of ±10% may be required, because the particle size will require higher injection volumes for the same performance (as measured by reduced retention time). In separate separations, when a change is made in particle diameters (±0.5 µm to ±1 µm), an additional increase in injection volume (±10%) may be required. A change in flow rate of ±5% may be required.

- **Injection Volume**
  - 1: initial column 2: minor column 3: flow 4: d: inner diameter dp: particle size

- **Peak Response**
  - May be decreased, provided detection and repeatability of the peaks are satisfactory, no increase permitted.
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Allowed Adjustments: **Liquid Chromatography** - Gradient Elution
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<table>
<thead>
<tr>
<th>Method Description</th>
<th>Pharamacoepia 2.0.1 draft</th>
<th>Pharamacoepia 2.0.1 current</th>
<th>USP 49-NF 12.0.1 current</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composition of the Mobile Phase + Gradient</strong></td>
<td>N/A</td>
<td>Minor adjustments to the composition of the mobile phase and the gradient are acceptable, if the system suitability requirements are met, the principle peaks elute(s) within ±15% of the indicated retention times, and the final elution power of the mobile phase is not swayed.</td>
<td>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.</td>
</tr>
<tr>
<td><strong>Mobile Phase Gradient</strong></td>
<td>±0.2 g units, unless otherwise prescribed</td>
<td>±10%</td>
<td>±0.2 units</td>
</tr>
<tr>
<td><strong>Concentration of salts in buffer</strong></td>
<td>No adjustment permitted</td>
<td>No adjustment permitted</td>
<td>No adjustment permitted</td>
</tr>
</tbody>
</table>

### 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 not be the same. A change from totally Porous Particle (TPP) (silica) to Superficially Porous Particle (SPP) volume is allowed provided these requirements are met.

| **Column Temperature** | ±10 °C | ±10 °C |
| **Wavelength of Detector** | No adjustment permitted | No adjustment permitted | No adjustment permitted |

### Column Length

- No change of particle size
- ±10% | no adjustment permitted |

### Column Internal Diameter

- No change of particle size and flow rate
- ±25% | no adjustment permitted |

### Particle Size

- The particle size and/ or length of the column may be modified, provided the ratio of the column length (l) to the particle size (d) remains constant in the range 25 to 64 if the prescribed LOD/LOQ for the application of particle size adjustment is to supercritical pure particles, when combinations of and d are used provided that the number of theoretical plates (N) is within 25% to 10% relative to the prescribed column. These changes are acceptable provided system suitability requirements are fulfilled, and sensitivity and selectivity of the specified impurities to be controlled is demonstrated to be acceptable.

### Flow Rate

- No change of flow rate
- N/A | no adjustment permitted |

### Injection Volume

- 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.

- Less peak broadening and loss in resolution due to the use of smaller volumes.
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:

\[ t_{G_2} = t_{G_1} \frac{F_1}{F_2} \times \frac{L_2}{L_1} \frac{dc_2^2}{dc_1^2} \]

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.
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<table>
<thead>
<tr>
<th>Variable</th>
<th>Original conditions</th>
<th>Adjusted conditions</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column length (L) in mm</td>
<td>150</td>
<td>100</td>
<td>User’s choice</td>
</tr>
<tr>
<td>Column diameter (dc) in mm</td>
<td>4.6</td>
<td>2.1</td>
<td>User’s choice</td>
</tr>
<tr>
<td>Particle size (dp) in μm</td>
<td>5</td>
<td>3</td>
<td>User’s choice</td>
</tr>
<tr>
<td>L/dp</td>
<td>30</td>
<td>33.3</td>
<td>-1</td>
</tr>
<tr>
<td>Flow rate (F) in mL/min</td>
<td>2</td>
<td>0.7</td>
<td>-2</td>
</tr>
<tr>
<td>Gradient adjustment factor</td>
<td></td>
<td></td>
<td>-3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gradient conditions</th>
<th>Time (min)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B (per cent)</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3 (3 × 0.4) = 1.2</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>13 [1.2 + (10 × 0.4)] = 5.2</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>16 [5.2 + (3 × 0.4)] = 6.4</td>
</tr>
</tbody>
</table>

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
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- 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”

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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.

Author
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