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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
Revision of European Pharmacopeia (EP) Chapter 2.2.46

Allowed Adjustments: **Liquid Chromatography** - Gradient Elution
<table>
<thead>
<tr>
<th>Method Transmission</th>
<th>EP Pharmacopeia 7.0.3 (draft)</th>
<th>N/A</th>
<th>OAP-48-MF-00 (D) - current</th>
</tr>
</thead>
</table>

**Revision of European Pharmacopeia (EP) Chapter 2.2.46**

### Composition of the Mobile Phase + Gradient
- N/A

**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 be the same; a change from totally Porous Particle (TPP) (silica) to Superficially Porous Particle (SPP) volume is allowed provided these requirements are met.
- No change of the identity of the substituent permitted (e.g., no replacement of CB by CM).

#### Column Temperature
- No change permitted.

### Wavelength of Detector
- No change permitted.
- No change of the maximum error in the detector wavelength to ± 3 nm.

### Column Length
- ± 10 % no adjustment permitted

### Column Internal Diameter
- ± 20 % 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 20 to 40 % of the prescribed value.
- No adjustment permitted no adjustment permitted

#### Flow Rate
- After an adjustment, due to a change in column dimensions, an additional change in flow rate of ± 10 % is permitted when the particle size is changed, the flow rate may be adjusted, because smaller particle columns will require higher linear velocities for the same performance (as measured by reduced plate height).

### Injection Volume
- Except for changes from TPP columns to SP columns when the column dimensions are changed, retention volume adjustment must be made by the resolution factor.
- May be decreased, provided detection and repeatability of the peak(s) are satisfactory; no increase permitted

Can be adjusted, in precision, linearity and detection limits are achieved. A low log volume can lead to broadening and loss in resolution.
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:

\[ tG₂ = tG₁ \left( \frac{F₁}{F₂} \right) \left( \frac{L × dc₂}{L₁ × dc₁} \right) \]

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.
<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>0.4</td>
<td>-3</td>
</tr>
</tbody>
</table>

**Gradient conditions**

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

1) 11% increase within allowed L/dp change of −25 per cent to +50 per cent; 2) calculated using \( F_2 = \frac{L_{12} \times (dc_{22} \times dp_1)}{(dc_{12} \times (F_1 \times F_2) \times dp_2)} \); 3) calculated using \( t_{G2} = \frac{L_{1} \times (dc_{22})}{t_{G1} \times (F_1 / F_2 \times (L_{2} \times dc_{22}) / (L_{1} \times dc_{12}))} \)

References

- European Pharmacopoeia 9.0, Volume 1, Strasbourg Cedex, France, 2016; General chapter <2.2.46>

- Pharmedica 29.3, European Directorate for the Quality of Medicines & Healthcare, Strasbourg Cedex, France, 2016; Reference: PA/PH/Exp. CST/T (17) 3 ANP
Revision of European Pharmacopeia (EP) Chapter 2.2.46

- 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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