Navigating US Pharmacopoeia changes in Empower

The US Pharmacopoeia, USP–NF 2022 Issue 3, Chapter 621 Chromatography, has been updated and harmonized with the 11th Edition of the European Pharmacopoeia (Ph. Eur.). As of 1 December 2022, the USP has changed the formulas for resolution and plate count and has updated the terminology for tailing; now called symmetry factor. Other changes such as allowable changes to the chromatographic method (and more) are defined; these do not directly impact the calculations within Empower.

The calculation changes call for using the width at 50% height approach (rather than the width at tangent approach) for the determination of resolution (R) and plate count (N or Plate Number).

The 1 December 2022 definition of signal-to-noise ratio defines the range of the noise in this calculation to be observed over a distance equal to 20 times the width at 50% height of the peak in a blank injection. This represents a change from the previous guidance that did not require a blank injection and required a distance of greater than or equal to 5 times the width at 50% height of the peak. *However, on 6 January 2023, in response to the 14 December 2022 announcement that Ph. Eur will continue using the 5x peak width[1], the USP announced that it too, will revert to the 5x peak width guidance, effective 1 April 2023[2].* The 1 April 2023 guidance continues to require that the noise be determined within a blank injection as stated in the currently official, 1 December 2022 USP Chapter 621.

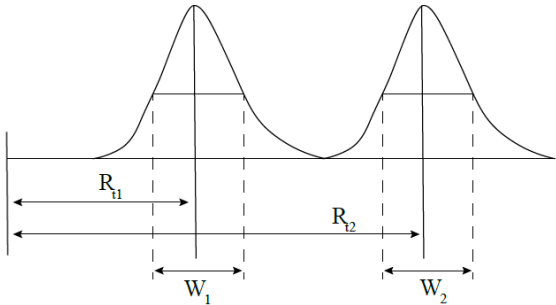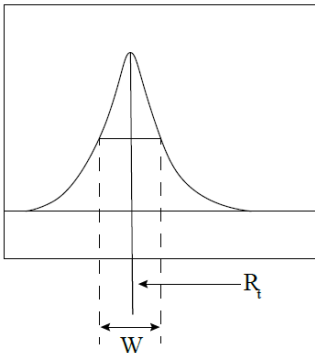This document contains information on the following:

Note: The formulas stated in this document pertain to Empower 3.7.0, 3.8.0 as well as other earlier versions. These calculations adhere to the Pharmacopoeias' definitions which have changed over the years. Therefore, calculations in earlier versions of Empower may differ from what is stated here. Reference Help to confirm the formulas for the specific version of Empower which you are using.

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[1] Ph. Eur. General chapter *2.2.46*, Supplement 11.3 which is to be published in July 2023 and implemented on 1 January 2024; see https://www.edqm.eu/en/-/signal-to-noise-ratio-revision-of-ph.-eur.-general-chapter-chromatographic-separation-techniques-2.2.46-
[2] USP Chapter <621> Chromatography, Intent to revise from 06 Jan 2023; see https://www.uspnf.com/notices/621-nitr-20230106

Empower fields that comply with the USP Chapter 621 Chromatography, effective December 2022

Resolution as defined by USP as of December 2022. Empower performs this calculation in the following fields: • **USP Resolution (HH)** • **Resolution**	Plate Number as defined by USP as of December 2022. Empower performs this calculation in the following fields: • **EP Plate Count** • **JP Plate Count** • **ChP Plate Count (HH)**
  $$R = \frac{1.18(R_{t2} - R_{t1})}{(W_2 + W_1)}$$ Where: R = Resolution (EP and JP) and USP Resolution (HH) R_{t1} = Retention time of the first peak R_{t2} = Retention time of the second peak W_1 = Width of the first peak at 50% peak height W_2 = Width of the second peak at 50% peak height	 $$N = 5.54\left(\frac{R_t}{W}\right)^2$$ Where: N = Plate count (the number of theoretical plates in a chromatographic column) R_t = Retention time W = Peak width at 50% of peak height

The terminology for Tailing is harmonized with that of the European and Japanese Pharmacopoeias and is called Symmetry Factor. The Symmetry Factor calculation has the same formula as in the previous USP version and also uses the width at 50% height approach.

Symmetry Factor as defined by USP as of December 2022. Empower performs this calculation in the following fields: • **USP Tailing** • **Symmetry Factor (for EP and JP)** • **ChP Tailing Factor**
Symmetry factor (A_s): The symmetry factor of a peak (also known as the asymmetry factor or tailing factor) $$A_s = \frac{W_{0.05}}{2d}$$ $W_{0.0}$ = width of the peak at one-twentieth of the peak height d = distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height

Based on feedback from USP, it is Waters' understanding that these changes apply to both existing and future methods, noting that USP Chapter 621 Chromatography applies specifically to compendial methods, however, may also be applied to other methods.

Solution within versions of Empower prior to 3.8.0

See also, System Suitability Quick Reference Guide, part number 715007659.

General information on Resolution, is provided in TECN134899112 - Resolution Values in Empower 3.

The calculations for resolution, relative resolution, plate count, and symmetry factor required by the USP are calculated and available in Empower.

When the Pharmacopoeia in the processing method is set to:					
	United States Pharmacopoeia (USP)	European Pharmacopoeia (EP)	Japanese Pharmacopoeia (JP)	Chinese Pharmacopoeia (ChP)[3]	All
These Empower fields use the desired width @50% peak height approach:					
Resolution	USP Resolution (HH)	Resolution	Resolution	ChP Resolution (HH)[4]	-USP Resolution (HH) -Resolution
Plate Number	While the USP Plate Count field is determined using the width @ tangent approach, the EP Plate Count field is determined when the Pharmacopoeia is set to 'USP' and the EP Plate Count uses the width @ 50% height approach.	EP Plate Count	JP Plate Count	ChP Plate Count (HH)	-EP Plate Count -JP Plate Count -ChP Plate Count (HH)
Symmetry Factor	USP Tailing	Symmetry Factor	Symmetry Factor	ChP Tailing Factor	-USP Tailing -Symmetry Factor -ChP Tailing Factor
Relative Resolution is determined using the desired width @ 50% peak height approach: [5]					
	Yes	Yes	Yes	No	No

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[3] ChP support, including ChP Resolution (HH) and ChP Plate Count (HH) is available in Empower 3.7.0 and above.

[4] ChP Resolution (HH) uses Width @ 50%, but it uses different constants in the formula (2.0/1.17 for ChP Resolution vs. 1.17 for USP Resolution (HH) and Resolution) so the calculated value will be different than the USP Resolution (HH) and Resolution values.
[5] EP and JP Relative Resolution are calculated using the formula for Resolution which uses the half height approach. USP Rel. Resol. Is calculated using the formula for USP Resolution (HH) which uses the half height approach. ChP Rel. Resol. is calculated using the formula for ChP Resolution which uses the width @ tangent approach. When All is selected, Rel. Resol. is calculated using the formula for USP Resolution which uses the width @ tangent approach.

Figure 1: Pharmacopoeia choice in the Empower processing method (Suitability tab).

Given the matrix above, it is possible to generate results for Resolution, Relative Resolution, and Plate Number using the width @ 50% peak height approach when USP, JP or EP is selected for the Pharmacopoeia parameter. When USP is chosen, use the USP Resolution (HH), Rel. Resol., and EP Plate Count fields.

However, it is not possible to generate results for Relative Resolution using the width @ 50% peak height approach while using Pharmacopoeia selections of 'ChP' or 'All'.

Custom fields can be created for Plate Count (Plate Number) and/or Relative Resolution. Refer to the sections for *Custom Fields* in this document for details.

Signal-to-noise

The Signal-to-noise ratio requires the noise value to be determined using a blank injection. This is accomplished by defining a blank injection in the sample set and specifying 'Use noise centered on peak region in blank injection in the processing method.

Figure 2: Sample table showing field in which to define a blank injection.

Figure 3: Processing method editor parameter - Use noise centered on peak region in blank injection parameter

Update Views and Reports

If you are transitioning to use the new USP guidance, existing processing methods, reports, views and view filters may need to be updated to include the appropriate fields/parameter settings. It may also be necessary to consider if any custom fields using the legacy fields for Resolution and/or Plate Count are present and need updating.

Custom Field for Plate Count/Plate Number

When the Pharmacopoeia setting is 'USP', the width at tangent approach is used by Empower to determine Plate Count in the USP Plate Count field[6]. If you are using 'USP' and have a desire to identify Plate Count using the width at 50% height approach, a custom field may be created. To calculate the updated determination of Plate Count, create a Peak Type custom field using the following formula:
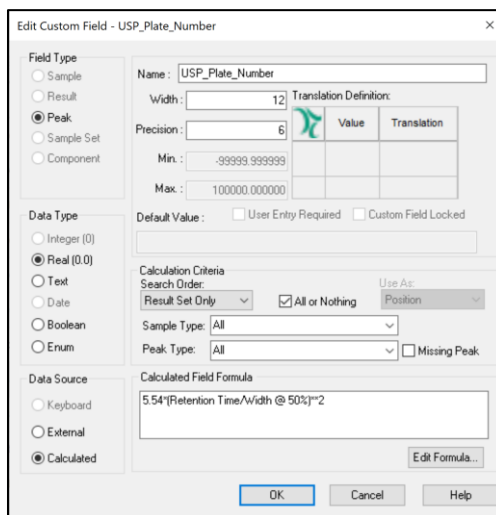
N = 5.54*(Retention Time/Width @50%)**2

Figure 4: Example of a custom field for Plate Number

Waters recommends that custom fields are appropriately validated as fit for purpose before putting into routine use.

Custom Field for Relative Resolution

When the Pharmacopoeia setting is 'All' or 'ChP', the width at tangent approach is used by Empower to determine Relative Resolution. If you are using 'All' or 'ChP' and have a desire to identify Relative Resolution using the width at 50% height approach, a custom field may be created. To calculate the updated determination of Relative Resolution, create a Peak Type custom field using the following formula:

R = 1.18*((Retention Time)-CCompRef1[Retention Time])/(Width @ 50%+CCompRef1[Width @ 50%])

In the processing method, define the CCompRef1 field as the component for your Relative Resolution Reference.

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[6] While the USP Plate Count field is determined using the width @ tangent approach, the EP Plate Count field is determined when the Pharmacopoeia is set to 'USP' and the EP Plate Count uses the width @ 50% height approach. Using EP Plate Count may be a viable workaround for you as it allows you to avoid creating, validating, and copying a custom field to all current and future projects.

Waters recommends that custom fields are appropriately validated as fit for purpose before putting into routine use.
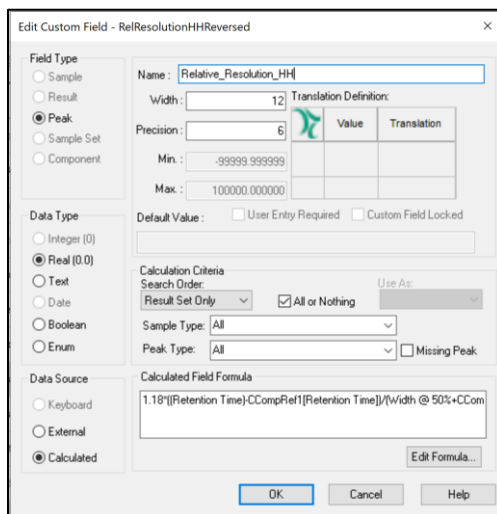

Figure 5: Example of a custom field for Relative Resolution

Solution within Empower 3.8.0

The request for support of these USP changes is documented as CRI-4304.

The following changes in Empower 3.8.0 have been made to address these USP changes.

1. The existing **Resolution** field is calculated for all the Pharmacopoeia choices.

2. A field called **Relative Resolution** is present and is calculated for all of the Pharmacopoeia choices. It uses the same formula as the **Resolution** field when a Relative Resolution Reference peak is defined in the processing method. Note: In Empower versions 3.7.0 and lower, the field for Relative Resolution in the English version of Empower is named Rel. Resol. In the Chinese, Korean, and Japanese languages, this field is called Relative Resolution. In Empower 3.8.0, the Relative Resolution field in the Asian languages has been renamed to Legacy Relative Resolution. With this approach, the field called Relative Resolution is available in all languages using the same name.

3. A field called **Plate Number** is present which is calculated for all the Pharmacopoeia choices. It is calculated using the same formula as the **JP Plate Count** and **EP Plate Count** fields.

4. The existing **Symmetry Factor** field is calculated for all the Pharmacopoeia choices.

5. For backwards compatibility with legacy data, all previously existing system suitability fields remain and are still calculated using the same formulas as in Empower 3.7.0.

This solution leverages the names harmonized amongst the various pharmacopoeias as shown in this table.

Empower 3.8.0 fields:				
	Resolution (existing field)	**Relative Resolution** (new in 3.8.0, English version)	**Plate Number** (new in 3.8.0)	**Symmetry Factor** (existing field)
Pharmacopoeia terminology:				
USP–NF 2022	Resolution (R_s)	Resolution (R_s)	Plate Number (N)	Symmetry Factor (A_s)
Ph Eur. 10	Resolution (R_s)	Resolution (R_s)	Plate Number (N)	Symmetry Factor (A_s)
JP 18	Resolution (R_s)	Resolution (R_s)	Number of Theoretical Plates	Symmetry Factor
ChP 2015 English Edition	Resolution (R_s)	Resolution (R_s)	Number of Theoretical Plates on the Column (n)	Tailing Factor (T)

## USP Chapter 621 Chromatography: previous calculations vs. current calculations

Resolution / Relative Resolution

Previous USP <621> USP41-NF36, 1 August 2017

- Use widths at the base of the peak obtained by extrapolating the relatively straight sides of the peaks to baseline (width @ tangent approach) [graphic on left]

- For electronic integrators, it may be convenient to user the widths at 50% height approach [graphic on right]
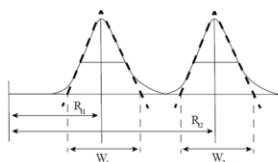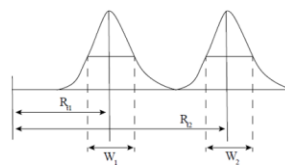
$$R = \frac{2.0(R_{t2} - R_{t1})}{(W_2 + W_1)}$$
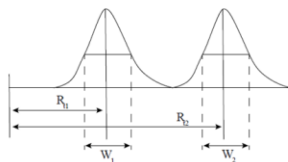
$$R = \frac{1.18(R_{t2} - R_{t1})}{(W_2 + W_1)}$$

Current USP <621> USP–NF 2022, Issue 3 Effective 1 December 2022

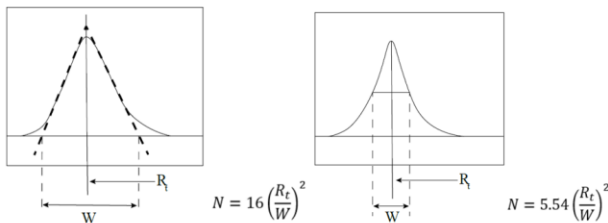- Peak width at 50% is the only approach mentioned

$$R = \frac{1.18(R_{t2} - R_{t1})}{(W_2 + W_1)}$$

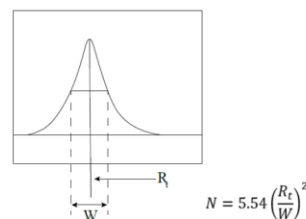### Number of theoretical plates (*N*) / Plate Count / Plate Number

Previous USP <621> USP41-NF36, 1 August 2017	Current USP <621> USP–NF 2022, Issue 3 Effective 1 December 2022
• For gaussian peaks, use width at the base of the peak obtained by extrapolating the relatively straight sides of the peaks to baseline (width @ tangent approach) [graphic on left] • For electronic integrators, it may be convenient to user the widths at 50% height approach [graphic on right]	• New terminology: Plate Number • Peak width at 50% is the only approach mentioned
$$N = 16\left(\frac{R_t}{W}\right)^2 \qquad N = 5.54\left(\frac{R_t}{W}\right)^2$$	$$N = 5.54\left(\frac{R_t}{W}\right)^2$$

Symmetry Factor (previously Tailing Factor)

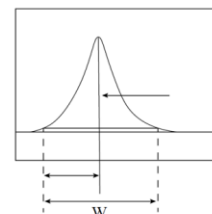| Previous USP <621> USP41-NF36, 1 August 2017	Current USP <621> USP–NF 2022, Issue 3 Effective 1 December 2022
• Width of the peak at 5% height • Height, f, is the distance from the peak max to the leading edge of the peak at 5% of the peak height	• No effectual change to formula • Terminology was harmonized previously • Width of the peak at one-twentieth of the peak height • Height, d, is the distance between the perpendicular from the peak max and the leading edge of the peak at one-twentieth of the peak height
$$A_S = W_{0.05}/2f$$	$$A_s = \frac{W_{0.05}}{2d}$$

Noise for Signal-to-noise

Previous USP <621> USP41-NF36, 1 Aug 2017	Compendial Notice: Intent to revise USP <621> Posting date: 06 January 2023, Effective Date: 1 April 2023
▪ Noise observed over a distance $\geq$ 5x the peak width at 50% height ▪ If possible, situated equally around the peak of interest $$s/n = \frac{2H}{h}$$	▪ Noise via injection of a blank observed over a distance $\geq$ 5x the peak width at 50% height ▪ If possible, situated equally around the place where this peak would be found $$s/n = \frac{2H}{h}$$

Future revision to USP Chapter 621 Chromatography

The following revision was posted on 28 Oct 2022 by the USP.
https://www.uspnf.com/sites/default/files/usp_pdf/EN/USPNF/revisions/gc-621-rb-notice-20221028.pdf

This revision delays the implementation of the subsections **System Sensitivity** and **Peak Symmetry** under System Suitability for one year. Please refer to the referenced section within the USP Chapter 621 Chromatography.

Given that this delay refers to the acceptable limit of quantitation and the acceptable symmetry factor and does not affect any calculations, Waters guidance and upcoming Empower changes are not affected by this revision.