Guidelines for Setting Auto-Peak Width in ApexTrack Processing Methods and its Effect on USP Resolution and USP Plate Count

The information in this article applies to both Empower and Empower 2 software with one exception, which is noted in this document.

Selecting an Appropriate Peak Width Setting

Ideally, you should leave the Peak Width parameter in an ApexTrack processing method blank because this allows the software to perform an auto-peak width calculation. The auto-peak width function allows Empower software to determine the optimal peak width setting for each chromatogram, and automatically uses this setting to process the chromatogram. If company policies prohibit a blank entry for this parameter, Waters recommends that you set the Peak Width parameter in the processing method equal to the auto-peak width setting calculated by the software.

Creating an ApexTrack Processing Method that Uses Auto-Peak Width

1. Open the Review window with the data channel of interest, and select View > Processing Method Layout. Both the chromatogram and the processing method are displayed.

2. In the ApexTrack processing method, enter values for the Start and End parameters. This is important – do not leave these blank. Select these values so that the peak(s) of interest lie between these times.

   NOTE: Do not include more of the chromatogram than is needed in this time range. When you use a blank setting for the Peak Width parameter, the software calculates the optimal Peak Width value using the chromatographic data in the time range defined by the Start and End settings.

3. Leave the Peak Width and Detection Threshold parameters blank.

4. Click the Integrate button.

   In the example shown in Figure 1, the auto-peak width value is 2.53 and the auto-detection threshold value is 3.800. This allows all peaks to be integrated. To eliminate the small peaks, set the Detection Threshold parameter larger than the automatically determined detection threshold. In the example shown in 2, we have increased the Detection Threshold to 200 (2.00e+002).

   NOTE: If your procedures prohibit a blank Peak Width value, enter the auto-peak width value in the Peak Width parameter (2.53, in this example).
Viewing the Auto-Peak Width Parameter

After a result has been determined using an ApexTrack processing method that has a blank entry for the Peak Width parameter, you can view the auto-peak width and auto-threshold values in the Review window. In the example shown in Figure 3, the auto-peak width value is circled in red, and the auto-threshold value is circled in blue. You can also view the auto-peak width value in the Peak Width result field of each result.
Tips for Creating an ApexTrack Processing Method that uses a Blank Peak Width Parameter (to invoke Auto-Peak Width)

- Always begin with blank Peak Width and Detection Threshold parameters.
- Always use a Start and End time in your method, including only the section of the chromatogram that needs to be integrated.
- After processing once using auto-peak width and auto-threshold, if small peaks of non-interest are integrated, increase the Detection Threshold parameter in increments of 10x until you achieve the desired integration.
- If a small peak of interest is not integrated, decrease the Detection Threshold parameter.
- Do not enter a Peak Width other than the auto-peak width value (either a blank entry or the statically-entered auto-peak width value) except as an absolute last resort to obtain the desired integration.
- If the chromatogram’s peaks are of significantly different widths (indicating that a single setting for the Peak Width parameter may not be appropriate for the entire chromatogram), determine what the auto-peak width setting is for each region as described in the next section of this document, “Determining the Auto-Peak Width Setting Within a Chromatogram Region.” Then, set the Start and End times to include all of the regions and enter the setting observed for the first region’s auto-peak width in the Peak Width parameter of the processing method. To set the Peak Width parameter appropriately for the other time regions, use the Set Peak Width integration event to change the peak width to the appropriate values for the time ranges.

Determining the Auto-Peak Width Setting Within a Chromatogram Region

When you integrate a chromatogram using a processing method that contains a blank Peak Width setting, Empower determines the Peak Width parameter based on the chromatographic data between the Start and End parameter settings. This occurs when you background process data, or in Review when you click the Integrate button.

Alternatively, in Review, when you click the Set Processing Method Peak Width button, Empower determines the Peak Width parameter based only on the chromatographic data that is displayed in the chromatogram plot, ignoring any entries in the Start and/or End parameters. If you have zoomed in on a region of the chromatogram, only the data in that region is used to determine the auto-peak width.

You can determine the auto-peak width setting for a region of the chromatogram in two ways:

- Set the Start and End processing method parameters to include only the desired time range, leave the Peak Width parameter blank, and integrate the chromatogram (in Review, click the Integrate button). View the calculated auto-peak width value in the Result Peak Width area of Review (red-circled area in Figure 3).

  or

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• In the Review Main Window, zoom in on the time region of interest within the chromatogram plot. With a blank Peak Width parameter in the processing method, click **Set Processing Method Peak Width**. Empower determines the peak width value based on the data in the zoomed chromatogram plot (ignoring the Start and End parameter values) and displays this value in the Result Peak Width area of Review (red-circled area in Figure 3).

For additional information about developing an ApexTrack processing method, see the following:

• Chapter 2 of the *Empower Software Data Acquisition and Processing Theory Guide*

• Chapter 3 of the *ApexTrack: Theory and Integration White Paper*

• Empower online help

**Differences Between Empower and Empower 2**

In Empower 2 Build 2154, a warning message is displayed in Review when you integrate a chromatogram with an ApexTrack processing method for which the Peak Width setting is more than a factor of 2 (higher or lower) than the auto-peak width value. This message is not displayed in Empower Build 1154.

Also in Empower 2, processing code I39 is displayed in all results where the Peak Width setting is more than a factor of 2 (higher or lower) than the auto-peak width value. This processing code is not displayed in Empower.

The purpose of the message and processing code in Empower 2 is to warn you that you may be using an inappropriate setting for the Peak Width parameter. You are still allowed to create methods and results with any Peak Width setting.

Note that the warning and processing code is used when the Peak Width setting differs by more than a factor of 2 from the auto-Peak width value. In a few situations, using a difference of less than a factor of 2 may be inappropriate. Likewise, there may also be a few situations where using a difference of more than a factor of 2 is appropriate.

**Appropriate Determination of USP Resolution and USP Plate Count**

In ApexTrack integration, the Width @ Tangent value is calculated as the width at baseline between two lines drawn tangent to the peak, at the peak’s inflection points. The location of the inflection points is determined from the second derivative chromatogram, which is smoothed according to the setting of the Peak Width parameter. Changing the Peak Width parameter changes the smoothing of the second derivative chromatogram, which in turn alters the location of the inflection points. This changes the way in which the tangent lines are drawn and also changes the Width @ Tangent value. Since the calculations for USP Plate Count, USP Resolution, and USP Relative Resolution (if the Pharmacopoeia choice is All and the processing method contains a Relative Resolution Reference) depend on the Width @ Tangent value, these calculations also depend on the Peak Width parameter in the ApexTrack processing method.
USP Plate Count and USP Resolution are both calculated using the Width @ Tangent value, as shown in the following formulas:

\[
\text{USP Resolution} = 2.0 \times \frac{(Rt_2 - Rt_1)}{(W_2 + W_1)}
\]

\[
\text{USP Plate Count} = 16 \left(\frac{Rt}{W}\right)^2
\]

where \(Rt\) = Retention Time
\(W\) = Width @ Tangent

Relative Resolution is also calculated using the USP Resolution equation when the Pharmacopoeia chosen in the processing method is All and there is a Relative Resolution Reference specified in the processing method.

An example of the way in which changing the Peak Width parameter changes the location of the inflection points is shown in Figure 4. Auto-peak width was used for the middle chromatogram, and was determined to be 8.16. The chromatograms on the left and right are integrated with Peak Width settings of 1.00 and 30.00, respectively. In all of these chromatograms, the Liftoff % and Touchdown % settings are 100%, which causes the baseline to be drawn between the data points closest to the peak’s inflection points. In a Gaussian peak, one would expect the inflection points to be at 61% of the peak height. This example shows that using the auto-peak width setting results in the most appropriate location for the inflection points. Therefore, using the auto-peak width setting will yield the most appropriate values for USP Plate Count and USP Resolution. If you set the Peak Width value statically, you need to be sure to set correctly in order for USP Plate Count and USP Resolution to be appropriately determined.

![Figure 2 - Examples of How the Peak Width Value Affects the Inflection Point Location](image)

The “correct” values for USP Plate Count, USP Resolution, and USP Relative Resolution are those that are calculated when the appropriate setting for Peak Width is used. This ensures that the second derivative chromatogram is not smoothed too much or too little, and that the inflection points and the Width @ Tangent are determined appropriately.

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System Suitability Processing Code S29

When you have the Suitability option enabled in the processing method and integrate with an ApexTrack processing method, all peaks are displayed with processing code S29. This serves as a reminder that the tangents were drawn at the peak’s inflections points instead of at a percentage of the peak height, as in Traditional integration. If you manually integrate, or manually alter a peak with an S29 code, the S29 code may be removed, indicating that the tangents for that peak are no longer drawn at the inflection points, but are drawn at the height percentage specified in the processing method. Because there are differences in how manual integration functions between Empower and Empower 2, you will notice that the S29 code is more often removed during manual integration in Empower than in Empower 2.