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EMPOWER 3 SOFTWARE

## ApexTrack Integration: Theory and Application

# [RESOURCE GUIDE]

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## 1. INTRODUCTION

The Millennium® Chromatography Manager Software was introduced in 1992, and since that time, the peak integration algorithm used in the software remained essentially unchanged. This algorithm is based upon the identification of a peak's liftoff point by comparison of the peak's slope against a fixed threshold. The algorithm then proceeds along the chromatogram finding features: apices, valleys, and so on, until a suitable slope threshold is met, indicating peak touchdown. The Millennium implementation of peak integration is referred to as Traditional integration in this paper.

In Empower® Software, in addition to Traditional integration, another integration algorithm called ApexTrack™ is available.

The goals of ApexTrack integration are to:

1. Provide an integration package that automatically integrates large and small peaks in noisy or sloping baselines
2. Reduce the time needed to develop an integration method
3. Reduce the need for manual integration of data

ApexTrack integration can be used in place of Traditional integration with any type of data, however the algorithm was specifically designed to improve integration for data that require time consuming manual manipulations which minimize productivity, or with data where required peaks are not detected.

ApexTrack integration implements a new approach to the basic peak detection and baseline determination algorithms. In ApexTrack, the feature used to detect a peak is the peak's apex and baselines are drawn using a search algorithm that starts from each peak's apex and works downward and outward.

ApexTrack makes a clean separation between peak detection and baseline location; the parameters that control these functions are independent. Changing parameters in the processing method that control which peaks are detected does not affect where the baselines for these peaks are drawn and vice versa. This is not true of Traditional integration.

The purpose of this paper is fourfold:

1. Discuss the finer points of ApexTrack integration and to point out differences between ApexTrack and Traditional integration when appropriate for clarification.
2. Discuss method development using ApexTrack integration.
3. Discuss method validation using ApexTrack integration.
4. Provide troubleshooting information relating to ApexTrack integration.

This paper does not discuss all aspects of ApexTrack integration theory in detail. For additional information, see the *Empower Data Acquisition and Processing Theory Guide* and the *Empower Online Information System*.

## 2. APEXTRACK PROCESSING

### 2.1 OVERVIEW

The goal of chromatographic peak integration is to obtain the retention times, heights, and areas of peaks contained in a separation. At the heart of peak integration are two key algorithms: the one that detects the peaks and the one that determines their baselines. Once a peak's baseline is known, it is straightforward to determine its retention time, height, and area.

The heights of peaks can vary and peaks can coelute. Such variations in height and resolution result in response profiles that exhibit a wide range of patterns, which in turn pose integration challenges. ApexTrack effectively overcomes these difficulties by using the second derivative of the chromatogram to detect peaks. The second derivative is an effective method to detect peaks of all sizes, including coeluted peaks and those on sloping or drifting baselines.

*Note: Except where noted, the ApexTrack processing methods in this section use AutoPeak Width to determine the peak width value, and AutoThreshold to determine the detection threshold. values are entered for the Start and End parameters to define the region of the chromatogram that is used to calculate the peak width and detection threshold values. The Liftoff % (0.00) and Touchdown % (0.00) parameters are left at their default values.*

The following discussion illustrates how ApexTrack integration is better than Traditional integration when integrating peaks on a sloping baseline, when using a skim event, and when detecting shoulder peaks.

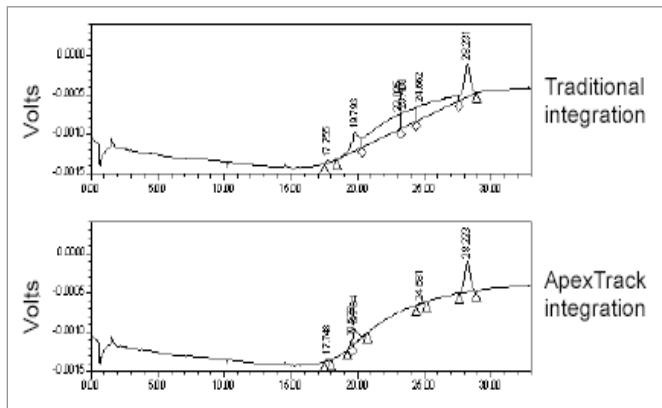


Figure 2-1. Traditional (top plot) versus ApexTrack (bottom plot) integration showing a sloping baseline.

### Sloping baseline

Figure 2-1 shows the data integrated using ApexTrack and Traditional integration. Traditional integration has a difficult time with such a sloping baseline, so it draws the baseline with several drop lines. ApexTrack integration can follow the sloping baseline, so it draws the baseline from peak to peak.

The processing method for ApexTrack sets the Start value at 15 minutes and the End at 30 minutes. The processing method for Traditional uses two Inhibit Integration timed events: the first from 0 to 15 minutes, and the second, from 30 minutes to the end of the chromatogram.

### Skimming

In Empower 3, both ApexTrack and Traditional integration support Tangential skimming. In addition, ApexTrack supports Gaussian skimming, and Traditional supports Exponential skimming. Although each type of skimming offers advantages, the following examples will explore the differences between Gaussian and Exponential skimming.

*Note: You must use a timed event to enable any type of skimming. The Gaussian Skim timed event does not require additional parameters. Both the Exponential Skim and Tangential Skim timed events require you to enter a value for the height ratio (ratio of the parent peak height to the rider peak height).*

Figure 2-2, compares the data integrated with ApexTrack using Gaussian skimming, and Traditional integration using Exponential skimming. The profile of the parent peak in the ApexTrack chromatogram follow a Gaussian shape. The profile of the parent peak of the exponential skim looks mathematically derived.

In the processing method for ApexTrack, the Gaussian Skim timed event is enabled at the beginning of the chromatogram. In the processing method for Traditional integration, the Exponential Skim timed event is enabled and a value of 0.00 is specified for the height ratio.

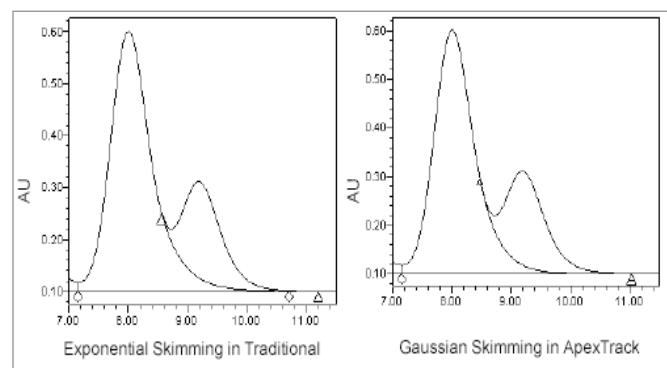


Figure 2-2. Gaussian Skimming in ApexTrack Versus Exponential Skimming in Traditional Integration.

## Shoulders

ApexTrack integration offers the Detect Shoulders timed event, which is not available in Traditional integration.

Figure 2-3 shows a chromatogram with shoulder peaks integrated with ApexTrack integration. With no timed events, shoulder peaks are not integrated. When the Detect Shoulders event is used, the shoulder peaks are integrated. When both the Detect Shoulders and Gaussian Skim events are used, both the valley drop lines and the shoulder drop lines are replaced by Gaussian skim profiles. Traditional integration has no event that will integrate shoulder peaks.

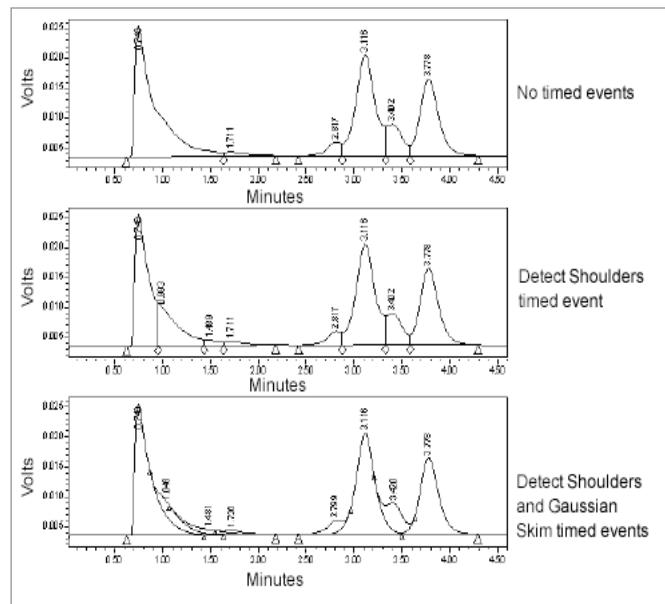


Figure 2-3. ApexTrack Integration with Detect Shoulders and Gaussian Skim Event enabled.

## 2.2 PEAK DETECTION

Peak detection is the process of defining what peaks are present in a chromatogram. The integration algorithm accomplishes this based on integration parameters set in the processing method. Both Traditional and ApexTrack integration have a Peak Width parameter. Traditional integration uses a Threshold parameter ( $\mu\text{V/sec}$ ) but ApexTrack uses a Detection Threshold parameter ( $\mu\text{V}$ ). Although these have similar names, they operate differently.

Where Traditional peak detection is based upon measuring the slope of the chromatogram at each point to determine the beginning of a peak, ApexTrack peak detection is based upon measuring the curvature of the chromatogram at each point to determine the apex of a peak. The curvature is measured using a plot of the second derivative of the chromatogram. The second derivative measures curvature. The definition of curvature is the rate of change of slope.

In Figure 2-4, the upper curve is a Gaussian peak profile, and the lower curve is its second derivative (multiplied by -1). Intuitively, it seems that it should be easier to detect an apex rising above the baseline (as in ApexTrack integration) rather than a peak start within the baseline (as in Traditional integration). Results obtained from the second derivative profile also have the advantage that they are independent of the underlying baseline slope.

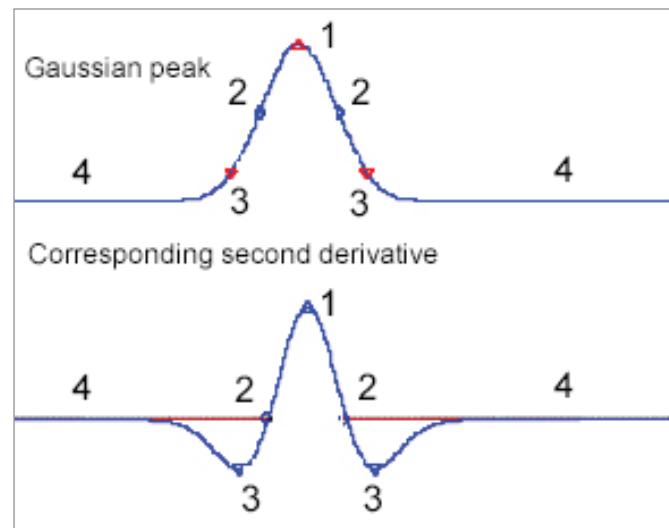


Figure 2-4. Plot of a Gaussian Peak (top plot) and the Corresponding Second Derivative Plot, multiplied by -1 (bottom plot).

The peak apex (#1) is a local maximum of curvature in the second derivative plot. The height of the maxima in the second derivative plot is roughly proportional to the peak height in the chromatogram plot. (More specifically, it is proportional to the peak height divided by the square of the peak width if the peak is not a shoulder.) The points where the curvature crosses zero are the inflection points (#2); two inflection points always straddle each apex. The upslope points (#3) are local minima in the second derivative. The baseline region (#4) has zero curvature.

Note that normally the curvature at the apex of a positive peak is negative, and vice versa. However, in this paper, the negative of the second derivative is employed for clarity; the second derivative plot is multiplied by -1, so the apex of a positive peak also appears as a positive peak in the second derivative and vice versa.

Because the second derivative of a chromatogram is affected by the baseline noise in the chromatogram, not all maxima in the second derivative are peaks. A threshold criteria is therefore applied to discriminate between real peaks and variations within the baseline; a local maximum of curvature whose magnitude is above the detection threshold value is

recognized as a detection of a positive chromatographic peak, and a local maximum of curvature whose magnitude is below the detection threshold is not recognized as a positive peak. The opposite is true for negative peaks.

Curvature detection (used in ApexTrack integration) has the following advantages over slope detection (used in Traditional integration):

- Curvature detection can detect shoulders, because a shoulder readily appears as a maximum of curvature in the second derivative.
- The curvature of a straight line is zero, even if it is sloped; hence a drifting baseline with a constant slope does not affect the curvature, and therefore detection, of a peak.
- Constant baseline noise appears as constant amplitude of noise in the second derivative plot and can be rejected as peaks by using a simple detection threshold.
- The effect of changing the detection threshold is easy to understand and gives predictable results: Raising (or lowering) the detection threshold will reduce (increase) the number of peaks found.
- Peak detection and baseline determination are decoupled. Baseline location is performed after peak detection and a baseline is located for every peak detected. Changing parameters that affect baseline placement do not affect which peaks are detected and vice versa.

*Note: Traditional integration detects a peak by finding the start of a peak; its liftoff. It does so by comparing the slope of the data against a fixed threshold value in  $\mu\text{V/sec}$ . This slope criterion successfully detects big peaks and small peaks, as well as co-eluted peaks separated by a valley. However,*

- *A slope criterion cannot be used to detect shoulder peaks because there is no valley between the peaks, so the slope never goes to zero.*
- *A sloping baseline can affect the detection of small peaks. If the baseline drifts downward, a small peak may not be detected since the baseline slope may contribute to the overall slope to which the threshold slope is being compared.*
- *Peak detection and baseline determination are coupled. The slope at liftoff determines if a peak is detected and is also used to determine baseline placement. Hence, if processing method parameters are modified with the intention of changing the baseline placement, this might also change which peaks are detected.*

## 2.2.1 ROLE OF PEAK WIDTH IN DETECTING PEAKS

During peak detection, the integration algorithm uses smoothed data. This allows peaks to be detected more efficiently since some of the baseline noise is eliminated. Both the Traditional and ApexTrack integration algorithms use smoothed data during this step, however, the actual smoothing occurs differently between the two algorithms.

*Note: For both Traditional and ApexTrack algorithms, unsmoothed data is used to determine baseline placement.*

In ApexTrack, modified Savitzky-Golay (MSG) filtering is used to smooth the data during peak detection. The entered value of the peak width, times the sample rate, divided by an empirical constant of 1.5, sets the width of the filter, which is used in the second derivative plot. For example, a peak that has a chromatographic peak width of 1 minute and is sampled at 1 point per second has a second derivative MSG filter that is about 40 points wide.

This filter also acts as a notch filter and effectively sets a minimum time between potential second derivative apices to about  $\frac{1}{2}$  the entered value of the peak width; peaks do not appear closer together than  $\frac{1}{2}$  a peak width. The peak detection algorithm also rejects noise peaks significantly narrower or wider than the entered peak width value.

ApexTrack uses the peak width value when performing peak detection as follows:

1. Sets the width of the MSG filters.
2. Smooths the second derivative chromatogram.
3. Locates each local maximum in the second derivative chromatogram.

The next step is to eliminate those local maxima whose second derivative is below the detection threshold.

## 2.2.2 ROLE OF DETECTION THRESHOLD IN DETECTING PEAKS

In Traditional and ApexTrack integration, the "Threshold" parameter and the "Detection Threshold" parameter, respectively play a role in determining which peaks are integrated and which are considered baseline noise. Each algorithm, however, uses this parameter differently.

In ApexTrack, the Detection Threshold value is a measure of peak-to-peak baseline noise in peak height units ( $\mu\text{V}$ ). It is used during peak detection and is applied to the second derivative chromatogram. The dashed horizontal line in Figure 2-5 represents the Detection Threshold. Each second derivative maxima is labelled as a peak if it is greater than the detection threshold. Second derivative maxima less than the detection threshold are not considered peaks.

## Example of peak detection via apex curvature (simulated data)

In Figure 2-5, the top plot is a simulated chromatogram. The first peak is baseline resolved, the second and third peaks cluster to form a valley. The final pair fuse at lower resolution such that there is no valley point. As a result, the last pair of peaks is shouldered. The lower plot in Figure 2-5 is the second derivative plot of the chromatogram (multiplied by -1).

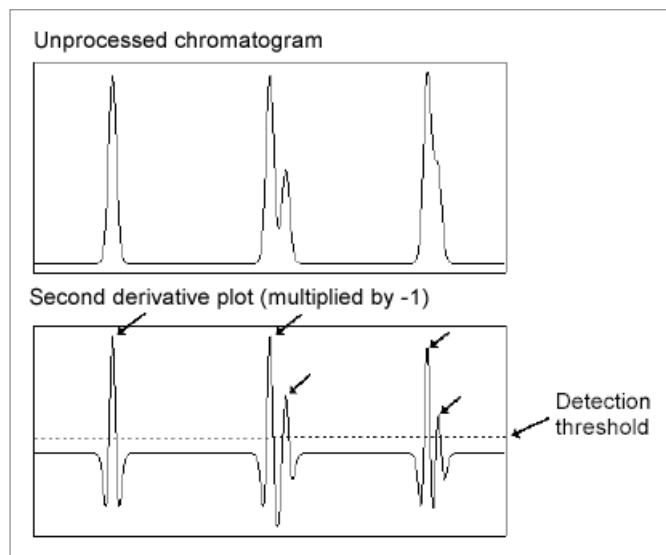


Figure 2-5. A simulated chromatogram (top plot) and the corresponding second derivative plot (bottom plot).

The local maxima of the second derivative are indicated by the arrows in the bottom plot. Note that the shouldered peak is easily detected in the second derivative profile. All local maxima are above the detection threshold (dashed horizontal line) and are therefore considered true peaks by the peak detection algorithm.

## Example of peak detection via Apex curvature (real data)

Figure 2-6 shows an integrated chromatogram where peaks are baseline resolved, but at differing heights, and the corresponding second derivative profile of this region.

Only two peaks are integrated in the chromatogram because the Detection Threshold is higher than the second derivative apex of the third peak.

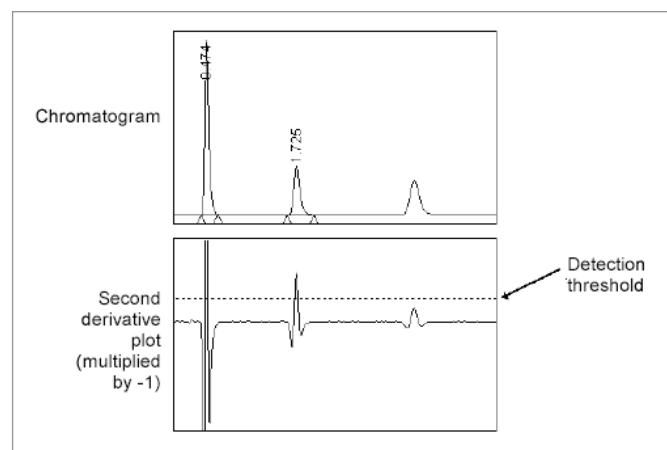


Figure 2-6. Chromatogram showing baseline resolved peaks of different heights.

## 2.3 BASELINE DETERMINATION

In ApexTrack integration, there are two parameters – Liftoff % and Touchdown % – that control the exact placement of the baseline start and end points, respectively. Unlike Traditional integration, changing these parameters may change the particular sample points that define a baseline, but will not change which peaks are detected.

Note: If the Detect Shoulders event is not enabled, increasing the Liftoff % and/or Touchdown % values can cause shoulder peaks that previously were not detected to be detected.

Apex detection is the starting point for the algorithm that locates the baseline. One characteristic of a second derivative apex is that it is always straddled by inflection points (#2 in Figure 2-4). In Figure 2-7, these inflection points are indicated by X's. During baseline determination, a tangent to the peak profile is drawn through each inflection point. A line also connects the inflection points. This line is referred to as the inflection point baseline and is the first approximation of the baseline for the peak.

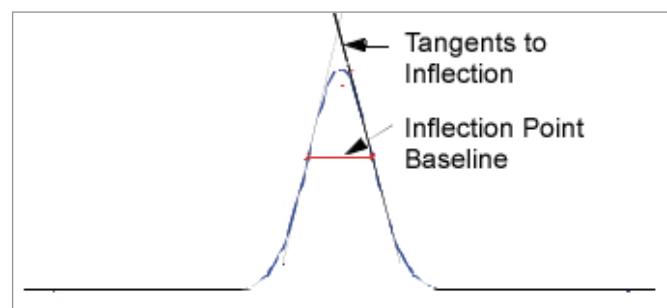


Figure 2-7. Example of baseline determination.

ApexTrack computes a slope difference value that is used to determine final baseline location. For each peak, ApexTrack measures the difference between the slope of the tangent at the upslope inflection point and the peak's inflection point baseline, as well as the difference between the slope of the tangent at the downslope inflection point and the inflection point baseline. These slope differences are proportional to the peak's height and are shown as  $\Delta m_1$  and  $\Delta m_2$  in Figure 2-8.

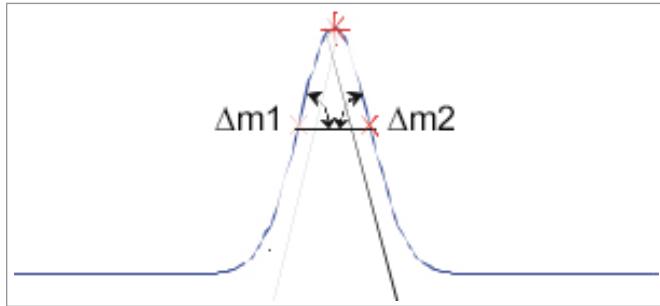


Figure 2-8. Computing slope differences.

The Liftoff % and Touchdown % values set in the processing method are then used to scale these initial slope difference values and determine two final slope difference criteria that define baseline placement. For liftoff, the final slope difference criteria used to determine baseline location for a given peak is the product of the Liftoff % times the  $\Delta m_1$  divided by 100. For touchdown, the final slope difference criteria is

the product of the Touchdown % times  $\Delta m_2$  divided by 100. Because the slope differences are proportional to peak height, tall peaks automatically have larger slope difference criteria and short peaks automatically have small slope difference criteria.

*Note: For more information regarding baseline location see the Empower Data Acquisition and Processing Theory Guide.*

Figure 2-9 shows the simulation of a tailed peak which illustrates how ApexTrack locates the baseline of a baseline-resolved peak. The initial baseline is the inflection point baseline. This baseline expands, data point by data point down the peak, testing the slope differences at each data point. With each expansion, the slope of the tangents at the end points of the baseline become less different than the baseline slope. The expansion stops when the slope difference criteria are met at both liftoff and touchdown. In this example the Liftoff % is set to 0.0 and the Touchdown % is set to 0.5. Note that the final slope differences (if a tangent were to be drawn at liftoff

and touchdown) are significantly smaller (zero in the case of liftoff) than the initial slope difference at the inflection point baseline (because the initial slope difference values are scaled by 0.0% and 0.5%, for liftoff and touchdown, respectively).

NOTE : the default values are 0.0 for both.

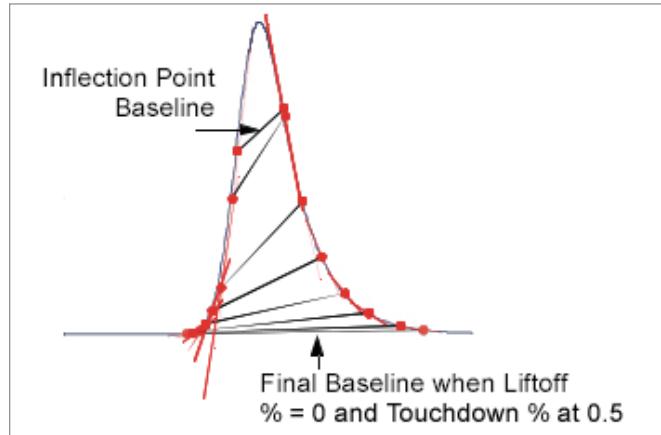


Figure 2-9. Example of expanding the baseline.

If fused peaks are involved, baselines similar to those in Figure 2-10 are found during baseline expansion.

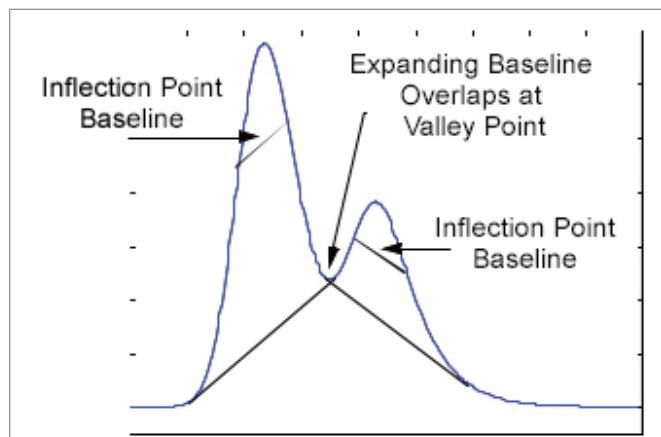


Figure 2-10. Initial baseline with fused peaks.

In such an instance, the baselines overlap at the valley point. When such an overlap is detected, the baseline is fused between the start and end of the cluster and the tangent search is then continued using the fused baseline to find the final baseline (Figure 2-11).

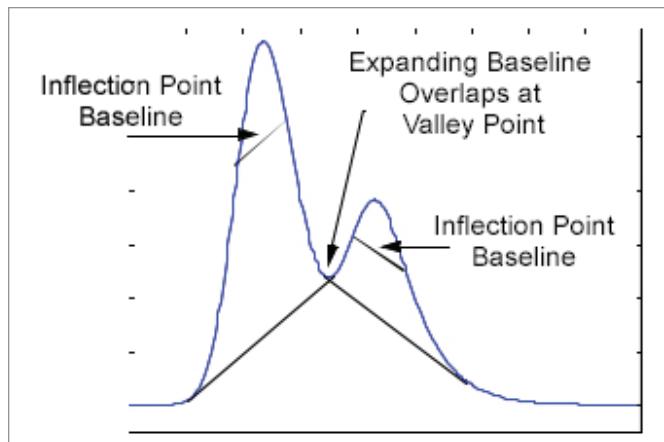


Figure 2-11. Fusing and then expanding the baseline with fused peaks.

*Note: In Traditional integration, once peaks and their start and end locations are identified within the chromatogram, the algorithm simply draws a baseline from the start to the end of each peak (or fused peak group).*

*If the projected baseline intersects the signal in the chromatogram, the software adjusts the baseline to the lowest point within the fused peak group, separating the peak group into individual and/or fused peaks, as appropriate. The software then rechecks the new baselines to make sure they do not intersect the chromatographic signal except at peak start or end points and readjusts the baseline as necessary.*

## 2.4 AUTOPARAMETERS

### Auto-Peak Width

Auto-Peak Width is the automatic determination of the peak width parameter. Measured in seconds, Auto-Peak Width sets the peak width to 5% height of the peak with the highest magnitude in the second derivative. This peak can be negative and may not be the highest peak in the chromatogram.

The width of the peak is determined by accurately measuring the time between the inflection points. This time is multiplied by a factor 4.89549/2, which gives the width at 5% for a Gaussian peak. It is this value that is reported and used by ApexTrack.

This peak width is determined for each peak. The value for the largest peak in the second derivative is then placed in either the Processing Method Peak width field or the Result Peak width field.

A minimum signal to noise of 10:1 is required in order for the Auto-Peak Width algorithm to work properly. Otherwise, the resulting peak width may be that of a noise artifact in the chromatogram.

Auto-Peak Width is enabled by default with ApexTrack integration (the peak width parameter is blank). To set the Peak Width using Auto-Peak Width, perform one of the following:

- Determining the Peak Width using the Integrate button in Review: In the Main Window of Review, clear the Peak Width parameter in the processing method and click the Integrate button. The software uses the data between any entered Start and End times and/or between any Inhibit Integration events that occur at the start or end of the chromatogram, to determine the Peak Width, and displays the calculated Peak Width value in the Result Peak Width box in the Main Window of Review. This value may be copied into the processing method by right-clicking on it.
- Determining the Peak Width using the Set Peak Width button in Review: In the Main Window of Review, click the Set Processing Method Peak Width button. The software ignores any entered Start and End times and Inhibit Integration events, and uses only the data seen in the plot (which may be zoomed) to determine the Peak Width. This value is displayed in Review and automatically entered into the Processing Method. Note: If the value for Peak Width is calculated from a zoomed portion of the chromatogram and that value is significantly different from the value that would be calculated from the entire chromatogram, when the chromatogram is integrated in Review, a message will be seen that the Peak Width parameter being used varies by more than a factor of 2 from the Auto Peak Width value. To prevent this message, enter the appropriate Start, End, or Inhibit Integration events to limit the section of the chromatogram from which the Peak Width is calculated.
- When background processing using a method with a blank for Peak Width, the software uses the data between any entered Start and End times and/or between any Inhibit Integration events that occur at the start or end of the chromatogram, to determine the Peak Width. The value used for Peak Width can be observed either by taking the result into Review and observing the Result Peak Width box in the Main Window, or in Report Publisher by printing the Peak Width field.
- Methods can be saved with a blank for Peak Width, so that the most appropriate value for Peak Width is used for each chromatogram processed with the method.

*Note: Traditional processing only sets the peak width and threshold values automatically if the system policy named Use v3.0X Style Peak Width and Threshold Determination is not enabled. When this system policy is active, the Peak Width and*

*Threshold buttons, the Processing Method Wizard, processing methods, and results all function as in versions of Millennium<sup>32</sup> prior to version 3.2. This system policy does not affect ApexTrack integration.*

## AutoThreshold

AutoThreshold is the automatic determination of the Detection Threshold parameter. Measured in microvolts, the AutoThreshold routine identifies regions of baseline noise within a chromatogram, which it then expresses as peak-to-peak baseline noise, scaled to a factor of 106. Peak-to-peak noise is defined as four times the root-mean-squared standard deviation. This value is then used by the ApexTrack integration algorithm in peak detection to distinguish between noise artifacts and real peaks.

Internally, AutoThreshold determines the second derivative threshold by examining the noise regions in the second derivative chromatogram. It is this value that is used by the ApexTrack algorithm. However, the noise in the second derivative is proportional to the noise in the baseline of the original chromatogram. Therefore, the threshold of the second derivative is proportional to the peak-to-peak noise in the original chromatogram. The threshold value that is used on the second derivative chromatogram is converted to the equivalent peak-to-peak noise threshold of the baseline in the original chromatogram. This is the value that is displayed in the processing method.

Hence, when a value is entered in the Set Processing Method Threshold field, the software converts it to an analogous measure of noise for the second derivative chromatogram where the value is actually applied during peak detection.

AutoThreshold is enabled by default with ApexTrack integration (the Detection Threshold parameter is blank).

To set the Detection Threshold using AutoThreshold, perform one of the following:

- Determining the Detection Threshold using the Integrate button in Review: In the Main Window of Review, clear the Detection Threshold parameter in the processing method and click the integrate button. The software uses the data between any entered Start and End times and/or between any Inhibit Integration events that occur at the start or end of the chromatogram, to determine the Detection Threshold, and displays the calculated Detection Threshold value in the Result Threshold box in the Main Window of Review. This value may be copied into the processing method by right-clicking on it.

- Determining the Detection Threshold using the Set Processing Method Threshold button in Review: In the Main Window of Review, click the Set Processing Method Threshold button. The software ignores any entered Start and End times and Inhibit Integration events, and uses only the data seen in the plot (which may be zoomed) to determine the Detection Threshold. This value is displayed in Review and automatically entered into the Processing Method.

*Note: You must have a peak width value specified before the Set Processing Method Threshold button is active.*

- When background processing using a method with a blank for Detection Threshold, the software uses the data between any entered Start and End times and/or between any Inhibit Integration events that occur at the start or end of the chromatogram, to determine the Detection Threshold. The value used for Detection Threshold can be observed either by taking the result into Review and observing the Result Threshold box in the Main Window, or in Report Publisher by printing the Threshold field.
- Methods can be saved with a blank for Detection Threshold, so that the most appropriate value for Detection Threshold is used for each chromatogram processed with the method.

*Note: Traditional processing only sets the peak width and threshold values automatically if the system policy named Use v3.0X Style Peak Width and Threshold Determination is not enabled. When this system policy is active, the Peak Width and Threshold buttons, the Processing Method Wizard, processing methods, and results all function as in versions of Millennium<sup>32</sup> prior to version 3.2. This system policy does not affect ApexTrack integration.*

## 2.5 CALCULATION OF RETENTION TIME

Depending on the peak boundaries and the properties of the peak profile, peak retention time is obtained using a 5-point quadratic fit, a 3-point quadratic fit, the time of the second derivative apex, or time of the highest point.

Retention Time is most commonly determined using a 5-point quadratic fit. For information regarding when each type of fit is used, see the Empower Data Acquisition and Processing Theory Guide.

When the 5-point quadratic fit is used, it is performed on the baseline-corrected peak profile.

## Baseline correction

In situations where the baseline is highly sloped, and the peak height is low, it may be that the highest point with respect to the baseline is not the data point with highest response.

In Figure 2-12, the highest data point follows the retention time by two sample points. Baseline correction subtracts the baseline data points from the signal data points to remove baseline effects such as this. In doing so, the integration results become immune from the effects of a sloping baseline.

In Traditional and ApexTrack, the estimation of peak height and retention time is obtained from the baseline corrected chromatogram. That is, the five (for a 5-point quadratic fit) or the three (for a 3-point quadratic fit) highest points are chosen after baseline correction is performed. Picking the highest point from baseline corrected data will yield the correct retention time and peak height, as indicated by the vertical line in Figure 2-12.

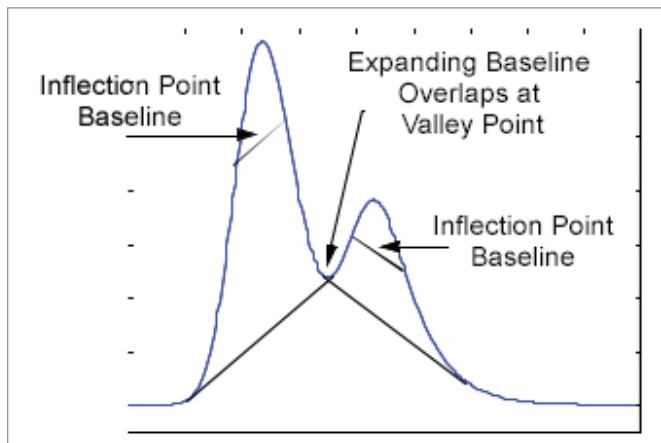


Figure 2-12. Peak on highly sloped baseline.

*Note: To determine retention time and height with Traditional integration, the software fits a quadratic curve to the five points at the top of the peak (the highest data point and the two data points on either side of this point after baseline correction) and sets the peak apex point to the inflection point of the fitted curve. The X value of the peak apex is the retention time of the peak. The 5-point fit in Traditional integration is performed using the uncorrected chromatogram. The zeroed baseline plot displays the baseline corrected peaks.*

*You can disable fitting a quadratic curve to the top of the peak by implementing a system policy named Use v2.xx Style Retention Time Calculations. When results are integrated with this system policy active, the processing code 109 is added to the Codes field in the result. This system policy does not affect ApexTrack integration.*

## 2.6 INTEGRATION EVENTS

Both Traditional and ApexTrack integration allow use of several integration events. The following table lists the events available with each integration algorithm:

Traditional Integration Event	ApexTrack Integration Event
Inhibit Integration	Inhibit Integration
Allow Negative Peaks	Allow Negative Peaks
Set Liftoff	Set Liftoff %
Set Touchdown	Set Touchdown %
Set Peak Width (sec)	Set Peak Width (sec)
Set Threshold	Set Detection Threshold
Set Minimum Area	Set Minimum Area
Set Minimum Height	Set Minimum Height
Set Maximum Width (sec)	Set Maximum Width (sec)
Set Maximum Height	Set Maximum Height
Valley to Valley	Valley to Valley
Exponential Skim	
Force Drop Line	
Force Baseline by Peak	
Force Baseline by Time	
Force Peak	
Forward Horizontal by Peak	
Forward Horizontal by Time	
Reverse Horizontal by Peak	
Reverse Horizontal by Time	
Tangential Skim	Tangential Skim
	Merge Peaks (GPC option only)
	Detect Shoulders
	Gaussian Skim

In this section, the following events are discussed:

- Allow Negative Peaks event
- Detect Shoulders event
- Valley to Valley event
- Gaussian Skim event

*Note: For information on other integration events, see Empower Online Information System*

## 2.6.1 ALLOW NEGATIVE PEAKS EVENT

ApexTrack detects negative peaks as apices that have curvatures whose sign is opposite that of positive peaks. Negative peaks can be isolated or part of a cluster. In a cluster, adjoining peaks can have the same or opposite signs.

Note: To ensure the best results, use this timed event sparingly. This event requires a period of stable, clean baseline (approximately the width of one peak) both before and after the peak or peak cluster. Always place the start time and end time within baseline regions, not within a peak or cluster.

This event works as follows:

- If you enable the Allow Negative Peaks event, ApexTrack detects the apices of both positive and negative peaks. (If you do not enable the Allow Negative Peaks event, ApexTrack detects only the apices of positive peaks.)
- If clusters contain both positive and negative peaks and you do not enable the Allow Negative Peaks event, ApexTrack often detects the negative peaks as valleys between positive peaks.
- Shoulder detection and Gaussian skims are compatible with Allow Negative Peaks. If these events are enabled, then shoulders of negative peaks are detected and vertical boundaries can be replaced by skim profiles.

See Section 3.6, Using the Allow Negative Peaks Timed Event for examples of the use of the Allow Negative Peaks event.

## 2.6.2 DETECT SHOULDERS EVENT

This event is only available when using ApexTrack integration. When the Detect Shoulders event is enabled in an ApexTrack method, shoulder boundaries and round boundaries may be seen between two peaks. Shoulder boundaries are labelled "S" and round boundaries are labelled "R".

The ApexTrack algorithm always detects shoulder and round boundaries, even when this event is not enabled. If the event is not enabled during processing, the shoulder and round boundaries are initially detected, but later each cluster of peaks is folded into one peak.

### Shoulder and round peaks

When peaks are not resolved from each other, they often have a valley between them. Shoulder peaks occur when two adjoining peaks (of unequal height) elute at low enough resolution that no valley appears between them. A pair of round peaks occurs when peaks of nearly equal height fuse at very low (but not zero) resolution.

The upper plot in Figure 2-13 shows a single peak (1) and pairs of peaks separated by a valley dropline (2 and 3), a shoulder dropline (4 and 5), and a round dropline (6 and 7). The 2nd derivative plot beneath it shows that the ApexTrack algorithm can easily discern both shoulder-fused and round-fused pairs of peaks, because each peak has a 2nd derivative apex above the Detection Threshold. In the 2nd derivative plot, the peaks' inflection points are located where the 2nd derivative plot crosses the zero line. Each peak has two inflection points, except for the pair of round peaks. Round peaks are unique in that the pair share the same pair of inflection points.

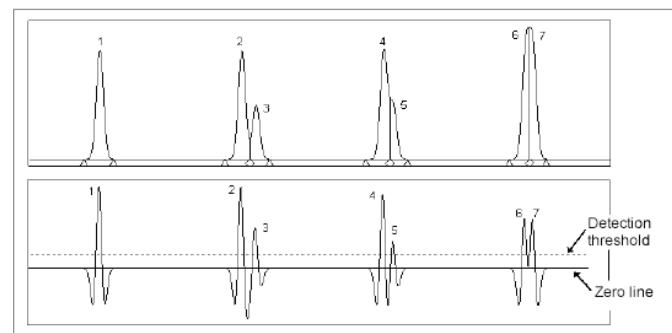


Figure 2-13. Isolated Peak (1), Fused Peaks (2 and 3), Shoulder Peak (4 and 5), and Round Peaks (6 and 7) (top plot) and the Corresponding Second Derivative Plot (bottom plot)

## 2.6.3 VALLEY-TO-VALLEY EVENT

The Valley-to-Valley event directs ApexTrack to replace valley boundaries with baseline boundaries. Shoulder and round boundaries are generally unchanged by the Valley-to-Valley event.

The Valley-to-Valley event directs ApexTrack to replace the valley V boundaries with baseline B boundaries. Thus if a peak cluster contains only V boundaries, this event will transform it into a series of individual, baseline-resolved peaks. The time of a B has the same time as that V it replaces. The Valley-to-Valley event is applied only after all peaks, baselines, and boundaries have been established.

Restriction: This event cannot overlap the Merge Peaks event or another Valley-to-Valley event, but it can overlap all other events.

The event is applied to all V boundaries that occur during the time the event is enabled. The event can be applied to a subset of V boundaries within a cluster. You can enable this event for a single V boundary but the event must encompass at least one V boundary to have an effect. Setting the event to encompass only a peak apex has no effect on the chromatogram.

When the event is enabled for only a subset of boundaries within a cluster, only those V boundaries during the time when the event is enabled will be converted to B boundaries.

## Tips:

- If the event is applied to only a subset of V boundaries within a cluster, additional V boundaries within a cluster may be changed to Bs to prevent a baseline from piercing the chromatogram.
- A new baseline end point causes the new baseline to pierce the chromatogram, the end point is indented to prevent piercing.

When the event is enabled, a low-level peak may disappear when its apex curvature is inconsistent with the new baseline. For example, if a peak with a positively curved apex has its apex drop below the new, valley-to-valley baseline, the peak will be omitted.

## 2.6.4 GAUSSIAN SKIM EVENT

When adjoining peaks elute at low resolution, the larger peak contributes to the baseline of the smaller peak. ApexTrack draws a gaussian curve of the larger peak's profile in order to better estimate the area and height of both peaks (Figure 2-15). A gaussian curve approximates the shape of an ideal chromatographic peak. The properties of the larger peak at its inflection point determine the parameters describing the Gaussian curve.

The boundary for valley or shoulder boundaries is a vertical drop line, which occurs in peak clusters when the Gaussian Skim event is not enabled. When gaussian skimming is activated by a timed event, an algorithm determines whether a gaussian skim should replace the valley or shoulder vertical drop line. Gaussian Skim boundaries are labeled with a G.

*Note: Enable the Shoulder Detection event in regions where the Gaussian Skim event is enabled. Shoulder Detection must be enabled in order for shouldered peaks to be skimmed by adjoining larger peaks.*

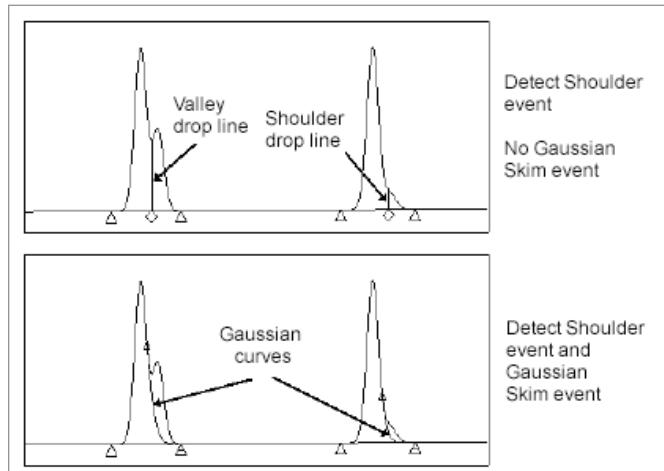


Figure 2-14. Valley boundary (left) and shoulder boundary (right).

ApexTrack supports both fronting and tailing skims. If Gaussian Skim is enabled, rules are applied to each pair of adjoining peaks in a cluster to determine which type of skim to use.

## How Gaussian Skimming works

The curve used for skimming is a portion of a simple Gaussian peak shape that starts and ends at the end point of the baseline of the next peak (for rear skims) or the start point of the baseline of the previous peak (for front skims).

Empower determines the curve used for skimming by searching for a suitable Gaussian profile of the parent peak that skims deepest into the child peak, and satisfies certain boundary conditions. The first boundary condition is that the Gaussian profile does not pierce the child peak. The second boundary condition is that Gaussian profile drops significantly close to the common baseline of the cluster at the far end of the next peak. Empower starts searching for the launching point of the Gaussian profile from the lowest point, or valley between the peaks, and moves the launching point towards the inflection point of the mother peak until the boundary conditions are satisfied. This results in the maximum possible Gaussian Skimming profile and preserves the most area for the mother peak. If Empower cannot draw a baseline at or below the inflection point that satisfies the boundary conditions, it will not skim the peak and will report a processing code to explain the reason Gaussian Skimming did not work.

Figure 2-15 and Figure 2-16 show chromatograms integrated with and without a Gaussian Skim event. Figure 2-16 has a Detect Shoulders event along with the Gaussian Skim event.

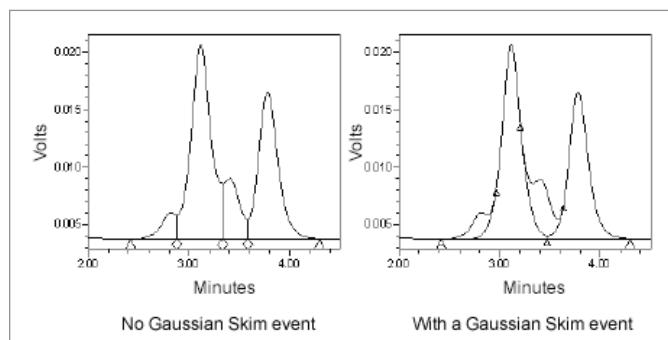


Figure 2-15. Example of the Gaussian Skim event.

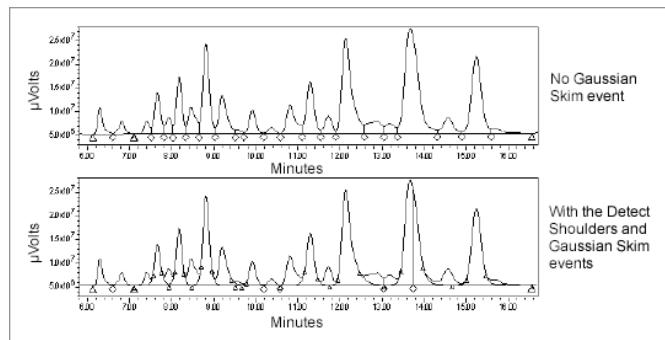


Figure 2-16. Chromatogram integrated without (top plot) and with (bottom plot) Gaussian Skim event.

The Gaussian Skim event is not available when using Traditional integration. When using Traditional integration, tangential and exponential skims may be used. For more information, see the Empower Online Information System.

Figure 2-17 is the same data as Figure 2-16, showing how Gaussian and Tangent Skim events can be used in the same ApexTrack method. No two skim events can overlap in time, but the use of several events can

produce a result such as that shown in Figure 2-17, with a tangent skim cluster at ~7.5 minutes, tangent skims at ~10.8 and ~14.5 minutes, and several Gaussian Skims.

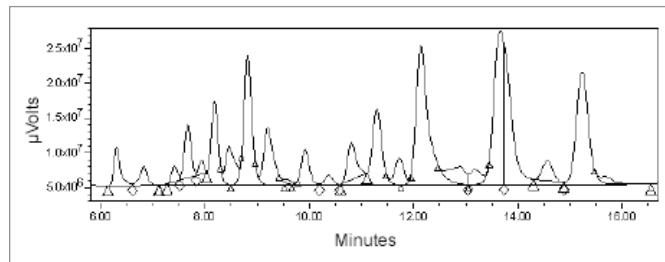


Figure 2-17. Example of a chromatogram with a Gaussian Skim and Tangent Skim events.

## 2.7 MANUAL INTEGRATION

### Traditional peaks in an ApexTrack result

In a result created with an ApexTrack method, all peaks, by definition, have a 2nd derivative apex and an inflection width (peaks in a result created with the Traditional algorithm, do not have these values). In an ApexTrack result, some manual changes to peaks have the potential to position the peak start and/or end such that the peak no longer includes the 2nd derivative apex or inflection width. When this occurs, peak type processing code I37 is used, and the peak is referred to as a "Traditional" peak in an "ApexTrack" result. Traditional peaks in an ApexTrack result do not have values for "2nd derivative apex" and "inflection width" in the Peaks Table.

The I37 code will never be seen in a result created with the Traditional algorithm.

*Note: Whether a peak is a Traditional peak or an ApexTrack peak determines how the "Width@Tangent" field is calculated, and will therefore affect all calculations based on "Width@Tangent". See Section 3.9, Using System Suitability with ApexTrack.*

### Adjusting peak and cluster baselines

The baselines of peaks or clusters may be adjusted by clicking on either the start or end point and dragging it to the desired time (either shorter or longer).

When a peak or cluster baseline is adjusted in ApexTrack integration, additional peaks may be added to the peak or cluster, peaks may be removed from a cluster, or the cluster may break into more than one cluster. If the adjusted region includes more or fewer 2nd derivative apices than the original region, more or fewer peaks will be seen accordingly. The adjusted endpoint is labeled "b", and the baseline may pierce the signal at that end of the cluster (if you choose to make the adjustment that way). However, unlike the Traditional algorithm, the opposite end of the peak or cluster is polished, i.e., moved, so that no piercing of the opposite end of the peak or cluster will occur. When it is polished, the opposite endpoint remains labeled "B", even when it moves.

*Note: Polishing only occurs when the opposite end of the cluster is originally labeled "B". A cluster whose opposite end point is a "b", or any other integration letter, will not polish, and will be labeled "b".*

To allow the addition of more peaks when a peak or cluster is expanded in ApexTrack integration, certain processing method parameters and events are ignored during ApexTrack manual integration. These are: Start, Stop, Minimum Height, Minimum Area, Inhibit Integration, Set Minimum Height, Set Minimum Area, Set Maximum Height, and Set Maximum Width.

To prevent the adjusted baseline from piercing the signal anywhere in the cluster, ApexTrack can also break the cluster into more than one cluster. When this happens, the breakpoints are labeled "B" and are also polished. If the Allow Negative Peaks event is on, ApexTrack may add, move or delete crossing points during the adjustment of the cluster's baseline.

Unlike a Traditional result, when manually adjusting an ApexTrack result, moving the baseline of a cluster undoes all manual peak adjustments that have already been made to the cluster, i.e., adding, moving, or deleting drop lines, adding Tangential skims, deleting peaks.

**Note:** In an ApexTrack result, if all peaks in a cluster have been adjusted so that they are Traditional peaks with the I37 code, adjustment of the cluster's baseline will then follow the Traditional rules – i.e., no new peaks are added or deleted during baseline adjustment, the cluster will not break, and no polishing will occur.

When working with an ApexTrack cluster, make cluster baseline adjustments prior to making peak adjustments within the cluster. If you inadvertently adjust the baseline after making peak manual adjustments, you can undo the baseline adjustment and return to the manually modified cluster.

## **Moving, adding, or deleting drop lines**

Drop lines can be moved by clicking on the drop line marker and dragging it to the desired location. Drop lines can be deleted by Control-clicking on the drop line marker. This is true with all drop lines labeled V, S, R, or v, except when the peak on either side of the drop line contains a G in its integration type. Moved drop lines are labeled v.

Drop lines can be added to any peak without a G in its integration type by Control-clicking on the signal where the drop line is desired. Added drop lines are labeled v. In an ApexTrack result, when a drop line is added or moved, the peaks on either side of that drop line become Traditional peaks, with the I37 code. Also, when a drop line is deleted, the peak formed by the deletion becomes a Traditional peak with the I37 code. When a drop line is added, moved, or deleted inside a child skim peak, the mother peak also becomes a Traditional peak with the I37 code.

## **Adding/deleting peaks and clusters**

Peaks that are not integrated can be integrated by clicking on the signal where the peak starts and dragging to the position on the signal where the peak ends.

The open ApexTrack processing method determines how many peaks and what types of peaks are added when you drag across a region of the chromatogram. The ApexTrack algorithm determines how many 2nd derivative apices are in the chosen region and integrates a peak for each one, using any events that may be turned on in the processing method (i.e., Skims, Shoulders, etc.). When a single peak is added, it is labeled "bb"; when a cluster is added, it starts and ends with a b.

**Note:** To allow the addition of ApexTrack peaks, certain processing method parameters and events are ignored during ApexTrack manual integration. These are: Start, Stop, Minimum Height, Minimum Area, Inhibit Integration, Set Minimum Height, Set Minimum Area, Set Maximum Height, and Set Maximum Width.

Single peaks, or peaks in clusters may be deleted by right-clicking either inside the peak, or on that peak's row in the Peaks table, and choosing "delete". Tangential skim mother peaks cannot be deleted. When Tangential child peaks are deleted, their area is added back to the mother peak. When Gaussian child peaks are deleted, their area is NOT added back to the mother peak.

When peaks are deleted, they are removed from the Peaks table. No changes are made to the integration types of the remaining peaks.

*Note: In an ApexTrack result, when peaks are added, if there is no 2<sup>nd</sup> derivative apex in the chosen region of the chromatogram, the added peak is a Traditional peak, with the I37 code. If the chosen region has a 2nd derivative apex, but does not have both of the peak's inflection points, it is a Traditional peak with the I38 code. Also, when a tangent skim child peak is deleted, the mother of that peak becomes a Traditional peak with the I37 code.*

## **Adding skim peaks**

When using ApexTrack integration, Tangential skims may be manually added by clicking and dragging between two points of a peak that is already integrated.

Tangential skims may be drawn in any peaks except in other child Tangential peaks or in peaks with the GG integration type. Added Tangential skims are labeled "tt".

*Note: Often, a drop line should be deleted before a skim is added.*

*Note: In an ApexTrack result, when tangent skim peaks are manually added, the skim peak plus that skim peak's mother peak become Traditional peaks with the I37 code.*

## **Adjusting skim baselines**

The baseline of a child skim peak may be adjusted if it is a Tangential skim. Click on the baseline marker and drag it to the desired location. The adjusted endpoint is labeled "t".

*Note: In an ApexTrack result, when the baseline of a tangent skim (or cluster of tangent skims) is adjusted, the tangent skim peak (or cluster of tangent skim peaks) plus the mother peak become Traditional peaks with the I37 code.*

### 3. PROCESSING METHOD DEVELOPMENT

The ApexTrack algorithm was developed with the intention of keeping the necessity for parameter changes minimal. With this in mind, many chromatograms can be suitably integrated using all default processing method parameters.

#### 3.1 OVERVIEW

The default ApexTrack method has blank values for Start, End, Peak Width, and Detection Threshold. The default value for Liftoff % is 0.000, and for Touchdown %, 0.000. No events are in the default method.

When processing using a method with blanks for Peak Width and Detection Threshold, the Auto Peak Width and Auto Detection Threshold algorithms determine optimum values for these parameters. The numerical values for Peak Width and Detection Threshold that were used for a particular result can be seen by observing the "Result Peak Width" and "Result Threshold" boxes in the Main Window of Review.

In most cases, when optimizing an ApexTrack method for a particular method, the parameters should be altered in the following order. Steps 1-3 determine which peaks are integrated; Step 4 fine tunes the start/end points of peaks that are already integrated. For many chromatograms, only steps 1 and 2 need to be addressed.

1. Start and End times
2. Detection Threshold
3. Peak Width
4. Liftoff % and Touchdown %

#### 3.2 ADDING START AND END TIMES TO THE APEXTRACK DEFAULT METHOD

Using the default ApexTrack parameters after entering an appropriate Start and End time usually integrates the desired peaks in a chromatogram. It is important to enter the Start and End times in the default method to define the region of the chromatogram to be integrated, since the Auto-Peak Width and AutoThreshold algorithms calculate the optimum values based on the region between the Start and End times.

Figures 3-1 shows a chromatogram integrated with the ApexTrack default parameters, without (Figures 3-1A) and with (Figures 3-1B) entering Start and End Times of 2 and 8, respectively. After entering appropriate Start and End times, the chromatogram is integrated correctly with the remaining default parameters.

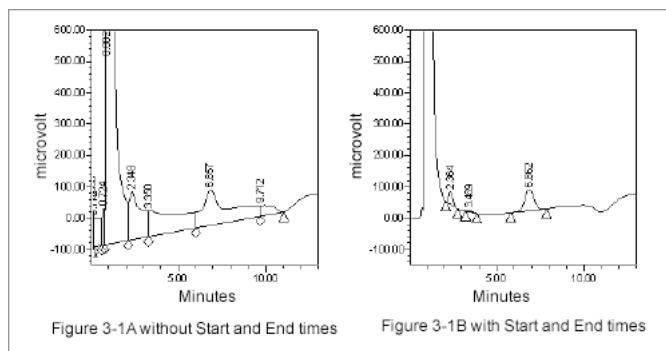


Figure 3-1. Chromatogram integrated with ApexTrack default parameters.

*Note: When choosing Start and End times, include a section of baseline in the region chosen to integrate. The ApexTrack algorithm uses a section of baseline when determining the Auto-Peak Width and AutoThreshold values.*

#### 3.3 CHANGING THE DEFAULT DETECTION THRESHOLD VALUE

Sometimes, when using the default parameters with appropriate Start and End times, the peak(s) of interest may not be integrated. Or, more commonly, they may be integrated, but several other undesired peaks are also integrated. The best way to change the number of peaks integrated is to change the Detection Threshold.

The example in Figures 3-2A shows a chromatogram integrated with the default ApexTrack parameters, with start time = 0.9 and end time = 5.5 minutes. The four main peaks are integrated, along with many minor peaks. The Main window in Review shows that the Detection Threshold that ApexTrack used was 0.980. Increasing the Detection Threshold will remove many of the smaller peaks. Figures 3-2B shows the result when the Detection Threshold was changed from a blank to 50 (Peak Width was left blank). Only the four main peaks are integrated. If the Detection Threshold is increased too much, the desired peak(s) may be not be integrated. In Figures 3-2C, the same data is seen, with the Detection Threshold set to 5000. Now, the last main peak (because it is the smallest) is not integrated. It is possible to increase the Detection Threshold so that no peaks are integrated.

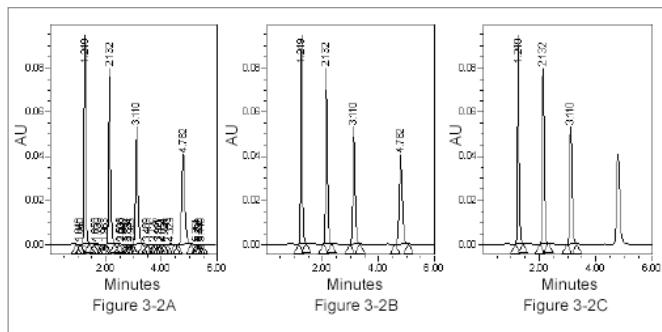


Figure 3-2. Effect of Increasing the Detection Threshold value.

To prevent small peaks from being integrated, it is usually better to raise the Detection Threshold than to add a Minimum Area or Minimum Height value to the method, as shown in Figures 3-3. Figures 3-3A shows a chromatogram integrated with the ApexTrack default parameters. In Figures 3-3B, the Detection Threshold was increased to eliminate the small peaks; in Figures 3-3C, a Minimum Area value was added to eliminate the small peaks.

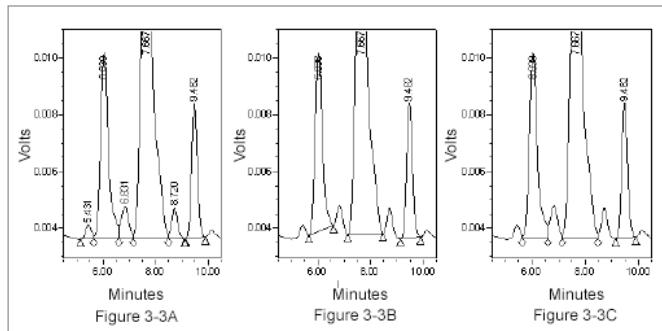


Figure 3-3. Eliminating Small Peaks using Detection Threshold (B) or Minimum Area (C).

The reverse issue is seen in Figures 3-4A where the small desired peak at ~9.5 minutes was not integrated with the default ApexTrack parameters, after entering Start and End times of 8 and 12 minutes. In Review, it is observed that the ApexTrack algorithm used a value of 32999.998 for Detection Threshold. Entering a lower value of 1000 gives the desired integration, in Figures 3-4B.

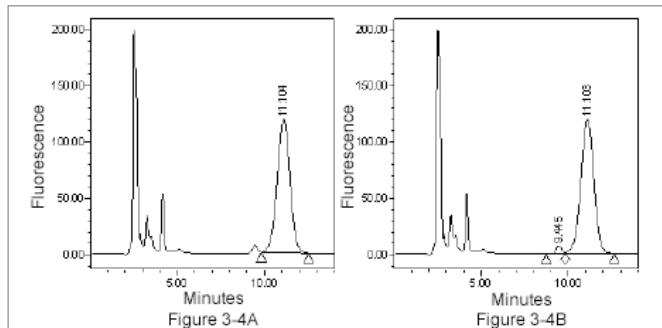


Figure 3-4. Lowering Detection Threshold to Integrate a Small Peak of Interest.

To summarize, the best way to set ApexTrack parameters for many chromatograms is to enter a Start and End time, integrate to see the results, and adjust the Detection Threshold to either add peaks that were not integrated, or remove peaks that are not desired. Since the value for Detection Threshold is a height threshold on the 2nd derivative chromatogram, the ApexTrack algorithm is simply integrating (or not integrating) peaks whose 2nd derivative apex is above (or below) this value.

*Hint: If you set the Detection Threshold to 0, ApexTrack will integrate every peak that has a second derivative apex. If the desired peak is not integrated using a Detection Threshold of zero, then either the Start and/or End times are set incorrectly, or the Peak Width value must be changed.*

### 3.4 CHANGING THE DEFAULT PEAK WIDTH VALUE

Changing the Peak Width should be the last resort to get your peaks of interest integrated. Using the Auto Peak Width (blank setting) for Peak Width (along with appropriate Start and End times) should automatically set an appropriate Peak Width.

Figures 3-5 is a chromatogram with varying peak widths. Using appropriate start and end times, a blank Peak Width and Detection Threshold gives good integration for both peaks, even though they have varying widths.

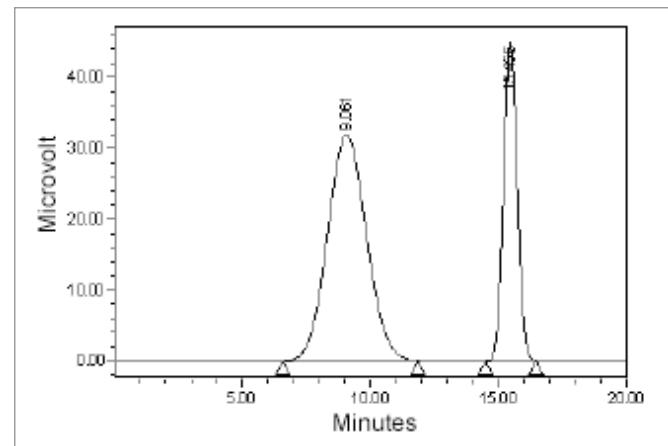


Figure 3-5. Chromatogram with Varying Peak Widths.

Occasionally, ApexTrack will not calculate the optimum Peak Width when the value is blank. An example is in Figures 3-6A, in which the small peaks starting at ~14 minutes are to be integrated. This has Start and End times of 10 and 30 entered. In Review, it can be seen that ApexTrack has used a value of 155.02 for the Peak Width. Eyeballing the chromatogram, it is obvious that our peaks of interest have much smaller widths. Entering a value of 80 for the Peak Width gives the desired integration in Figures 3-6B.

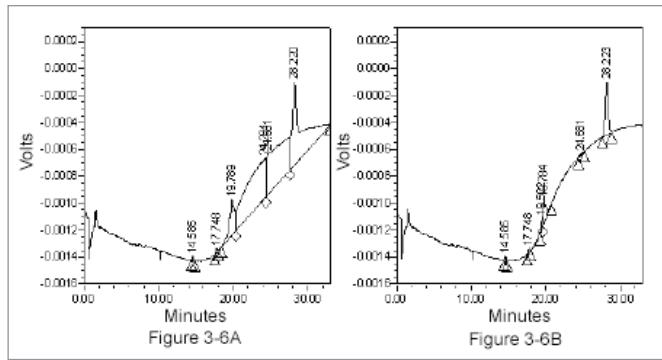


Figure 3-6. Effect of changing the Peak Width.

**Note:** When changing Peak Width, it is recommended that you remain within a factor of two of the automatically determined Peak Width. This is further explained in Section 3.9, Using System Suitability with ApexTrack.

### 3.5 CHANGING VALUES FOR LIFTOFF % AND TOUCHDOWN %

The Liftoff % and Touchdown % parameters are only changed after the Start, End, Peak Width, and Detection Threshold parameters have been optimized to find and integrate the desired peaks, and are used to fine-tune the start and/or stop times of peaks. Because of the unique aspect of ApexTrack integration that peak detection is decoupled from baseline determination, altering the Liftoff % and Touchdown % parameters will only affect the baseline starts and ends of peaks that are already integrated. When Liftoff % and/or Touchdown % are changed, they will never cause more or fewer peaks to be integrated (with the potential exception of "hidden" shoulder peaks, as explained below).

**Note:** If the desired peaks are integrated and their start and stop times are significantly incorrect, recheck that all other parameters are correctly optimized before changing Liftoff % and Touchdown %.

Figures 3-7A through Figures 3-7D show a peak where the Liftoff % was changed from 0, to 20, to 50, and to 100. The start point of the peak is moved up the peak, and the endpoint is adjusted so that the baseline does not pierce the peak. Corresponding chromatograms in which the endpoint would move up the peak would be obtained when changing only the Touchdown %.

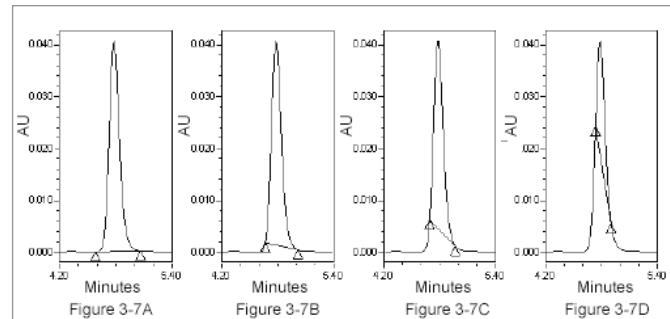


Figure 3-7. Effect of Varying the Liftoff % Value.

Both the Liftoff % and Touchdown % can be altered, moving the baseline up the peak. The maximum value for Liftoff % and Touchdown % (in all methods except GPC-type methods) is 100%. When these are set to 100%, the start and end of the peak are both set to the data point closest to the peak's true inflection points. The chromatogram in Figures 3-8 has both Liftoff % and Touchdown % set to 100%.

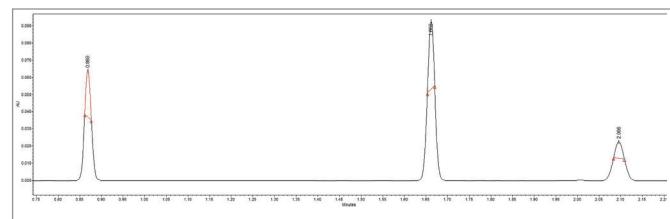


Figure 3-8. Liftoff % and Touchdown % Set to 100.

Figures 3-9 illustrates how an additional peak may be found when the Liftoff % and Touchdown % values are changed. In Figures 3-9A, default Liftoff % and Touchdown % values are used, and three peaks are integrated. In Figures 3-9B, both Liftoff % and Touchdown % were set to 100% and now four peaks are integrated. This is because there was a "hidden" shoulder peak that was not seen in Figures 3-9A, since the Detect Shoulders event was not on. In Figures 3-9C, returning to the parameters of Figures 3-9A and adding the Detect Shoulders event shows that there are, indeed, four integrated peaks in this region.

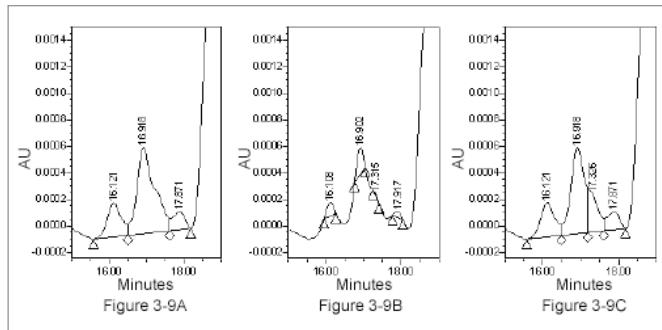


Figure 3-9. Changing Liftoff % and Touchdown % Values.

Increasing the Liftoff % and Touchdown % values can cause integrated peak clusters to break; however, if this is desired, it is better to use the "Valley-to-Valley" event to break the cluster. Figures 3-10A shows a chromatogram with default Liftoff % and Touchdown % parameters; in Figures 3-10B the Touchdown % was set to 20% to break the cluster; in Figures 3-10C, Touchdown % was returned to the default value and a Valley to Valley event was added. Figures 3-10C has better start and end points for the broken cluster than Figures 3-10B.

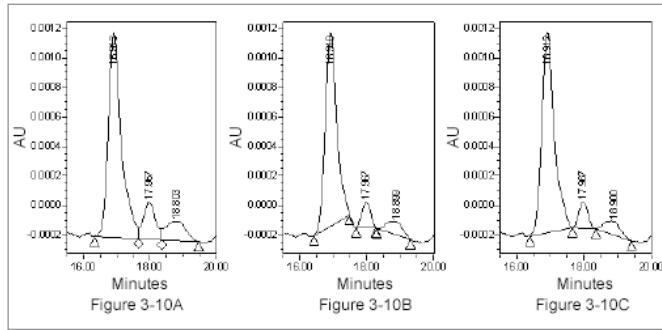


Figure 3-10. Examples of Breaking a Peak Cluster.

## 3.6 USING THE ALLOW NEGATIVE PEAKS TIMED EVENT

The "Allow Negative Peaks" event in ApexTrack should only be used when negative peaks are actually present in the chromatogram and are desired to be integrated. The event's start and end times should be set to include the negative peaks of interest.

Figures 3-11A shows the integration obtained using the default values (with Start and End set to 15 and 40 minutes respectively) in an ApexTrack GPC-type method and an "Allow Negative Peaks" event starting at 0 minutes. A few small negative peaks are seen in the noise before the first large positive peak. In Figures 3-11B, setting the event to start at 30 minutes prevents the small negative noise peaks from being integrated.

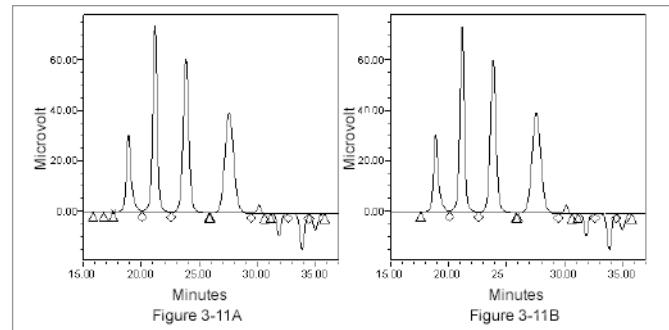


Figure 3-11. Integrating Negative Peaks.

## 3.7 USING THE DETECT SHOULDERS TIMED EVENT

When the "Detect Shoulders" event is added, Shoulder and/or Round drop lines may be added to the chromatogram in the region defined by the event. This event does not change the integration in any other way. Figures 3-12A and Figures 3-12B show a chromatogram before and after a "Detect Shoulders" event was added. In Figures 3-12B, both a Shoulder and a Round drop line were added (at 20.140 and 23.117 minutes respectively).

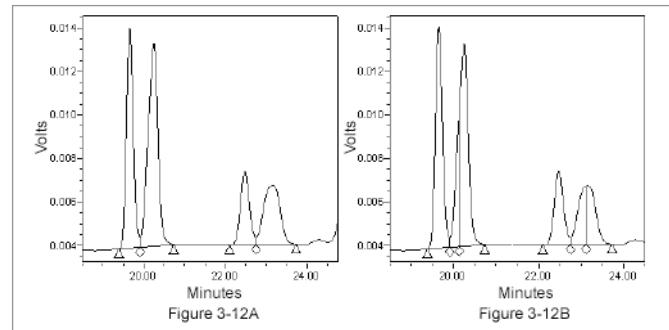


Figure 3-12. Integrating Shoulder and Round Peaks.

## 3.8 USING THE GAUSSIAN SKIM TIMED EVENT

When the "Gaussian Skim" event is added, Valley and/or Shoulder drop lines already present in the chromatogram may change into skim profiles (in the region defined by the event). If present, Round drop lines will never change to Gaussian skim profiles. If a Valley or Shoulder drop line does not change into a Gaussian Skim profile, one or more peak processing codes (I26 – I36) are added to provide an explanation. No "value" is needed when using an ApexTrack Gaussian Skim event. The ApexTrack Gaussian Skim algorithm draws all skims that are possible in the region identified by the start and stop times of the event. If certain skims are not desired, they can be omitted by changing the event's start and/or stop time.

Figures 3-13A and Figures 3-13B show a chromatogram before and after the Gaussian Skim event is added. In Figures 3-13B, when the event is added, the Valley drop line at 7.905 minutes changes to a Gaussian Skim; the Valley drop line at 10.238 minutes remains (processing code I26 is seen because the valley height between these two peaks was too low, so it does not skim).

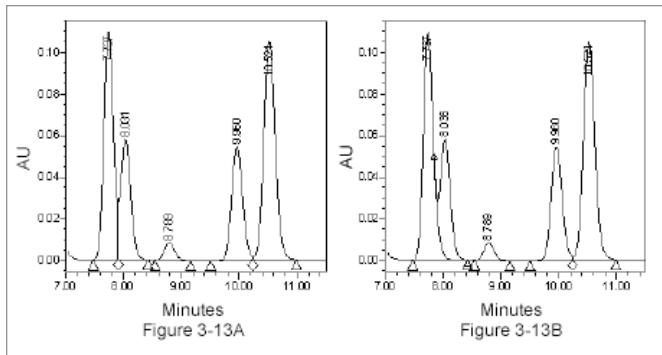


Figure 3-13. Example of Gaussian Skimming and Processing Code I26.

In Figures 3-14A, there are several Valley drop lines, a Round drop line at 20.051 minutes, and two Shoulder drop lines at 20.381 and 23.079 minutes. In Figures 3-14B, a Gaussian Skim event is added across the entire region and all Valley drop lines change to Gaussian Skim drop lines. The Shoulder drop line at 20.381 minutes also changes to a Gaussian Skim drop line, but the Shoulder at 23.079 minutes does not change and has the processing code I36 (no suitable Gaussian Skim profile could be drawn). The Round drop line does not change.

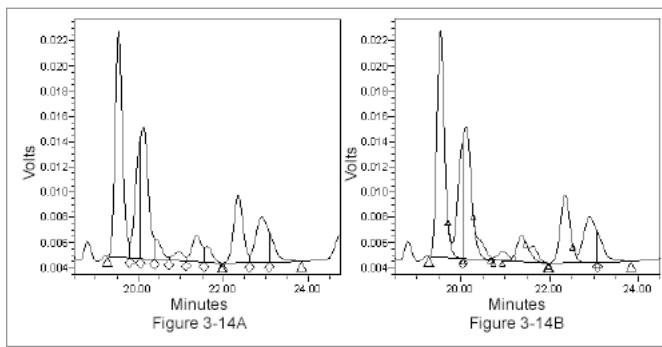


Figure 3-13 Example of Gaussian Skimming and Processing Code I26.

## 3.9 USING SYSTEM SUITABILITY WITH APEXTRACK

If your ApexTrack processing method includes System Suitability calculations involving "Width@Tangent" (such as Resolution and Plate Count), the following should be noted.

When a chromatogram is integrated using the ApexTrack algorithm, each peak's "Width@Tangent" value is calculated using the tangents at the peak's true inflection points, as defined by the ApexTrack algorithm. If you have manually entered an incorrect value for Peak Width into an ApexTrack method, the algorithm will not correctly determine the peak's inflection points, and therefore will not calculate "Width@Tangent" appropriately.

*Note: This differs from Traditional methods in that the "Width@Tangent" is calculated based on a percentage value entered into the processing method.*

Figures 3-15A through Figures 3-15C show a peak integrated with ApexTrack using a blank Peak Width (which is seen to be 9.79), a Peak Width of 1.0, and a Peak Width of 50. In each example, the Liftoff % and Touchdown % have been set to 100%, so that the baseline is drawn at the data points closest to the peaks' inflection points, as ApexTrack has defined them. In Figures 3-15A, the inflection points are where they would be expected to be (at ~61% of peak height in a Gaussian peak). In Figures 3-15B and Figures 3-15C, the inflection points are incorrect because an incorrect Peak Width value was used. When inflection points are incorrect, the value for Width@Tangent will also be incorrect, as will all fields calculated using the Width@Tangent value.

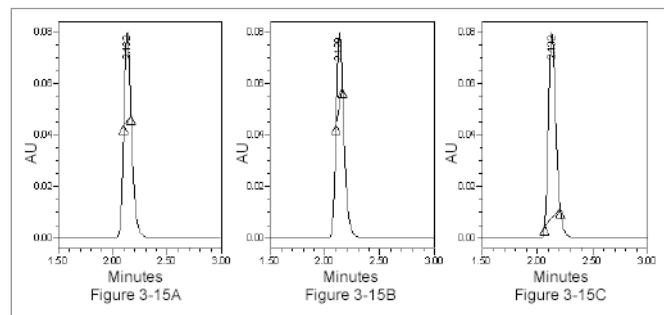


Figure 3-15. Location of the Inflection Points with Different Peak Width Values.

To warn of serious departures from the correct Peak Width, when processing in Review, the following message is seen when the entered Peak Width differs from the automatically calculated Peak Width by more than a factor of 2 in either direction. After clicking "OK", the entered value for Peak Width will be used for integration even if it differs by more than a factor of 2. A result code of I39 is added to the result. When results are created by background processing, the warning is not seen, but result code I39 is added to the result.



Figure 3-16.  
Warning message.

## 4. METHOD VALIDATION

When using an ApexTrack processing method (or any other type of processing method) in a validated environment, it is necessary to validate the method. The procedure to validate an ApexTrack processing method is no different than that for any other integration method. If you are transferring a method from Traditional to ApexTrack integration it may only be necessary to do a cross validation study.

Note: If you are currently using Traditional integration and are satisfied with the performance, Waters does not recommend automatically switching to an ApexTrack method, mainly due to the time involved in validating a new method. If, however, the Traditional method involves time-consuming manual manipulations or does not integrate peaks as desired, it may be worthwhile to assess your data using an ApexTrack method. If ApexTrack integration outperforms Traditional integration, or saves time in any way, this benefit may outweigh the time it takes to validate a new method.

### 4.1 VALIDATION

In order to validate a method, it is required to perform specific tests and, in general, determine that the reliability of the method is within an acceptable range.

The following parameters must be tested:

- Linearity
- Range
- Accuracy
- Precision
- Specificity
- Limit of Quantitation
- Limit of Detection
- Robustness

For complete information on method validation, see Chapter 1225 of the US Pharmacopoeia (USP 24) or [www.fda.gov/cder/guidance](http://www.fda.gov/cder/guidance). (The USP also includes calculations for EP and JP.)

### 4.2 CROSS VALIDATION

If you have previously validated a method and then convert to ApexTrack (or to any other integration method), you may be able cross validate the method and avoid the complete validation procedure. A cross validation study involves comparing results produced using both the old and new methods and ensuring that the results of the new method are the same as those of the old method, within an acceptable margin of experimental error.

If the results obtained using the new method are not within an acceptable margin of error, it then becomes necessary to fully validate the new method.

## 5. TROUBLESHOOTING

### 5.1 PROBLEMS SPECIFIC TO APEXTRACK INTEGRATION

Symptom	Problem	Resolution
Either too many or too few peaks are detected due to an improper Detection Threshold value when AutoThreshold was used.	ApexTrack requires a region of baseline noise in the chromatogram that is at least one peak width wide in order to set the Detection Threshold parameter. If this criteria is not met, an inaccurate Detection Threshold value may be determined.	In Review, zoom in on a region of the chromatogram that contains a representative section of the baseline.
Too few peaks of interest are detected, and it has been determined that the Detection Threshold value used is appropriate.	The Peak Width value (set via Auto-Peak Width) is too high. The Auto-Peak Width routine, which uses the largest peak in the second derivative chromatogram, used a saturated peak or a round peak to determine the peak width value.	In Review, zoom in on a region of the chromatogram that contains representative peaks of interest but does not contain the saturated peak and then set the Peak Width using the Set Processing Method Peak Width button. Alternatively, manually set the Peak Width value.
No Detection Threshold value was obtained when using AutoThreshold. The following message appears: Cannot calculate Threshold, region has less than 20 data points.	Less than 20 data points were present in the region in which AutoThreshold was being set.	<p>Depending on your data, one of the following may resolve the problem:</p> <ol style="list-style-type: none"> <li>1. In Review, zoom in on a region of the chromatogram that contains sufficient baseline and then set the Detection Threshold using the Set Processing Method Threshold button.</li> <li>2. Change the Start and End parameters or the Inhibit Integration regions in your processing method and determine the Detection Threshold again.</li> <li>3. Acquire data at a higher data rate.</li> <li>4. Extend your run time so that there is sufficient baseline at the end of the chromatogram for the algorithm to use when setting this parameter.</li> <li>5. Manually set this parameter.</li> </ol>
Either too many or too few peaks are detected due to an incorrect Peak Width value.	A minimum signal-to-noise of 10:1 is required in order for the Auto-Peak Width algorithm to work properly. Otherwise, the resulting peak width may be that of a noise artifact in the chromatogram.	<ol style="list-style-type: none"> <li>1. Optimize your sample or your chromatographic conditions to yield signal-to-noise greater than 10:1.</li> <li>2. Manually set this parameter.</li> </ol>
Auto-Peak Width yields a Detection Threshold value of 0.00.	This can occur when a region of the chromatogram contains only peaks and no baseline. Although this value appears odd, a Detection Threshold value of 0.0 results in the detection of all second derivative apices. Hence, this value gives correct results for such crowded regions.	None required. If too many peaks are detected, zoom in on the baseline and set the Detection Threshold to the height of the smallest peak.
A peak(s) is not detected, regardless of the integration parameters used.	To produce a result, ApexTrack needs a minimum of 5 points per peak (at 5% peak height).	Acquire the data at a data rate sufficient to produce a minimum of 5 data points per peak at 5% peak height. The optimal value is 15 points per peak at 5% peak height.

**APPENDIX A****QUICK REFERENCE: DIFFERENCES BETWEEN TRADITIONAL AND APEXTRACK INTEGRATION**

This appendix summarizes key differences between ApexTrack and Traditional processing.

**A.1 PROCESSING METHOD PARAMETERS THAT DIFFER BETWEEN TRADITIONAL AND APEXTRACK INTEGRATION**

Parameter	Traditional	ApexTrack
Peak Width Parameter	Used to average the data in bunches during peak detection. Measured in seconds.	Used to smooth the data (Modified Savitsky Golay) during peak detection. Measured in seconds.
Auto-Peak Width	Measures the peak width at 1% peak height or 6 standard deviations.	Measures the peak width at 5% peak height or 4.9 standard deviations.
Threshold Parameter	Used as a slope threshold to determine which peaks are detected as well as their liftoff and touchdown points. Measured in $\mu$ volt/s/seconds.	Called Detection Threshold. Used as a threshold to determine which peaks are detected. Measured in microvolts.
AutoThreshold	Measures an average baseline noise in $\mu$ volt/s/seconds.	Measures an average baseline noise in microvolts.
Liftoff %, Touchdown %	N/A	Used to determine baseline placement.
Start and End Parameters	N/A	Used to define the range of the chromatogram where integration occurs. Functions effectively as an Inhibit Integration event.

**A.2 INTEGRATION TYPES**

The following table lists the integration types that are found with Traditional and ApexTrack integration. Capital letters indicate automatic integration. Lower case letters indicate manual integration.

B = Baseline

V = Valley

T = Tangential Skim

E = Exponential Skim

G = Gaussian Skim X = Crossing Point R = Round

S = Shoulder

For more information regarding integration types see the Empower Online Information System.

	Traditional Integration Int Types	ApexTrack Integration Int Types
Traditional Integration	BVTE	BVRXSGT
Manual Integration	bvte	bvt

## [RESOURCE GUIDE]

### A.3 INTEGRATION RESULTS THAT DIFFER BETWEEN TRADITIONAL AND APEXTRACK INTEGRATION

The following table lists result fields that are calculated differently between Traditional and ApexTrack integration under specific conditions.

Result	Traditional	ApexTrack
Retention Time (if both boundaries are any combination of B, V, or X)	5-point quadratic fit (an interpolated value, (if the Use v3.0X Style Peak Width and Threshold Determination system policy is enabled) or highest data point above the integrated baseline (if the Use v3.0X Style Peak Width and Threshold Determination system policy is not enabled).	5-pt quadratic fit if 4 or more data points are present within the inflection point width. 3-pt quadratic fit if fewer than 4 data points are present within the inflection point width. (An interpolated value.)
Retention Time (if either (or both) boundaries are R)	N/A	Time of the second derivative apex. (An interpolated value.)
Retention Time (if either (or both) boundaries are S)	N/A	Time of one of the following: 5-pt quadratic fit if 4 or more data points are present within the inflection point width. <ul style="list-style-type: none"><li>■ 3-pt quadratic fit if fewer than 4 data points are present within the inflection point width.</li><li>■ Time of the second derivative apex if the peak is the child peak.</li></ul>
Height	Height above baseline at the Retention Time (baseline corrected, if the Use v3.0X Style Peak Width and Threshold Determination system policy is enabled) or the highest data point in the peak (if the Use v3.0X Style Peak Width and Threshold Determination system policy is not enabled).	Height at the Retention Time (baseline corrected).
Width	Number of points between peak boundaries, measured in seconds and scaled by the data rate.	Number of points between peak boundaries, measured in seconds and scaled by the data rate.
Inflection Width	N/A	The width, measured in seconds, between inflection points.

### A.4 PEAK BOUNDARIES

Boundaries	Int Type	Location (Traditional)	Location (ApexTrack)
Baseline Start	B, b	Snapped to closest data point	Snapped to closest data point
Baseline Stop	B, b	Snapped to closest data point	Snapped to closest data point
Valley Boundary	V, v	Snapped to closest data point	Snapped to closest data point
Shoulder Boundary	S	N/A	Snapped to closest data point
Round Boundary	R	N/A	Snapped to closest data point
Crossingboundary	X	N/A	Interpolated
Gaussian Skim Boundary	G	N/A	Snapped to closest data point
Tangential Skim Boundary	T, t	Snapped to closest data point	Snapped to closest data point
Exponential Skim Boundary	E, e	Snapped to closest data point	N/A

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Waters Corporation  
34 Maple Street  
Milford, MA 01757 U.S.A.  
T: 1 508 478 2000  
F: 1 508 872 1990  
[www.waters.com](http://www.waters.com)