

Resolution Values in Empower 3

This Technical Note describes how to calculate Resolution values in Empower 3.

Resolution is the measurement of separation quality based on a theory of Gaussian shaped peaks which are baseline resolved.

Resolution values, along with other System Suitability results, help to demonstrate the suitability of the chromatographic separation for its intended purpose. Failures to meet System Suitability requirements may be caused by instrumental or chemistry issues related to system preparation. When these requirements are not met, this may lead to a decision to reject results run on that system / in that analytical run.

Calculated Resolution and other System Suitability values are rarely used to create actual sample results.

As mentioned above, the system suitability calculations are mathematically based on the theory of perfect or almost perfect Gaussian peaks. Some values, such as Tailing or Asymmetry are a measure of how much a peak varies from the Gaussian ideal. Some values cannot be calculated reliably for non-Gaussian and specifically, unresolved peaks. As Resolution calculation relies on peak pairs, both peaks should be well resolved. See the troubleshooting section for specific examples.

Definition of terms

Retention time

Retention Time is the time that elapses between the injection of a sample and the appearance of the peak maximum (apex) of a component in the sample.

Width at Tangent (Width @ Tangent (USP Resolution))

Using ApexTrack integration

Width @ Tangent (USP Resolution) is the width of the peak at the detected peak's inflection points.

Using Traditional integration

Width @ Tangent (USP Resolution) is defined by the width of the peak at the height percent defined by the **Tangent Percent for USP Resolution** parameter in the processing method.

Width at 50% Height (Width @ 50%)

Width at 50% Height is the width of the peak drawn at 50% of the height of the peak.

Resolution definition based on USP <621> Chromatography Chapter

Resolution (R_s): The resolution is the separation of two components in a mixture, calculated by:

$$R_s = 2 \times (t_{R_2} - t_{R_1}) / (W_1 + W_2)$$

where t_{R_1} and t_{R_2} are the retention time of the two components; and W_1 , W_2 are the corresponding widths of the bases of the peaks obtained by extrapolating the relatively straight sides of the peaks to the baseline.

When electronic integrators are used, it may be convenient to determine the resolution, by the equation:

$$R_s = 1.18 \times (t_{R_2} - t_{R_1}) / (W_{1,h/2} + W_{2,h/2})$$

where $W_{1,h/2}$ and $W_{2,h/2}$ are the widths of peaks 1 and 2 at 50% height (see Figure 1).

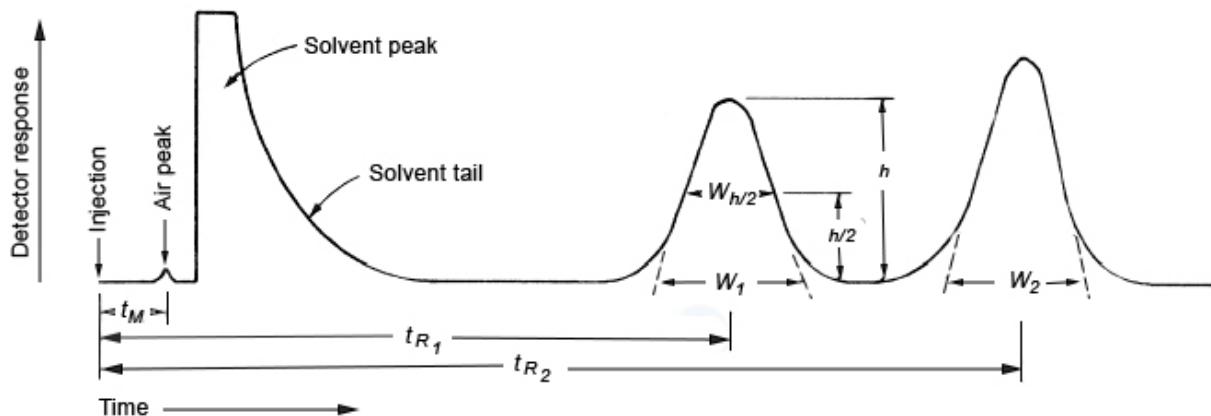


Figure 1 – Chromatographic separation of two substances

Resolution definition based on EP 9.0 and JP 17

The resolution (R_s) between peaks of two components (Figure 2) may be calculated using the following equation:

$$R_s = 1.18 \times (t_{R_2} - t_{R_1}) / (W_{h_1} + W_{h_2})$$

where:

$$t_{R_2} > t_{R_1}$$

t_{R_1}, t_{R_2} = Retention time of the peaks.

W_{h_1}, W_{h_2} = Peak widths at half-height.

In quantitative planar chromatography, using densitometry, the migration distances are used instead of retention times and the resolution between peaks of 2 components may be calculated using the following equation:

$$Rs = 1.18a \times (R_{f_2} - R_{f_1}) / (W_{h_1} + W_{h_2})$$

where:

R_{f_1}, R_{f_2} = Retardation factors of the peaks.

W_{h_1}, W_{h_2} = Peak widths at half-height.

a = Migration distance of the solvent front.

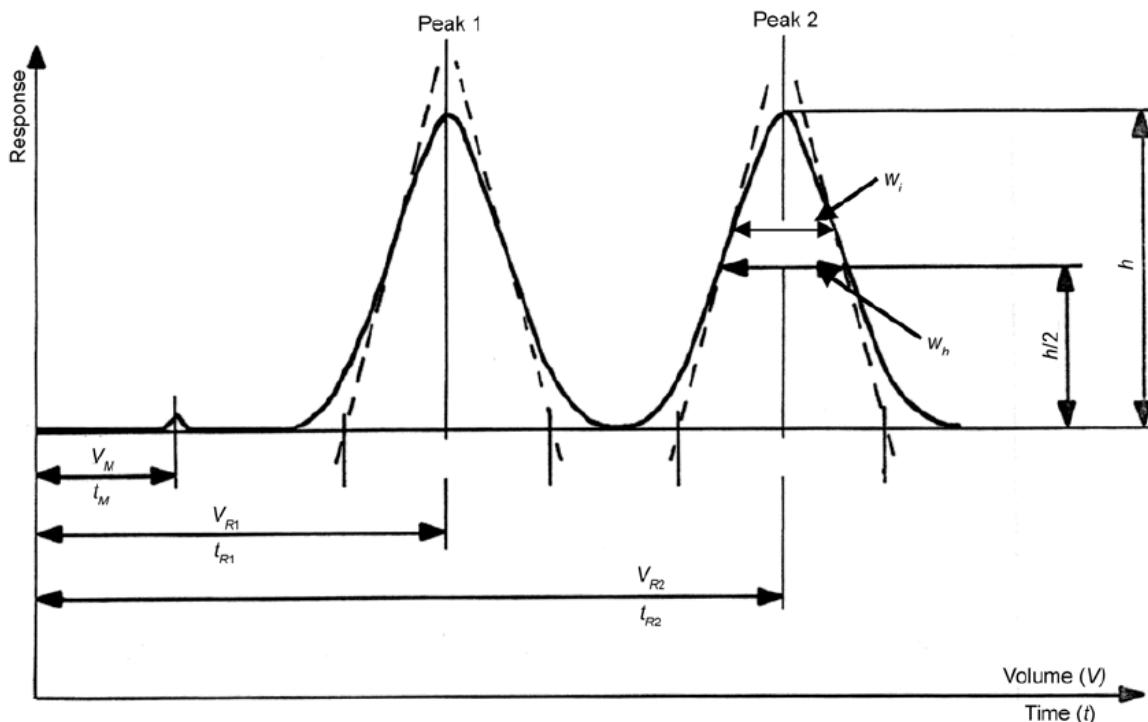


Figure 2 – Resolution determination

Resolution values in Empower 3

USP Resolution

In Empower 3, the USP Resolution field is reported based on the formula from the USP:

$$Rs = 2 \times (t_{R_2} - t_{R_1}) / (W_1 + W_2)$$

where:

t_{R_1}, t_{R_2} = Retention time of the two components.

W_1, W_2 = Corresponding widths at the bases of the peaks referred to as Width @ Tangent (USP Resolution).

USP Resolution (HH) and Resolution

In Empower 3 Feature Release 2 (FR2), the USP Resolution (HH) field is reported based on the alternative formula from the USP and the Resolution field is reported based on the formula from EP and JP. Both fields use the same formula:

$$Rs = 1.18 \times (t_{R_2} - t_{R_1}) / (W_{1,h/2} + W_{2,h/2})$$

where:

$W_{1,h/2}$, $W_{2,h/2}$ = Widths of peaks 1 and 2 at 50% height referred to as Width at 50%.

Starting in Empower 3 FR2, the numerical constant used to calculate USP Resolution (HH) is changed from (2.0/1.70) to 1.18, in accordance with the USP 35 NF 30 harmonized formula. (See web posting for [PCS #50238](#) – USP Resolution (HH) numerical constant).

System Suitability results based on Pharmacopoeia

Table 1 shows which formulas are calculated and available based on the selection of US Pharmacopoeia, Japanese Pharmacopoeia, European Pharmacopoeia or All in the processing method.

Table 1 - System Suitability results based on Pharmacopoeia

European Pharmacopoeia (EP)	Japanese Pharmacopoeia (JP)	United States Pharmacopoeia (USP)	All
---	---	USP Resolution	USP Resolution
---	---	USP Resolution (HH)	USP Resolution (HH)
Resolution	Resolution	---	Resolution
---	---	Width @ Tangent	Width @ Tangent
Width @ 50%	Width @ 50%	Width @ 50%	Width @ 50%

Relative Resolution values in Empower 3

Relative Resolution (Rel. Resol.) is measured between named peaks in the component table and their referenced peaks when you specify a relative resolution reference peak (Rel Resol Reference) in the processing method.

When calculating Relative Resolution for a peak, the RT and peak width values of the peak's Rel. Resol. peak are used in place of the previous peak's RT and peak width values. Different resolution formulas are used to calculate Relative Resolution, depending on the Pharmacopoeia setting in the processing method.

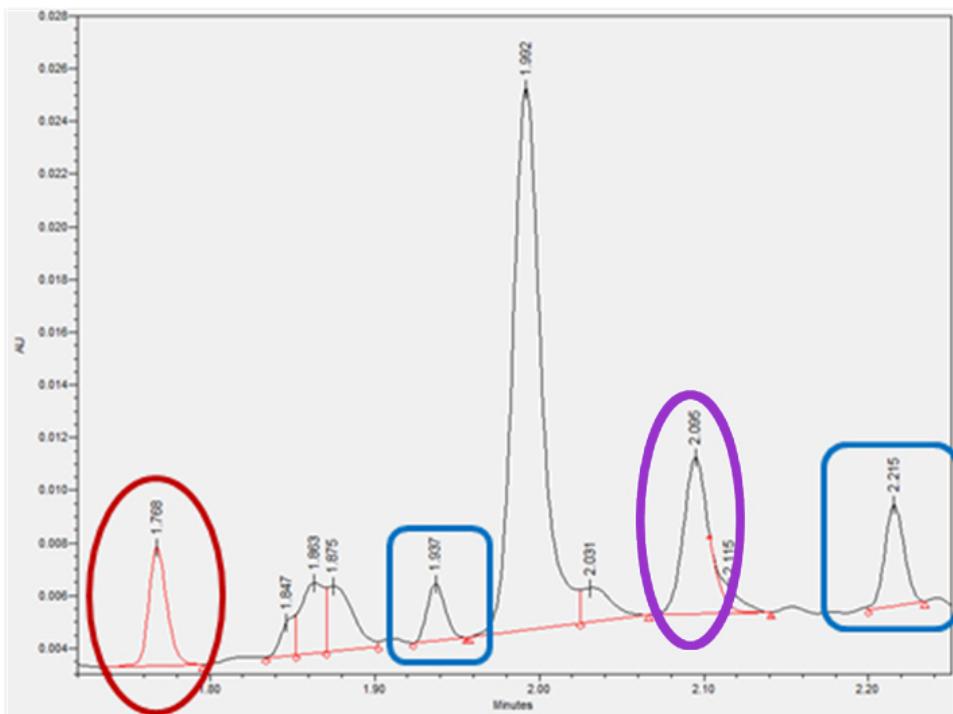
This method of resolution calculation is specifically useful to eliminate resolution calculation anomalies due to new or emerging peaks, which may appear between critical pairs. Other resolution calculations only consider adjacent peaks and so new unknown peaks will be included in these resolution calculations and may yield unexpected results. This may drive the suppression of unknown peaks and could lead to incorrect integration of the critical peaks.

Relative Resolution requires that the method clearly specifies the peak pairs using the relative resolution reference parameter (Rel Resol Reference) for each peak of interest.

The resolution equation used to calculate Relative Resolution (Rel. Resol.) depends on the pharmacopoeia you choose in the processing method. These are the four Pharmacopoeia options and their corresponding resolution formulas:

- US Pharmacopoeia (USP) – uses formula for Resolution, which is the same as USP Resolution (HH)
- European Pharmacopoeia (EP) – uses formula for Resolution, which is the same as USP Resolution (HH)
- Japanese Pharmacopoeia (JP) – uses formula for Resolution, which is the same as USP Resolution (HH)
- All – uses formula for USP Resolution

In the example shown in Figure 3, the peak highlighted in red and the peak highlighted in purple are reference peaks used to assess the resolution between themselves and the later peaks highlighted in blue. The use of this relative resolution criteria ensures that there is ample space for other peaks to elute between the two isolated peaks, without affecting this criteria.



	Retention Time (min)	Width @ 50%	USP Resolution (HH)	Width @ Tangent (USP Resolution)	USP Resolution	Rel. Resol.
14	1.768	0.012	2.719	0.020	2.789	
15	1.847					
16	1.863			0.081		
17	1.875			0.247	0.070	
18	1.937	0.012		0.021	0.466	8.332
19	1.992	0.017	2.203	0.030	2.175	
20	2.031			0.242	0.285	
21	2.095			0.028	0.474	
22	2.115					
23	2.215	0.013		0.022		4.819

Figure 3 – Calculating Relative Resolution (Rel. Resol.)

Calculating Width @ Tangent when manually integrating peaks

Using Traditional integration

Width @ Tangent is calculated the same way for both manual and automatic integration.

Using ApexTrack integration

Width @ Tangent can be calculated differently when using manual integration, as compared to automatic integration. This is because ApexTrack peak detection results in the determination of the peak's inflection points.

When adding a new manually integrated peak, Empower uses the Traditional integration method for measuring Width @ Tangent for this manually integrated peak. There will not be an S29 Processing Code associated with this peak. Instead, an I37 Processing Code will be present.

After a peak has been detected using ApexTrack integration, manually integrating that peak may result in the removal of the detected inflection points. In this case, Empower will revert to using the Traditional integration method for measuring Width @ Tangent for this manually integrated peak. There will not be an S29 Processing Code associated with this peak. Instead, an I37 Processing Code will be present.

After a peak has been detected using ApexTrack integration, if manually integrating that peak does not result in the removal of the detected inflection points, then Empower will use the ApexTrack integration method for measuring Width @ Tangent for this manually integrated peak. There will be an S29 Processing Code associated with this peak. There will not be an I37 Processing Code.

Troubleshooting Resolution calculations

Missing values

To ensure that resolution calculations are calculated:

- Ensure that **Calculate Suitability Results** is enabled.
- Check that the correct **Void Volume Time (min)** is entered.
- Select the required **Pharmacopoeia** selection. This will calculate system suitability values for all peaks.

If some peaks are not showing any system suitability results:

- For unknown peaks, ensure that **Calculate Suitability Results for Unknown Peaks** is selected.
- For named peaks, ensure that **Calculate Suit Results** is not disabled in the Limits tab.
- Note that re-integrating a chromatogram in Review will clear the peak identity so that all peaks become unknown. If **Calculate Suitability Results for Unknown Peaks** is NOT checked, then System Suitability fields will also be cleared. Calibrating or quantitating the chromatogram will then restore peak identification and System Suitability results.

Figure 4 shows the above selections in the Suitability tab of the processing method.

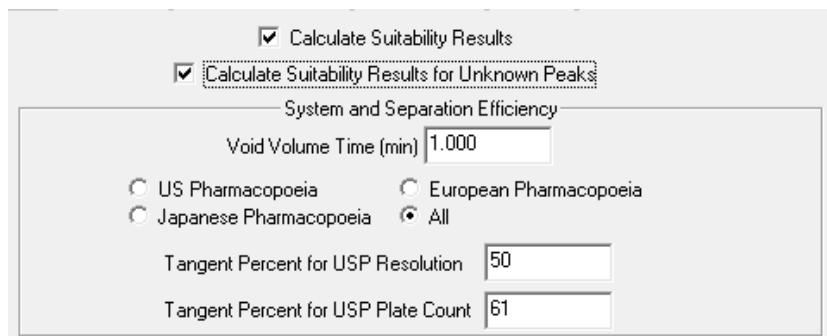


Figure 4 – Processing Method requirements for Resolution calculations

First peak

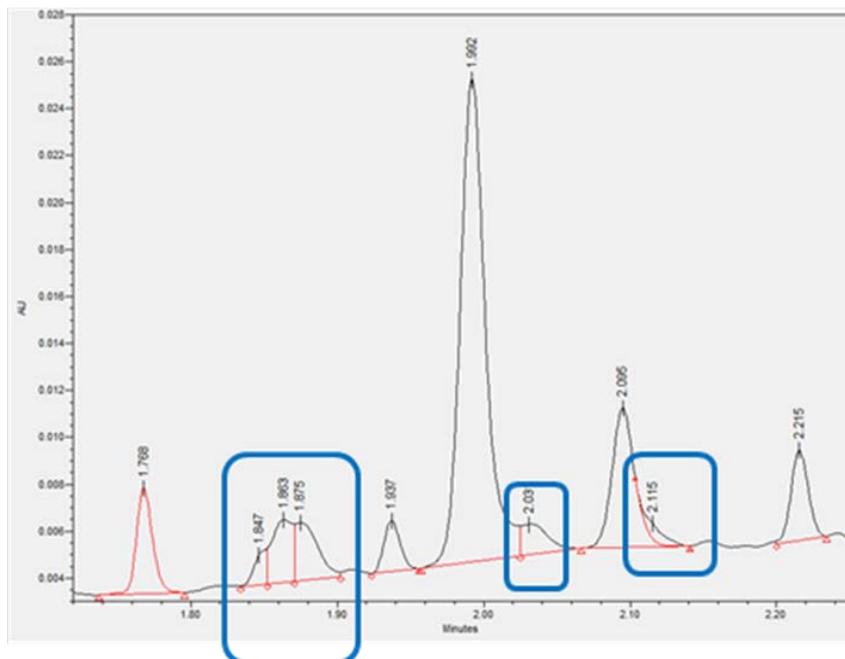
Since Resolution calculations compare fields from two peaks, if necessary information is missing, Empower leaves the affected calculated fields blank.

The first integrated peak on each chromatogram will never have Resolution, USP Resolution or USP Resolution (HH) values calculated, since it does not have a preceding peak.

Unresolved peaks

For any of the Resolution calculations, both the peak of interest and the preceding peak need to be present with enough resolution to be able to calculate the applicable peak widths (Width @ Tangent (USP Resolution) or Width @ 50%).

Figure 5 shows an example of ApexTrack integration with unresolved peaks (highlighted in blue) where it is not possible to calculate Width @ 50% for both peaks, and therefore, USP Resolution (HH) is also not calculated.

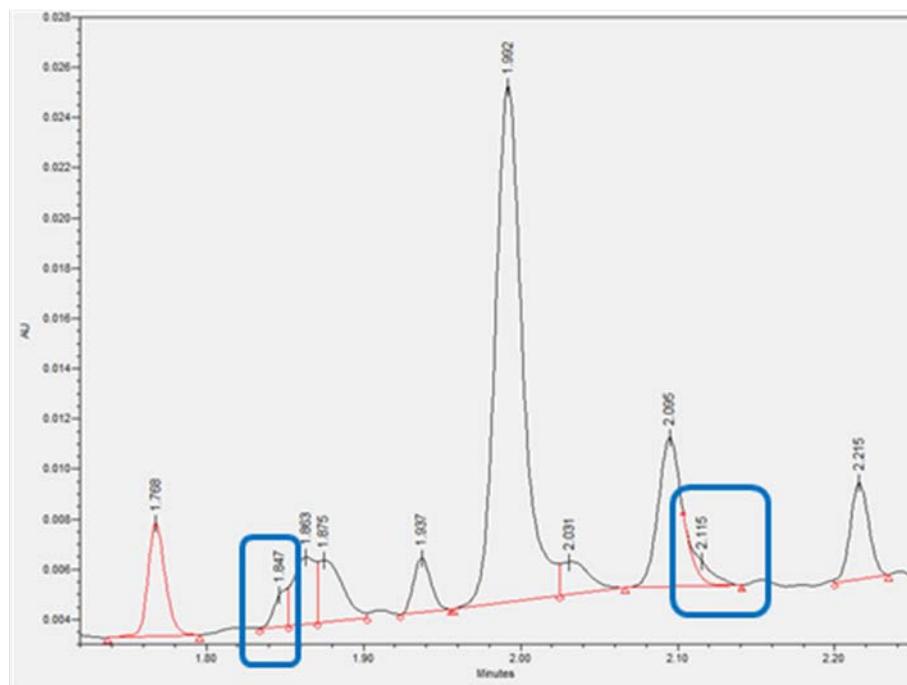


E	Retention Time (min)	Width @ 50%	USP Resolution (HH)	Width @ Tangent (USP Resolution)	USP Resolution
14	1.768	0.012	2.719	0.020	2.789
15	1.847				
16	1.863			0.081	
17	1.875			0.247	0.070
18	1.937	0.012		0.021	0.466
19	1.992	0.017	2.203	0.030	2.175
20	2.031			0.242	0.285
21	2.095			0.028	0.474
22	2.115				
23	2.215	0.013		0.022	

Figure 5 – Width @ 50% and USP Resolution (HH) calculations for unresolved peaks

Note in Figure 5 above that there are peaks where Width @ Tangent (USP Resolution) is calculated, even though Width @ 50% is not calculated. This is due to the fact that the inflection points were determined using ApexTrack.

Figure 6 highlights the peaks where it is not possible to calculate Width @ Tangent (USP Resolution), and therefore, USP Resolution is also not calculated.



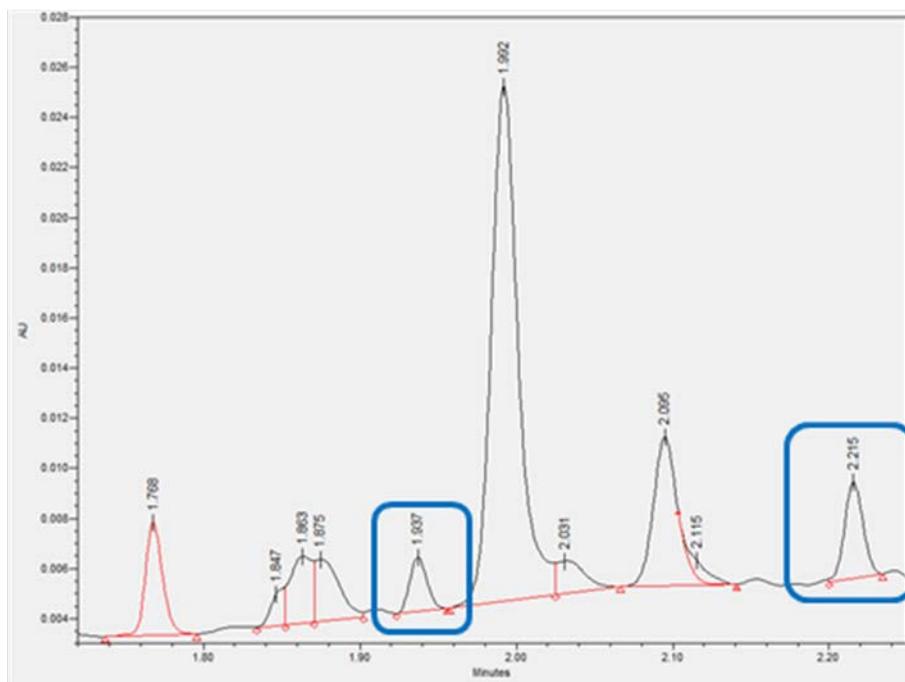
E	Retention Time (min)	Width @ 50%	USP Resolution (HH)	Width @ Tangent (USP Resolution)	USP Resolution
14	1.768	0.012	2.719	0.020	2.789
15	1.847				
16	1.863			0.081	
17	1.875			0.247	0.070
18	1.937	0.012		0.021	0.466
19	1.992	0.017	2.203	0.030	2.175
20	2.031			0.242	0.285
21	2.095			0.028	0.474
22	2.115				
23	2.215	0.013		0.022	

Figure 6 – Width @ Tangent (USP Resolution) and USP Resolution calculations are missing for unresolved peaks

Unresolved preceding peaks

Even if a peak is resolved and has width calculations itself, resolution calculations may not be completed or relied on because the preceding peak, being unresolved, may have issues calculating an accurate width.

Figure 7 shows an example where resolution calculations are missing or incorrect on peaks that follow unresolved peaks.



Y	Retention Time (min)	Width @ 50%	USP Resolution (HH)	Width @ Tangent (USP Resolution)	USP Resolution
14	1.768	0.012	2.719	0.020	2.789
15	1.847				
16	1.863			0.081	
17	1.875			0.247	0.070
18	1.937	0.012		0.021	0.466
19	1.992	0.017	2.203	0.030	2.175
20	2.031			0.242	0.285
21	2.095			0.028	0.474
22	2.115				
23	2.215	0.013		0.022	

Figure 7 – Resolution calculations missing or incorrect on peaks that follow unresolved peaks

PCS #54414 Issue on USP Resolution calculations

PCS #54414 applies to those using Empower 2 Feature Release 5 (FR5) up to and including Empower 3 Service Release 2 (SR2) Hotfix 2. This defect is fixed in Empower 3 FR3. See [TECN134972097](#) for more details.

Auto-Peak Width

Prior to the release of Empower 3, Auto-Peak Width setting changes will affect the calculation of Width @ Tangent and USP Resolution. For a more detailed explanation, refer to [TECN10008147](#) – Guidelines for Setting Auto-Peak Width In ApexTrack Processing Methods and its Effect on USP Resolution and USP Plate Count.

Processing Codes

Whenever resolution values are not calculated, take note of the Processing Codes for the peak or result to get a better understanding of why resolution values were not calculated. Table 2 lists the Processing Codes that may have an effect on resolution calculations in Empower.

Table 2 - Processing Codes that can impact calculating and reporting Resolution values

Code	Description
S10	Problem calculating peak width at 50% height.
S12	Problem calculating all peak widths. Retention time is the same the same as the start or end time.
S14	Problem calculating resolution. Check peak widths.
S22	Problem calculating tangent widths. Cannot calculate the tangent lines.
S23	Problem calculating tangent width. Cannot calculate the intercepts.
S27	Problem calculating USP Resolution. Check the peak widths.
S28	Problem calculating USP Resolution (HH). Check the peak widths.
S29	Width @ Tangent (USP Resolution) and Width @ Tangent (USP Plate Count) are not calculated using the percentages in the processing method. They are both calculated using lines tangent to the ApexTrack inflection points.
S32	Problem calculating a valid tangent width for USP Resolution. The Width at Tangent (USP Resolution) is negative.
S53	The peak's inflection points were recalculated using a peak width value that is optimized for the peak. The Width at Tangent is calculated using these recalculated inflection points.
S54	Width @ Tangent (USP Resolution) and Width @Tangent (USP Plate Count) are not calculated. The baseline is drawn at or inside inflection points. The peak will also have an I38 and I40 processing code.
I37	The result was processed with ApexTrack integration, but the peak with the processing code was processed with Traditional integration.
I38	ApexTrack: One or both inflection points are outside the peak region. Therefore, USP Resolution and USP Tailing values will be incorrect.

Code	Description
I39	This ApexTrack result was obtained using a Peak Width value that differs significantly from the Auto-Peak Width value calculated for the channel.
I40	ApexTrack: Baseline is drawn at inflection points.

References

United States Pharmacopeia and National Formulary (USP 38-NF 33). Vols 1 and 2. Rockville, MD: United States Pharmacopeial Convention; 2015.

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