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A4, Empower3
Processing Tips and Tricks

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- Processing Workflow
- Integration Theory
  - Traditional Integration Algorithm
  - ApexTrack Algorithm
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- Break
- System Suitability & Noise and Drift Calculations
- Managing Manually Adjusted Results
- Exercise 2

Basic Chromatography Workflow

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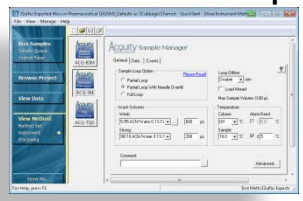
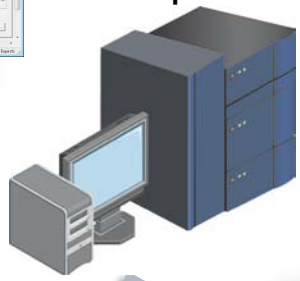
Sampling/Login

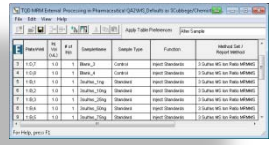
Method Setup



Sequence/Run Samples

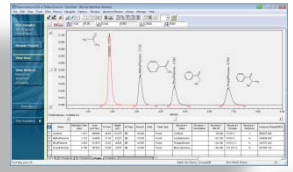


Process Data

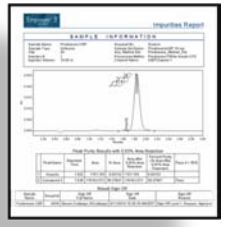


Empower Database

Review/Preview



Print Report



Processing Workflow

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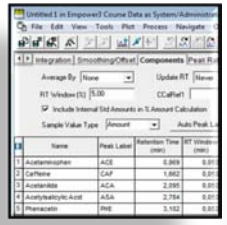
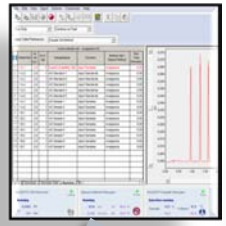
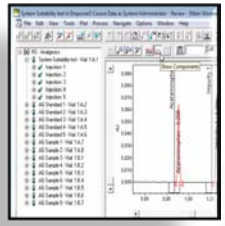
Create Processing Method



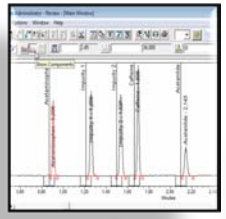
Run Sample Set



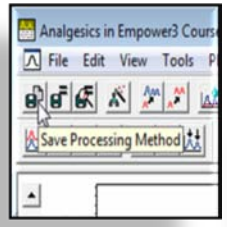
Bring Sample Set To review



Check/ Adjust Method



Save the Changes



Process Sample Set

Processing Workflow (continued)

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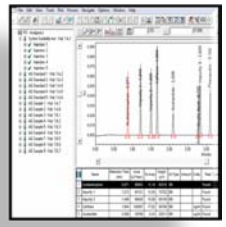
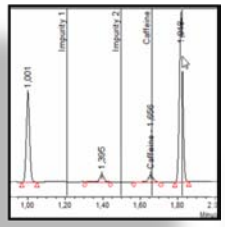
Bring Results Set To review

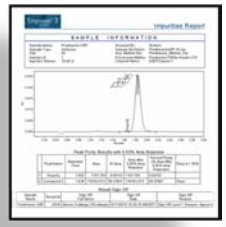


Adjust Integration/ Identification



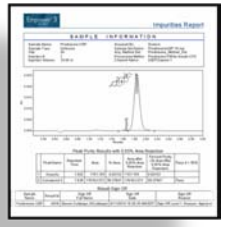
Save Changes

Preview Results Set



Sign Off Results

Print Results Set



Creating a Processing Method

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- Optimizing Integration
- Component table
- System Suitability Calculations
- Noise & Drift Calculations

Integration

- Integration requires three operations:
  1. Find the peak (peak detection)
  2. Find the baseline of the peak
  3. Compute the peak's area and height
- The first two are the challenge
- Empower has two different algorithms to perform integration
  - Traditional
  - Apex Track

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Traditional Integration

- Peak Width and Threshold work together to detect the peaks from the baseline.

4 Global Parameters

- Peak Width
- Threshold
- Minimum Area
- Minimum Height

Traditional Integration
Peak Width Determination

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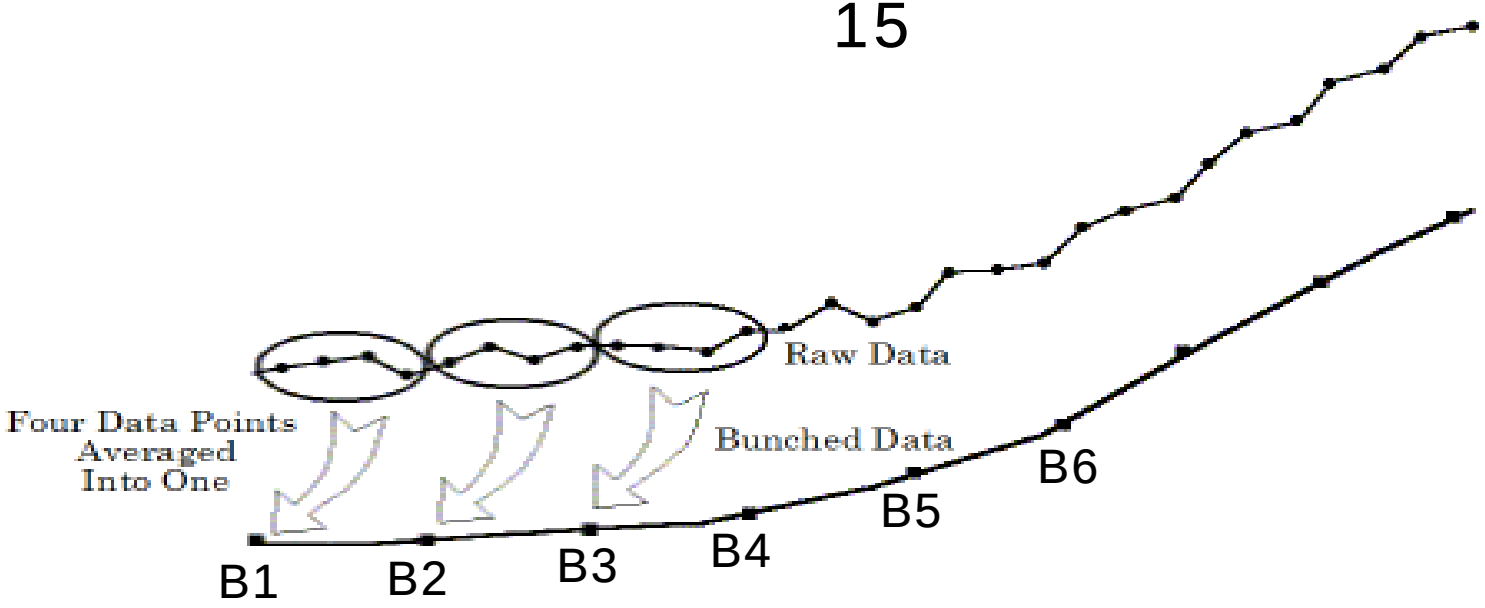
Peak Width

- Peak width is measured at the baseline of the narrowest peak of interest and is used to determine a bunching factor.

- $$\text{Bunching Factor} = \frac{\text{Peak Width} \times \text{Sampling Rate}}{15} = \frac{60 \times 1}{15} = 4$$



Traditional Integration
Determining peak start

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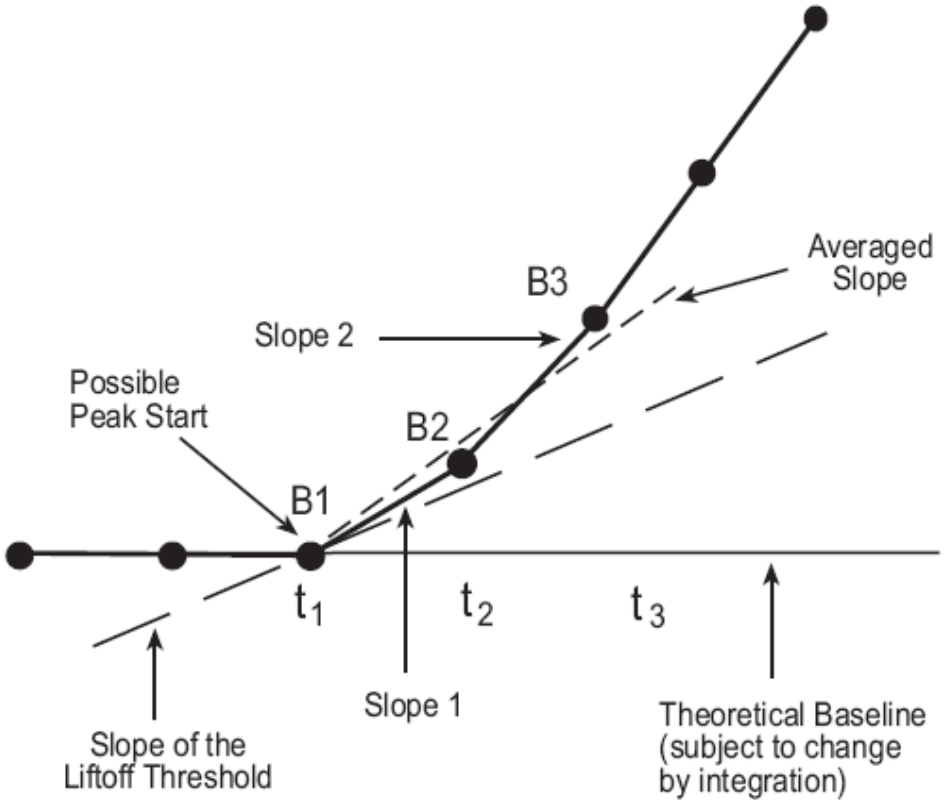
Threshold

- Specifies the liftoff and touchdown values (minimum rate of change of the detector signal) for peak detection.
- Empower averages the signal slope across 3 data bunch intervals and compares to the liftoff threshold
- When the average slope of the signal between the 3 bunches is ≥ the liftoff threshold value, point B1 is flagged as possible peak start
- Individual points in bunch B1 is then examined to determine peak start = data point with lowest Y-value



$$\text{slope}\,1 = \frac{B2 - B1}{t_2 - t_1} \qquad \text{slope}\,2 = \frac{B3 - B2}{t_3 - t_2} \qquad \text{average slope} = \frac{\text{slope}\,1 + \text{slope}\,2}{2}$$

Traditional Integration
Determining peak apex

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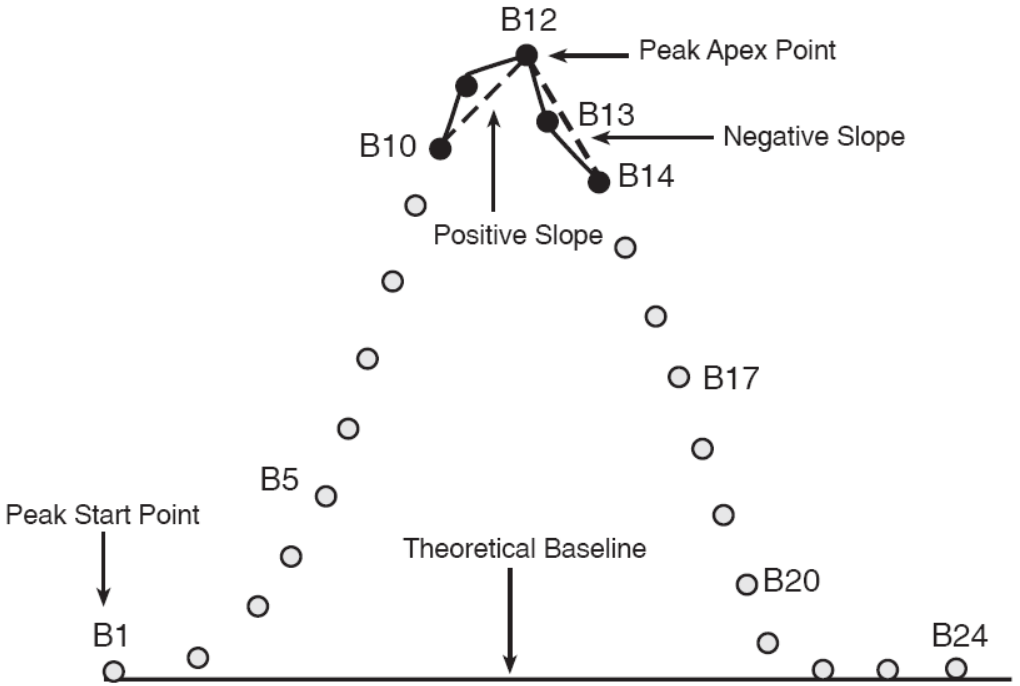
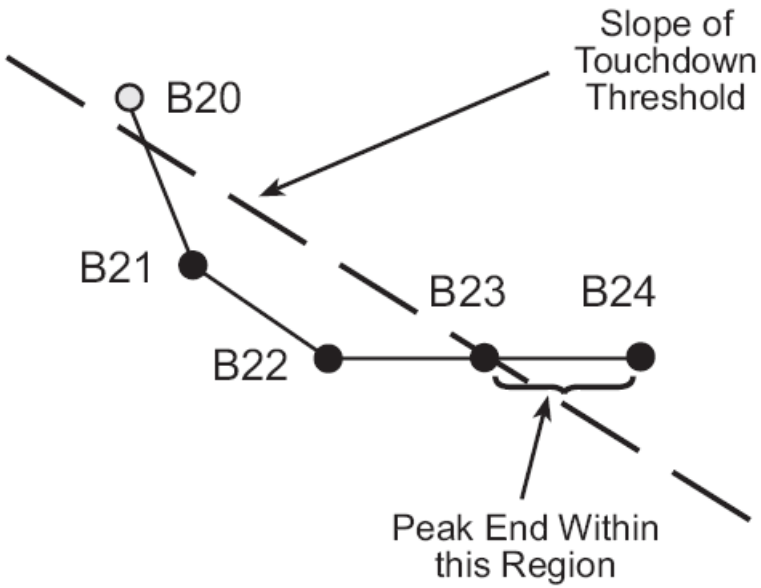
- Signal is monitored until slope sign changes from positive to negative
- Bunch where the slope change occurs (B12 in the figure) is analyzed.
- Data point which is farthest away from the baseline is tentatively assigned as peak apex
- Final apex is determined after integration and baseline assignment



Traditional Integration Determining peak end

- Slope of the signal is compared to the touchdown threshold
- When 2 consecutive slopes are < threshold, last point in the last bunch is flagged as possible peak end
- Individual points in this bunch and the next bunch to determine actual peak end = data point with lowest Y-value



Traditional Integration
Minimum Height or Minimum Area

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Minimum Height or Minimum Area

- Defines minimum peak area (mV*sec) or minimum peak height (µV) that Empower will report
- Used to reject unwanted peaks once integration has been optimized
- Empower use 95% of the peak's area/ height so that it can report peaks that approach the selected peak's size

Traditional Integration
Timed Events Parameters

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Timed Events

- a **time-based action** to adjust peak detection and/or integration in specified sections of a chromatogram
- There are 20 integration events that can be used to fine-tune integration across selected regions of a chromatogram
- You might need to apply one or more timed events when the default peak detection and integration parameters do not adequately detect and integrate all peaks in the chromatogram.

Traditional Integration Timed Events

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- II – Inhibit Integration
- SPW – Set Peak Width
- SLO – Set Liftoff
- STD – Set Touchdown
- SMA – Set Minimum Area
- SMH – Set Minimum Height
- SMxA – Set Maximum Area
- SMxH – Set Maximum Height
- VV – Valley to Valley
- ES – Exponential Skim
- TS – Tangential Skim
- ANP – Allow Negative Peaks
- FDL – Force Drop Line
- FBT – Force Baseline by Time
- FBP – Force Baseline by Peak
- FHP – Forward Horizontal by Peak
- FHT – Forward Horizontal by Time
- RHP – Reverse Horizontal by Peak
- RHT – Reverse Horizontal by Time
- FP – Force Peak

ApexTrack Integration

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A New Approach to the Integration of Chromatographic Peaks

- Easier than traditional integration
- Better than traditional integration
- Based on measuring the curvature (the rate of change of slope) of the chromatogram (2nd derivative)
- Traditional integration detects peaks by initially looking for a peak start
- ApexTrack integration detects peaks by initially looking for the peak apex

ApexTrack Integration

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Easier:

- Automatically determines appropriate integration parameter settings
  - Auto Peak Width
  - Auto Threshold
- Usually integrates well at first pass using default and automatic parameters

Better:

- Integrates negative peaks effectively
- Integrates small peaks in noisy or drifting baseline effectively
- Peak shoulders are easily detected
- Gaussian skimming available

Basis of ApexTrack: Curvature Threshold

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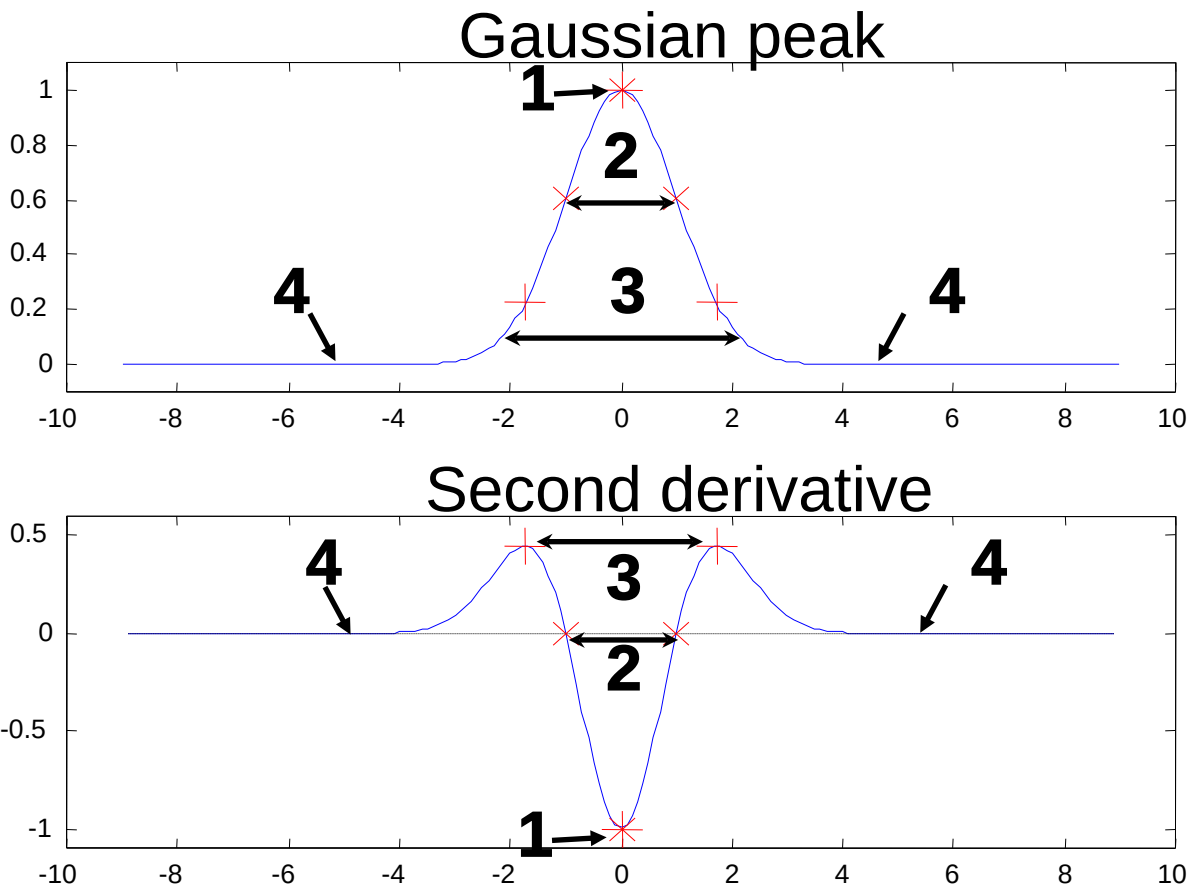
- Detects the peak apex when the *curvature* is above the threshold
- Effective:
  - Detects shoulders
  - Baseline slope does not affect detection of peaks
  - Peak detection and baseline determination are decoupled
    - Baseline placement can be modified without affecting the number of peaks detected and vise versa

Second Derivative Measures Curvature



	Curvature
1. Apex	High (-)
2. Inflection points	Zero
3. Liftoff / TD	High (+)
4. Baseline	Zero

Apex Track Integration

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Apex detection parameters

- Start (min) (Start Detection/Integration Time)
- End (min) (End Detection/Integration Time)
- Peak Width (sec) (Peak Width @ 5% Height)—**AUTO**
  - Recommended range= 0.5 to 2 times Auto PW value
- Detection Threshold (Peak Detection Threshold)—**AUTO**

Baseline determination parameters

- Liftoff %
  - Baseline start threshold %. Default:0
- Touchdown %
  - Baseline end threshold %. Default:0.5

Peak acceptance criteria

- Minimum Area (works in the same way as in traditional int.)
- Minimum Height (works in the same way as in traditional int.)

ApexTrack Peak Detection

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- Peak detection is controlled by the Peak Width and Threshold parameters
- Peak Width: measured in seconds, Auto Peak width sets it to 5% height of the largest peak in the second derivative (determined by using the inflection point width and calculating the gaussian peak width); used as a filter similar to traditional integration.
- Threshold: measured in units of height, Auto Threshold sets it to the peak to peak noise; used as a threshold for peak detection in the 2nd derivative

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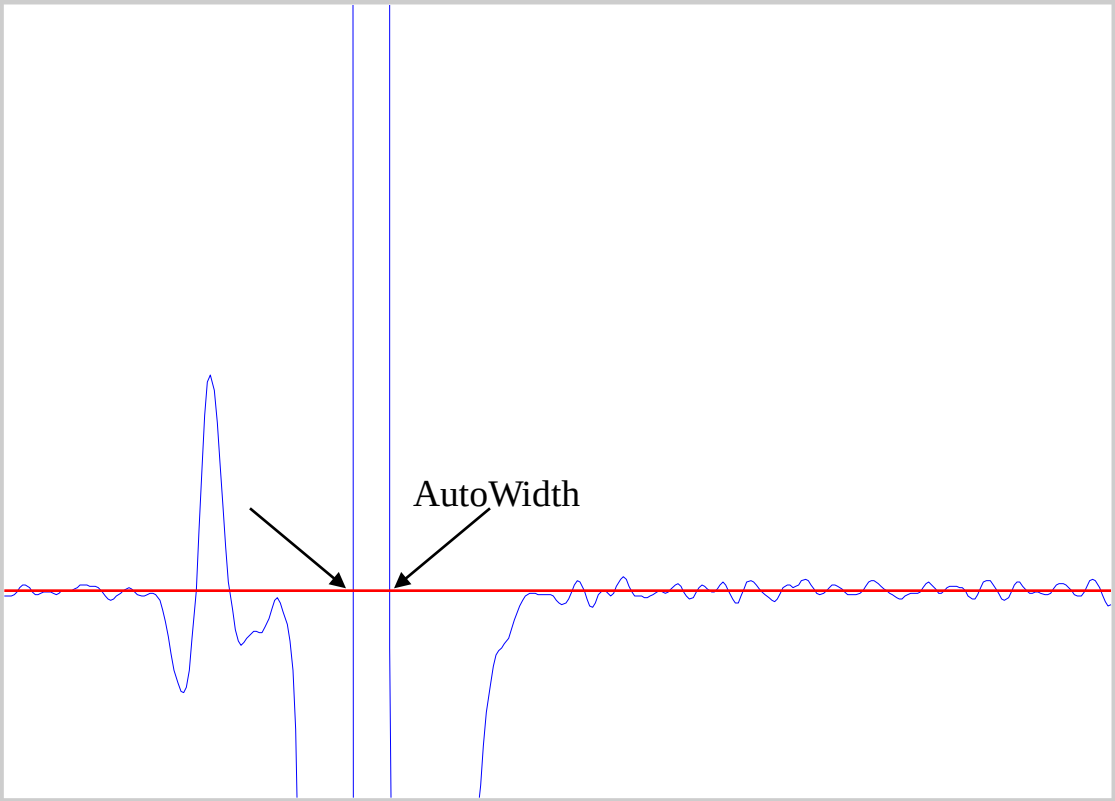
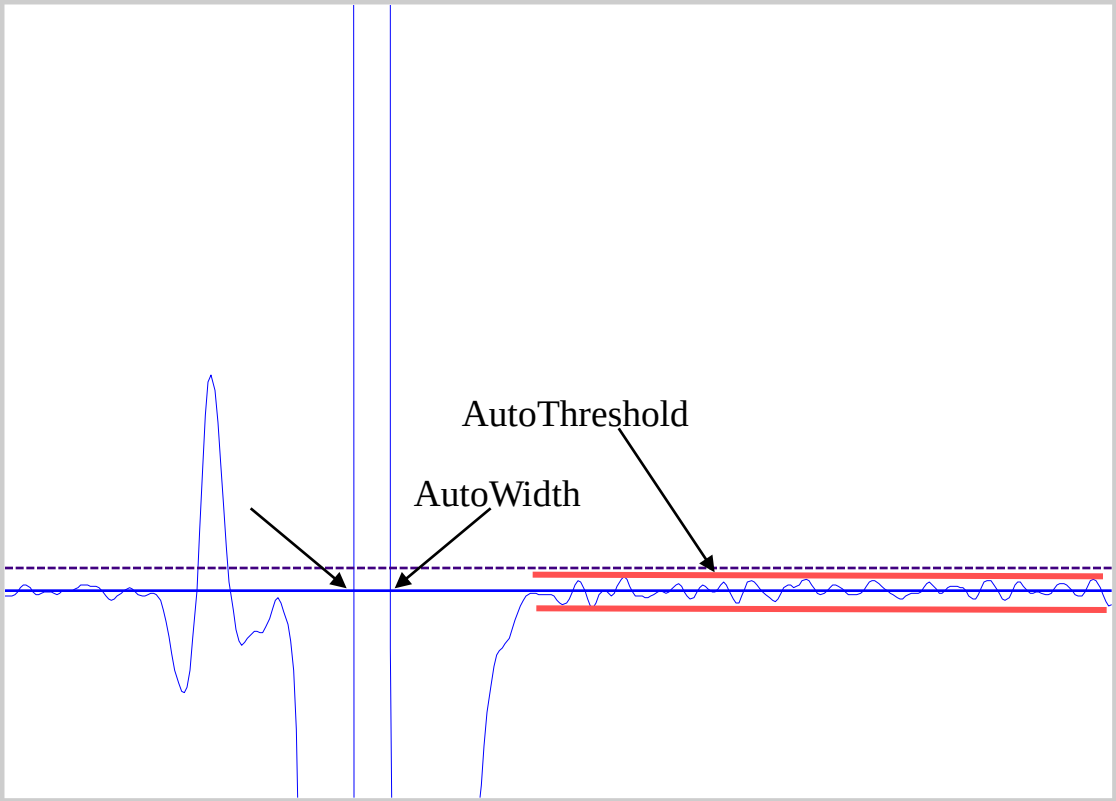
2nd derivative plot



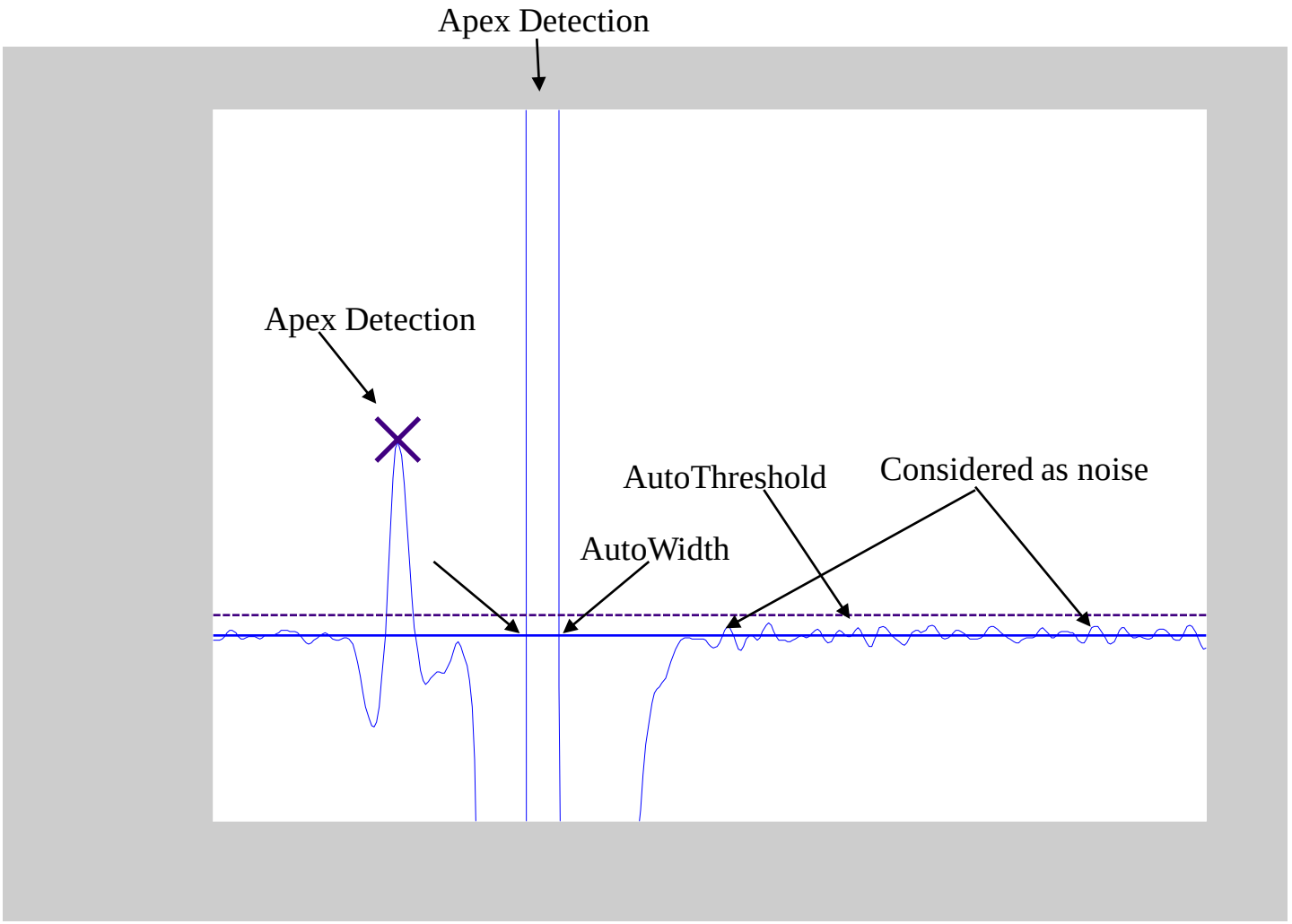
Threshold

2nd derivative plot



Apex detection

2nd derivative plot



Apex Track Integration

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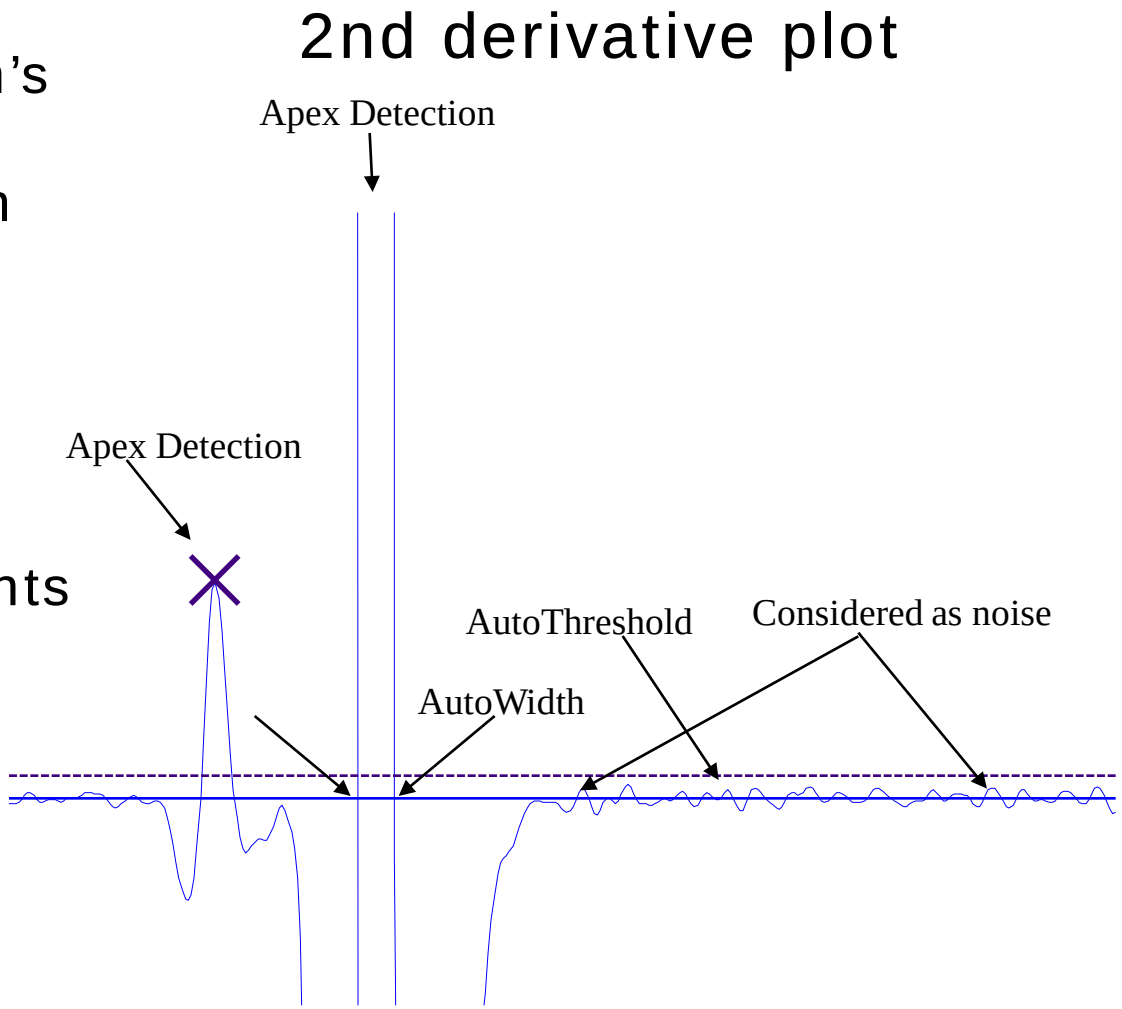
What happens?

1. Acquire the data
2. Obtain chromatogram's second derivative
3. Determine peak width (AutoPeakWidth)
4. Determine threshold (AutoThreshold)
5. Detect peaks
   - Second Derivative
6. Identify inflection points



Apex Track Integration

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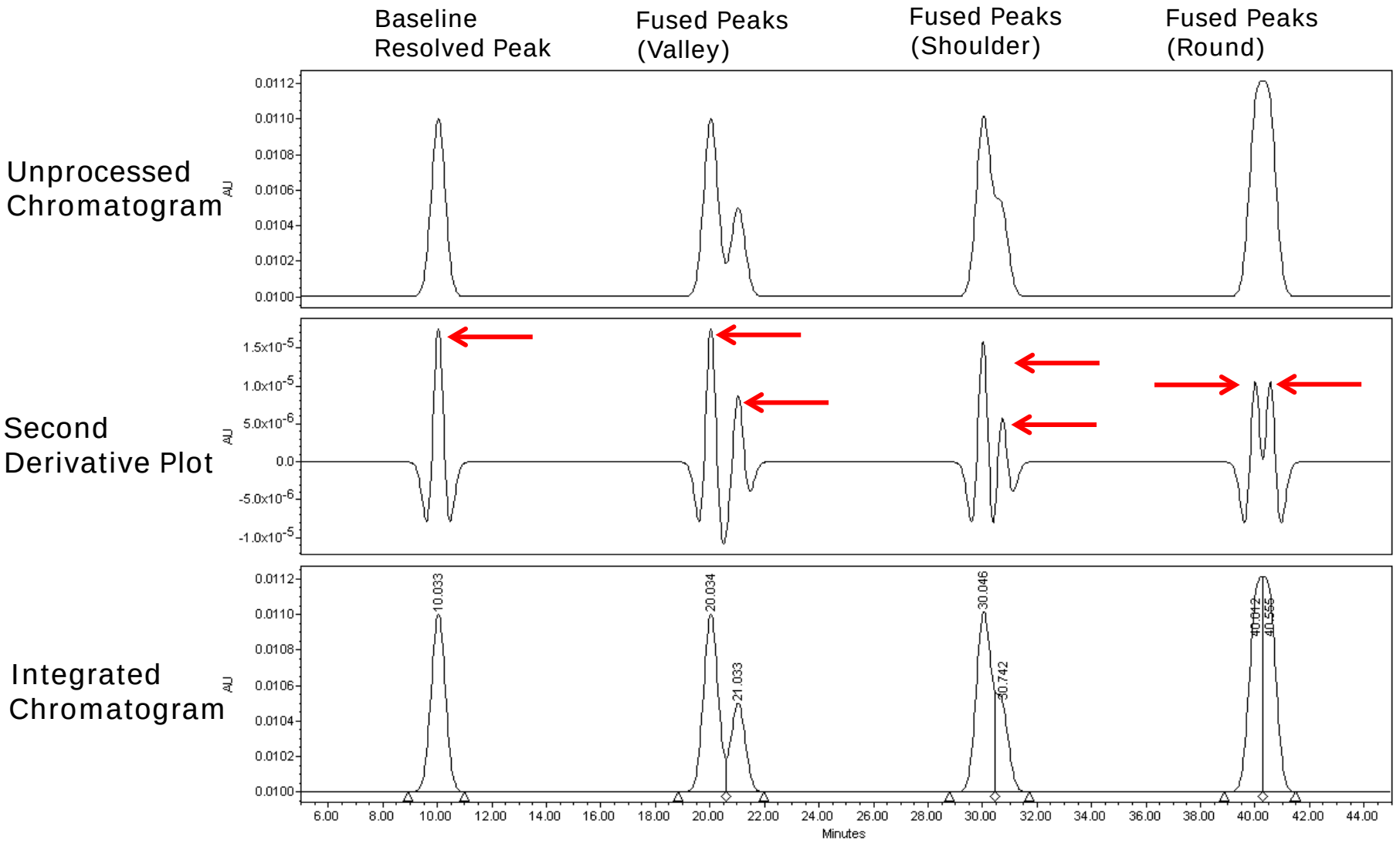

Apex Track Integration
Baseline Determination

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What about Baseline determination?

- ApexTrack uses *percentage* slope threshold.
  - The slope threshold depends on peak height
  - The baseline is the same for all peaks

Why?

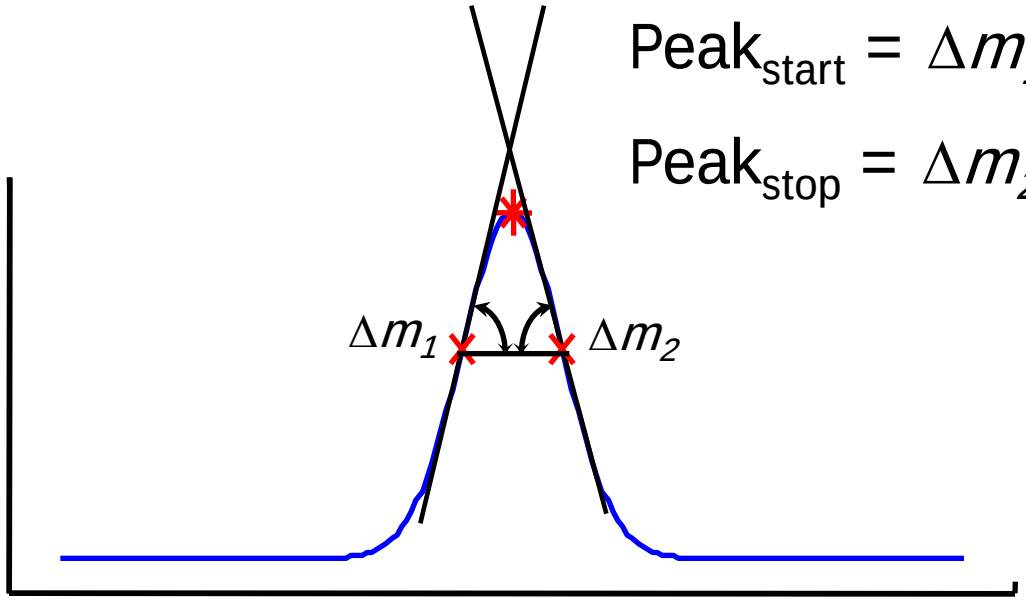
- Baselines change when concentration changes and the location of touchdown is most sensitive.

What happens?

- User specifies baseline threshold as a *percentage* of peak height.
- Algorithm computes a separate slope threshold for each peak
- Slope threshold is then *proportional* to peak height
  - Big peaks have big threshold
  - Small peaks have small threshold

Baseline Determination

1. Initially draws baseline between the inflection points
2. Determines slope differences (Δm)using tangents to the inflection points



$$Peak_{start} = \Delta m_1 \text{ x Liftoff\%/100}$$

$$Peak_{stop} = \Delta m_2 \text{ x Tuchdown\%/100}$$

3. Determines slope thresholds using Baseline % Thresholds from processing method and slope differences.

   Baseline % Thresholds scale inflection point slope differences to determine liftoff and touchdown points.

Baseline Determination

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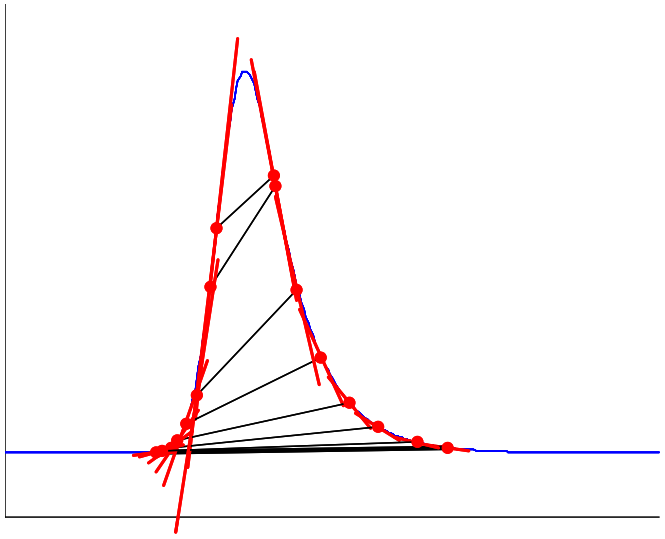
4. Baselines start at the “inflection point” baseline
5. Baselines are expanded until the slope threshold criteria are met



6. A Baseline % Threshold of 100 % yields baseline at inflection points
7. A Baseline % Threshold of 0 % yields baseline that is tangent to baseline noise

Concentration Change: Traditional Approach

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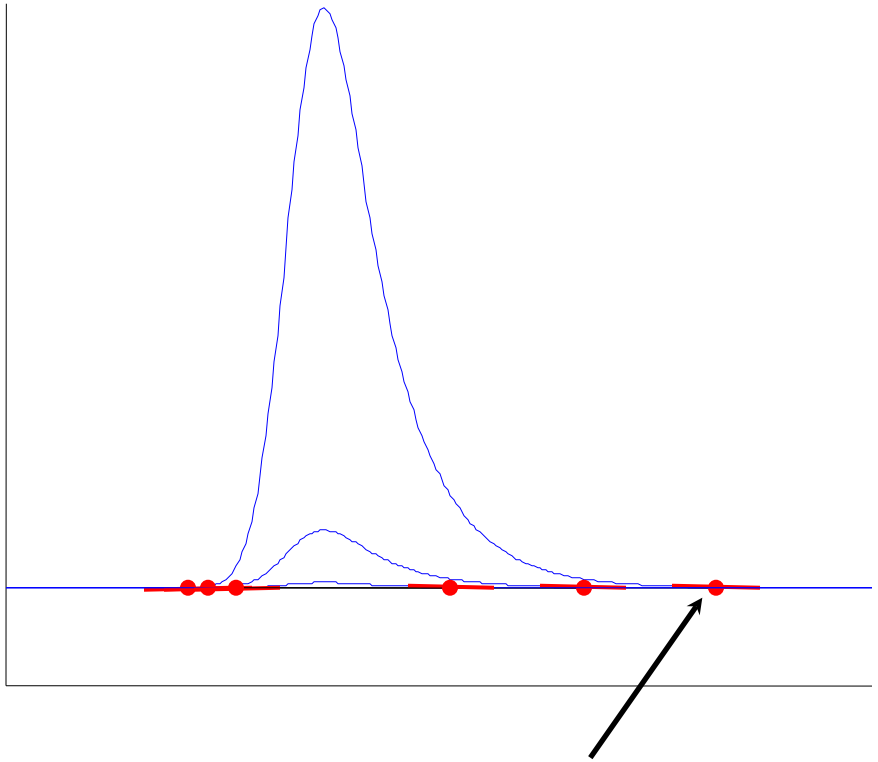
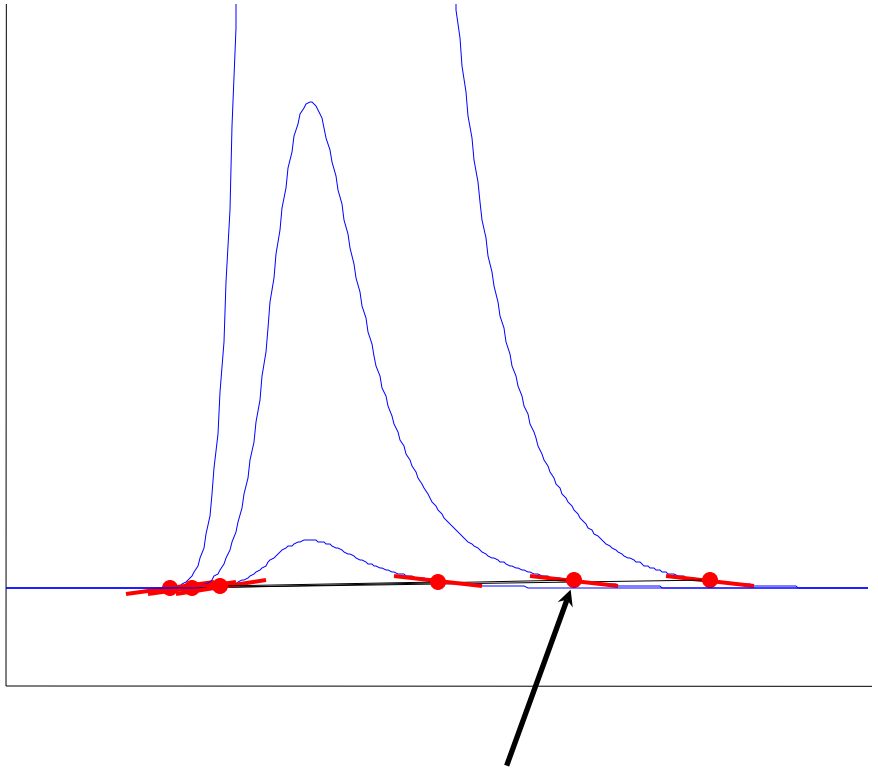
- Height ratios of 1: 1/10 : 1/100
- Times of liftoff and touchdown change
- Biggest peak: Touchdown far down in tail



Concentration Change: Zoom In

- Focus on 1/10 peak
- Middle peak: Touchdown is well positioned



Concentration Change: Zoom In Again

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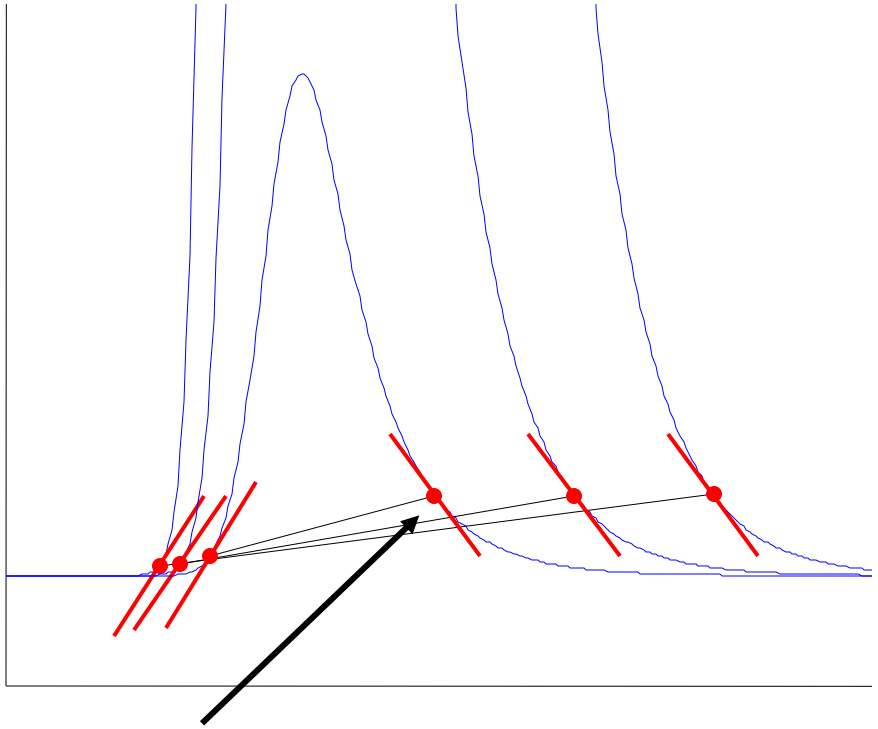
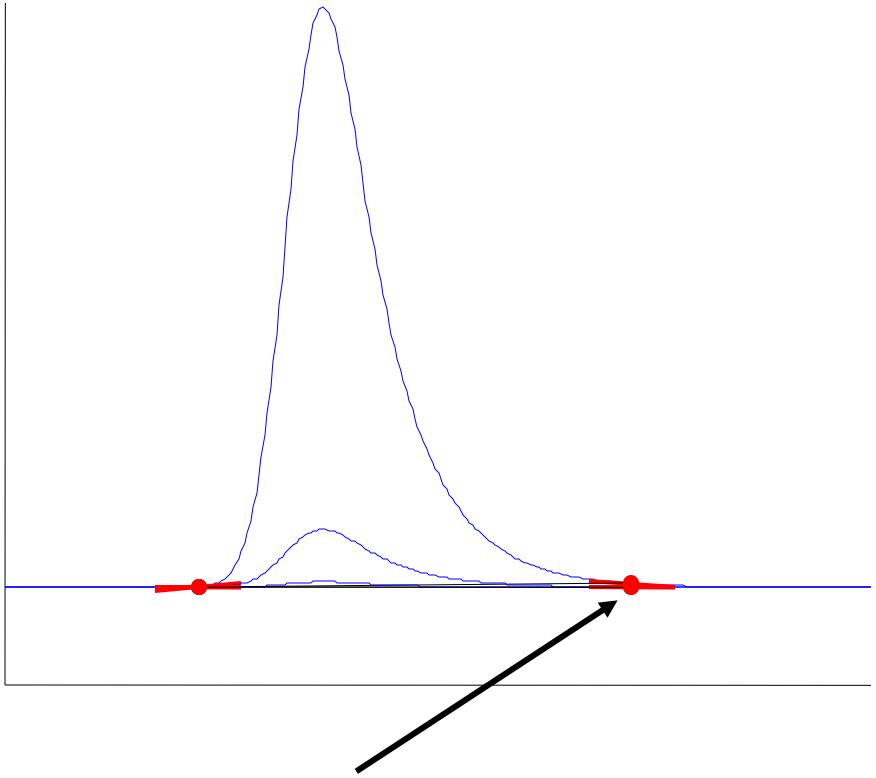
- Focus on 1/100 peak
- Smallest peak: Touchdown is high up the tail
- Relative area of smallest peak is reduced!



Concentration Change: ApexTrack

- Height ratios of 1: 1/10 : 1/100
- Liftoff is the same for each peak.
- Touchdown is the same for each peak
- Biggest peak: Touchdown is well positioned



Concentration Change: Zoom In

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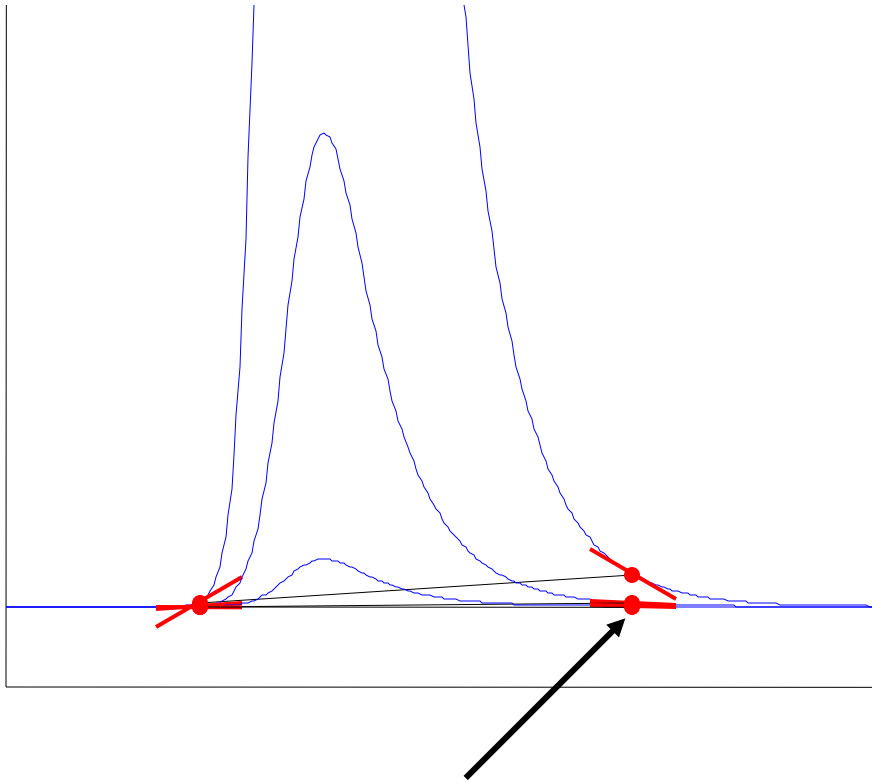
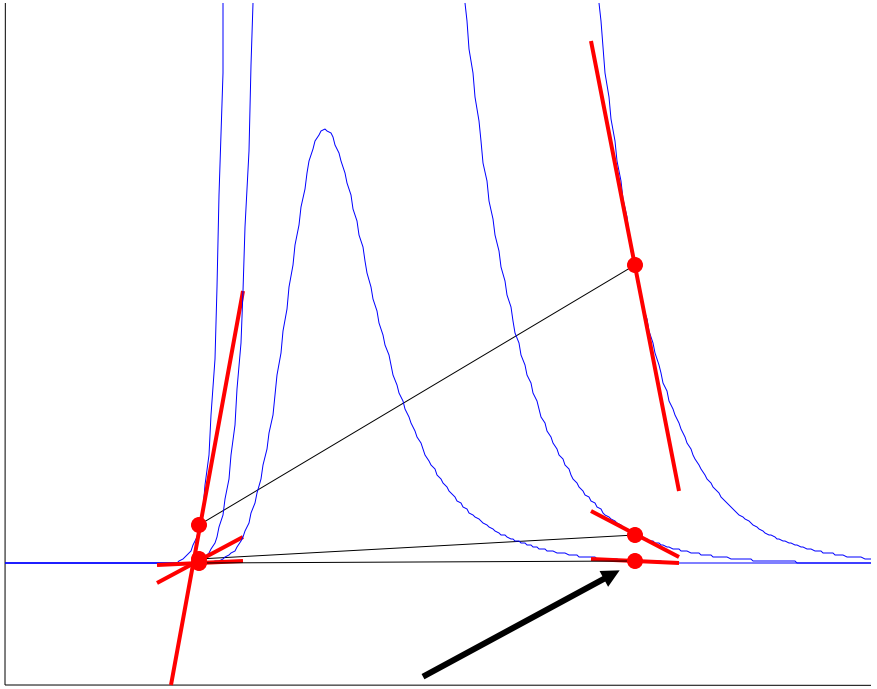
- Focus on 1/10 peak
- Middle peak: Touchdown is well positioned



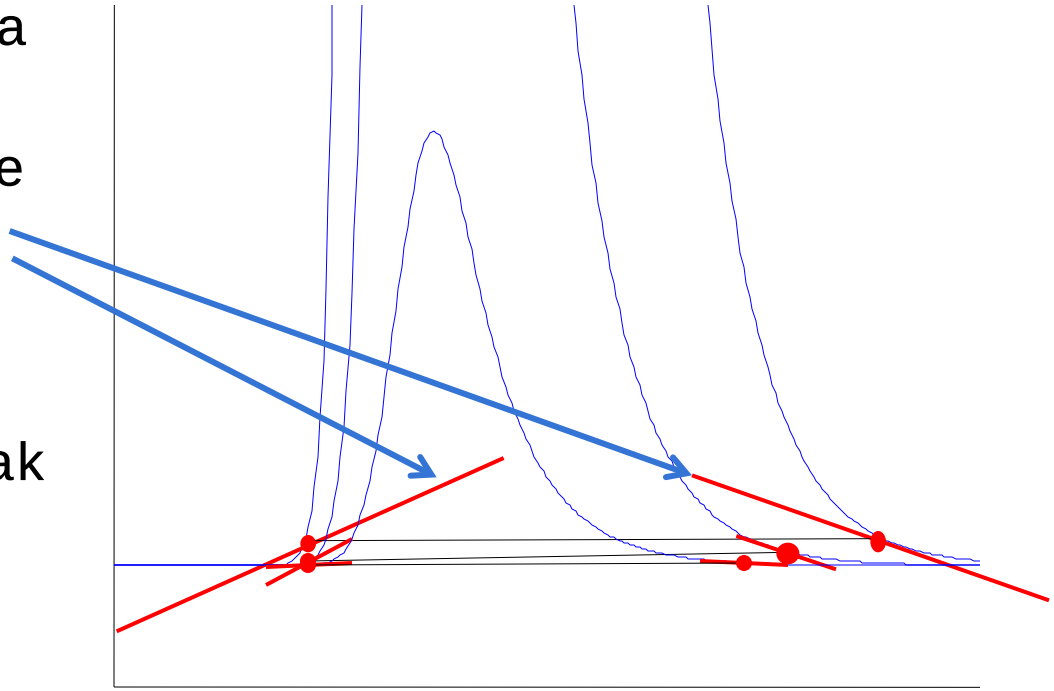
Concentration Change: Zoom In Again

- Focus on 1/100 peak
- Smallest peak: Touchdown is well positioned
- Note different slope thresholds



Changing %Touchdown

- Focus on Big peak
- A small change in the %Touchdown will have a big impact on the slope for the big peak because it is a percentage of the peak height
- This will have very little effect on the middle peak and NO effect on the small peak



Apex Track Integration
Timed Events

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- ANP - Allow Negative Peaks
- DS - Detect Shoulders
- GS - Gaussian Skim
- TS - Tangential Skim
- II - Inhibit Integration
- MP - Merge Peaks (for GPC only)
- SL% - Set Liftoff %
- ST% - Set Touchdown %
- SMA - Set Minimum Area
- SMH - Set Minimum Height
- SMxH - Set Maximum Height
- SMxA - Set Maximum Area
- VV - Valley-to-Valley
- SPW - Set Peak Width
- SDT - Set Detection Threshold

Integration events Comparison: Traditional –Apex Track

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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

Conclusions

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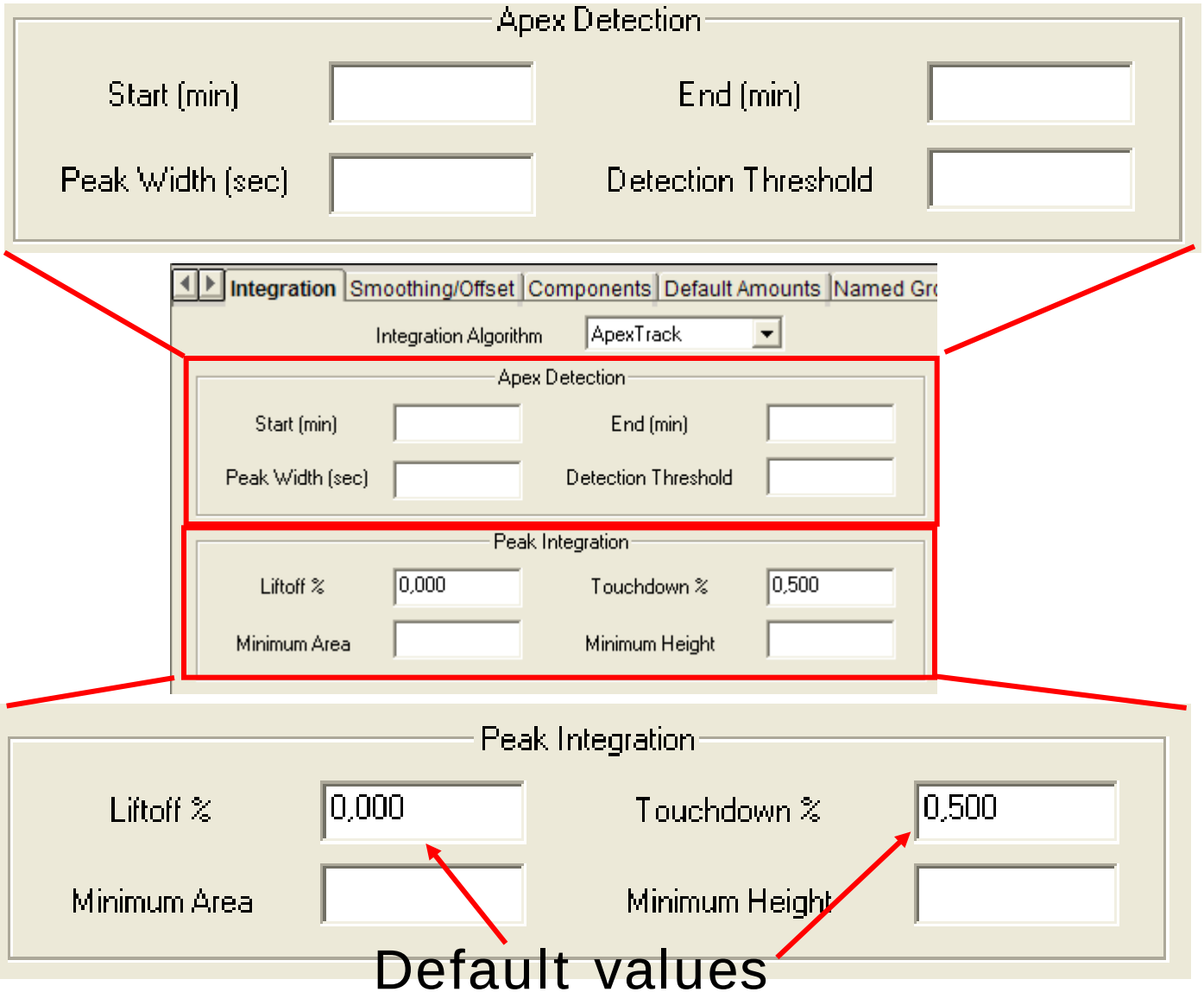
Advantages over other Integration Packages

1. Automatic parameter determination, for rapid method development
2. Default parameters superior to those of Traditional
3. Curvature detection, for reproducible detection of difficult peaks and shoulders
4. Internally adjusted slope threshold, for accurate baseline determination, does not affect peak detection
5. Gaussian Skimming

Processing Method



Developing an ApexTrack method

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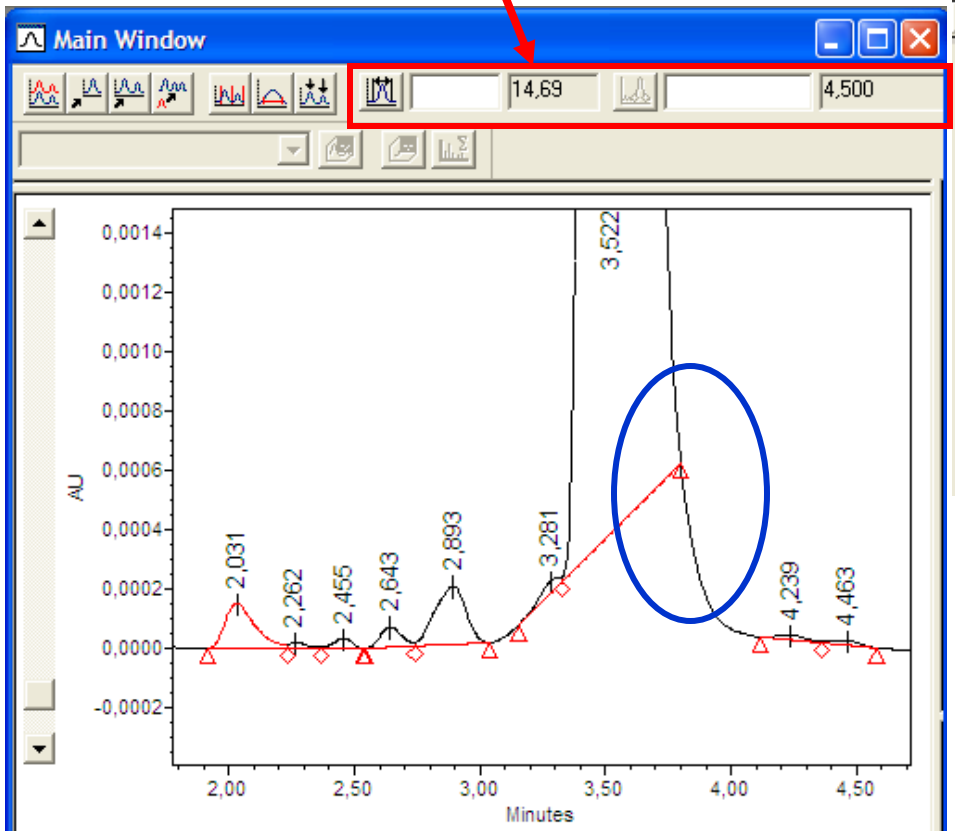
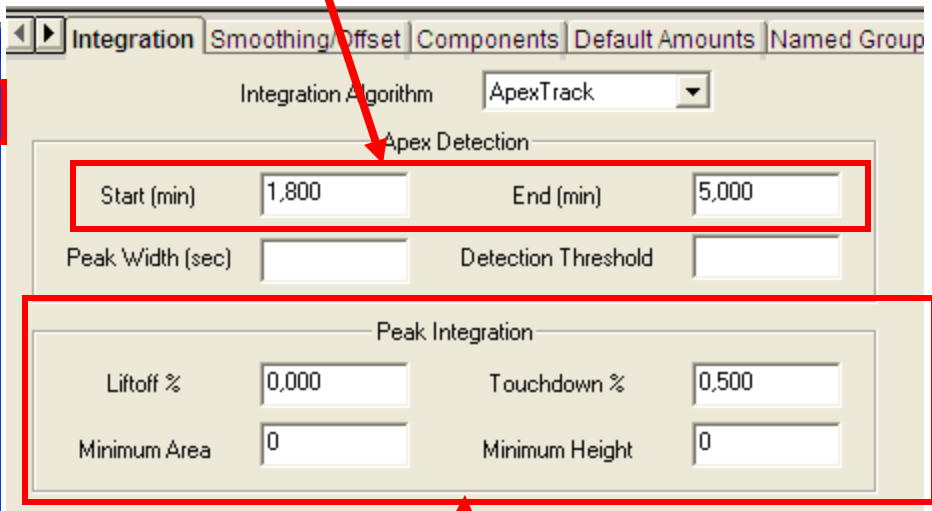
Peak width & Detection Threshold is automatically Determined (14.69 & 4.5)



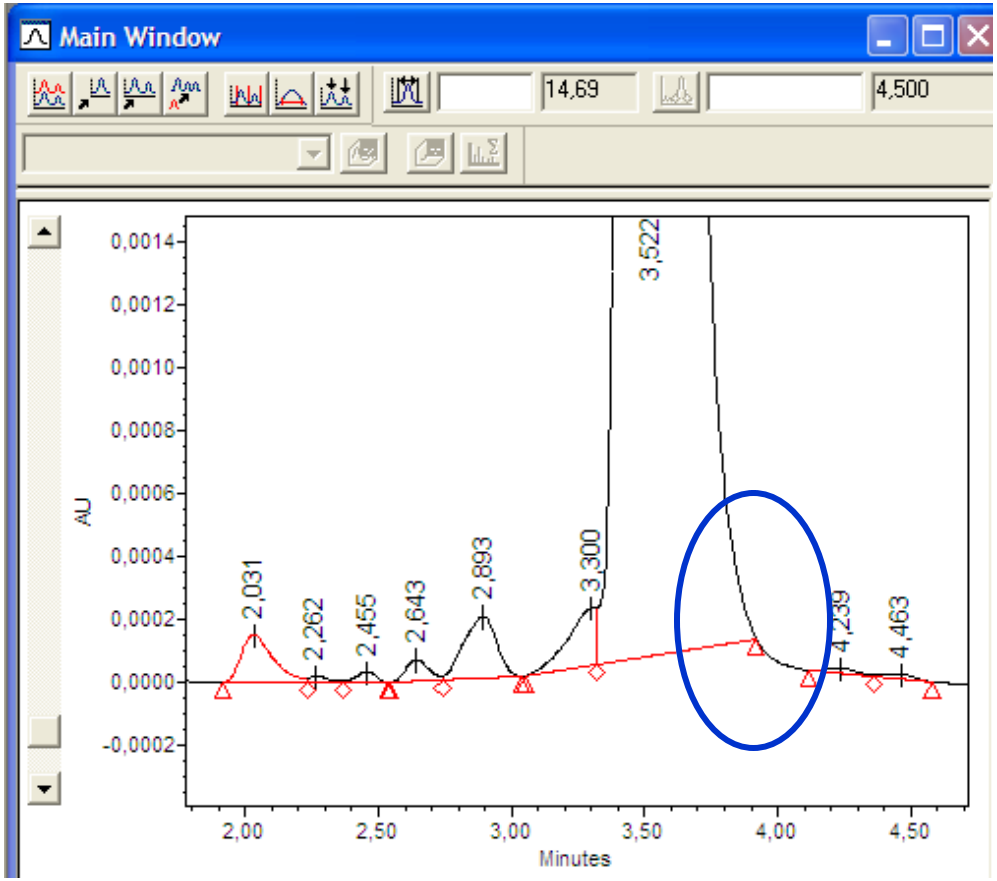
Limit the Apex Detection time zone (1.8 – 5.0 min)

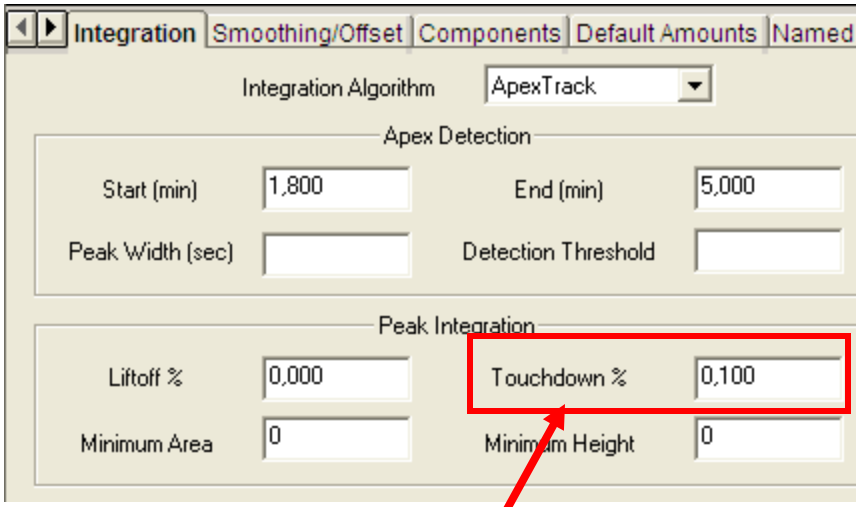


Default settings

41

Developing an ApexTrack method





Lower the Touchdown% to 0.1

42

Developing an ApexTrack method

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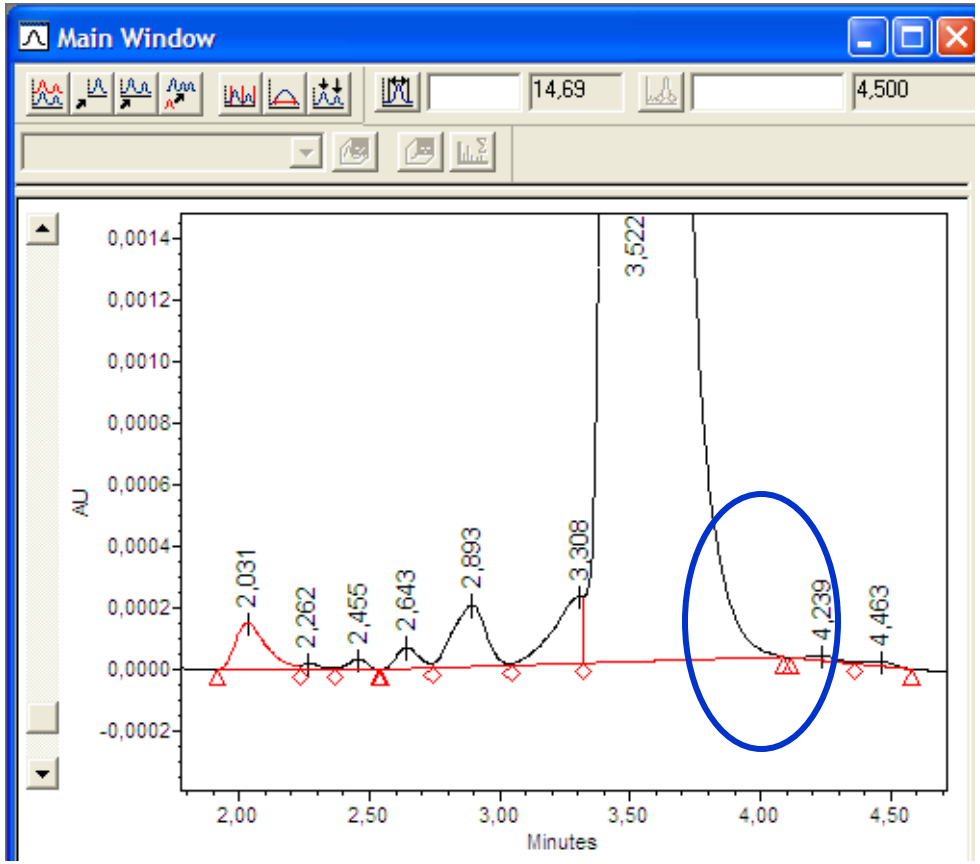
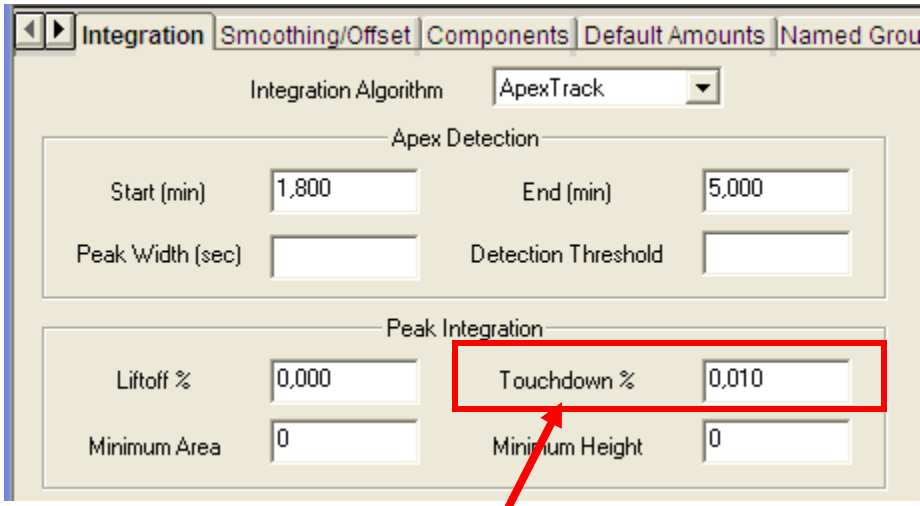
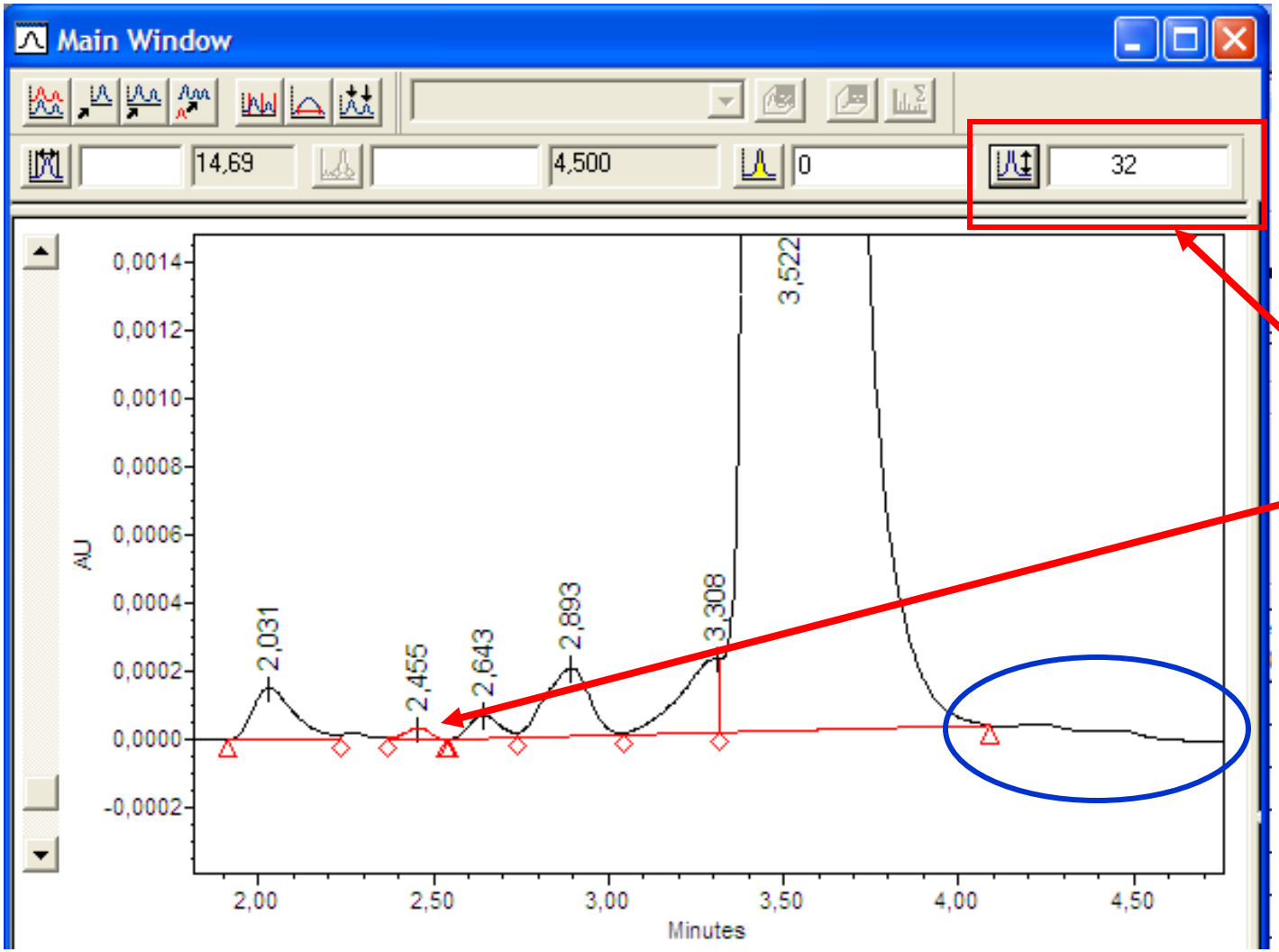




Lower the Touchdown% to 0.01

43

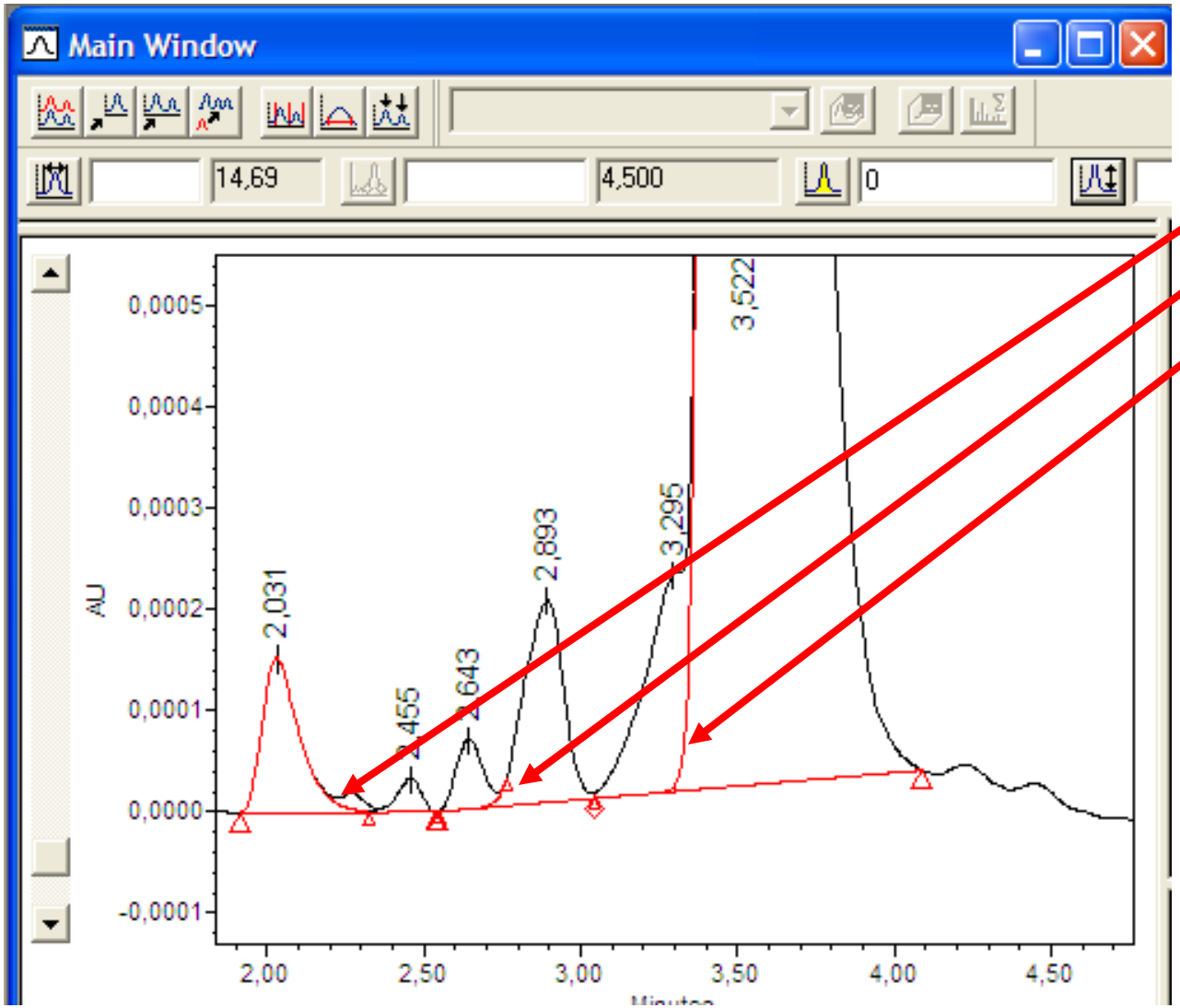
Developing an ApexTrack method



Setting min. Area

44

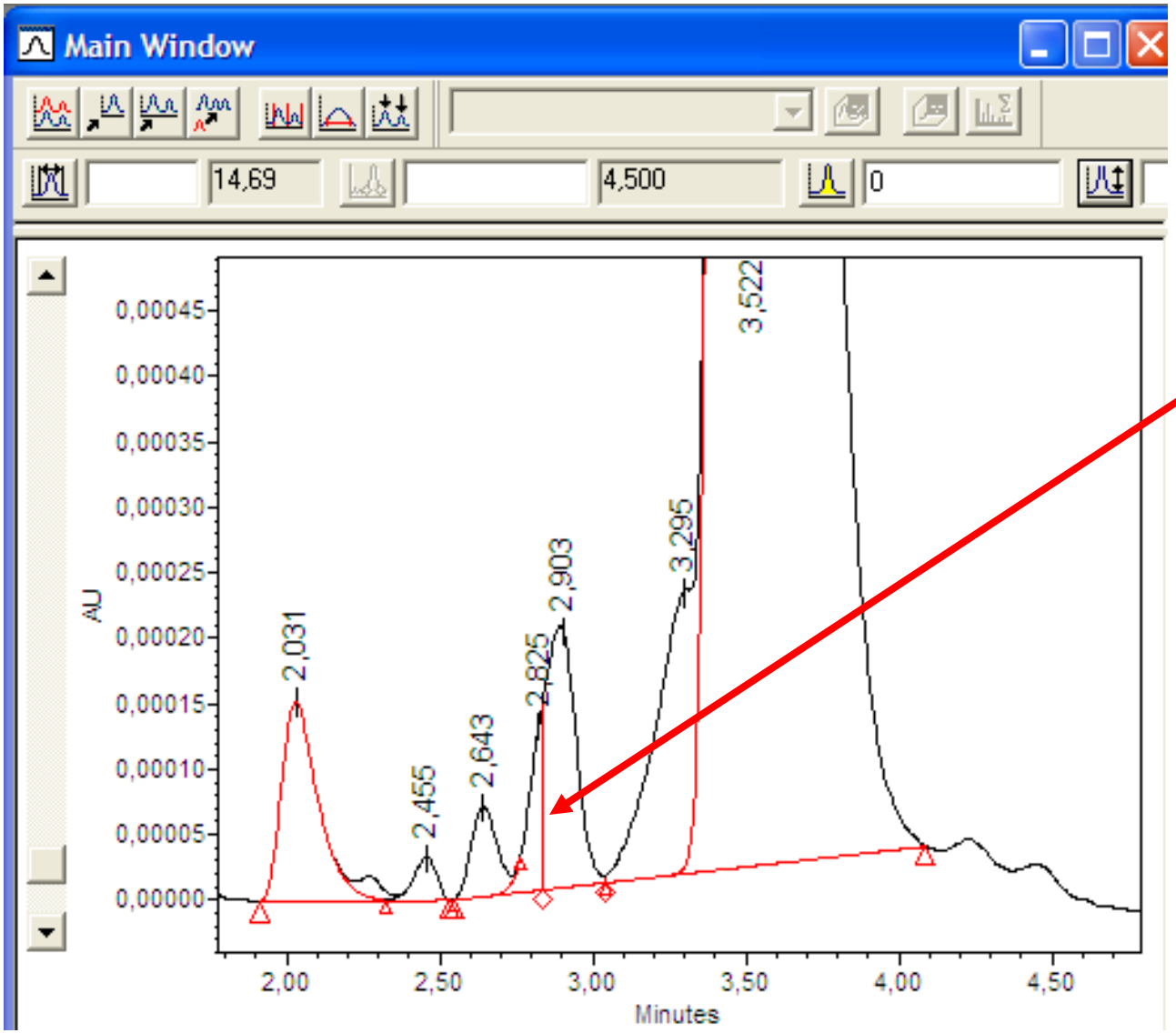
Developing an ApexTrack method



Adding Gaussian skim event

45

Developing an ApexTrack method



Adding
Detect shoulders
event

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Questions

Exercise 1

- Optimize existing processing method
  - Optimize integration
  - Adjust retention times

System Suitability Calculations

System Suitability Tab

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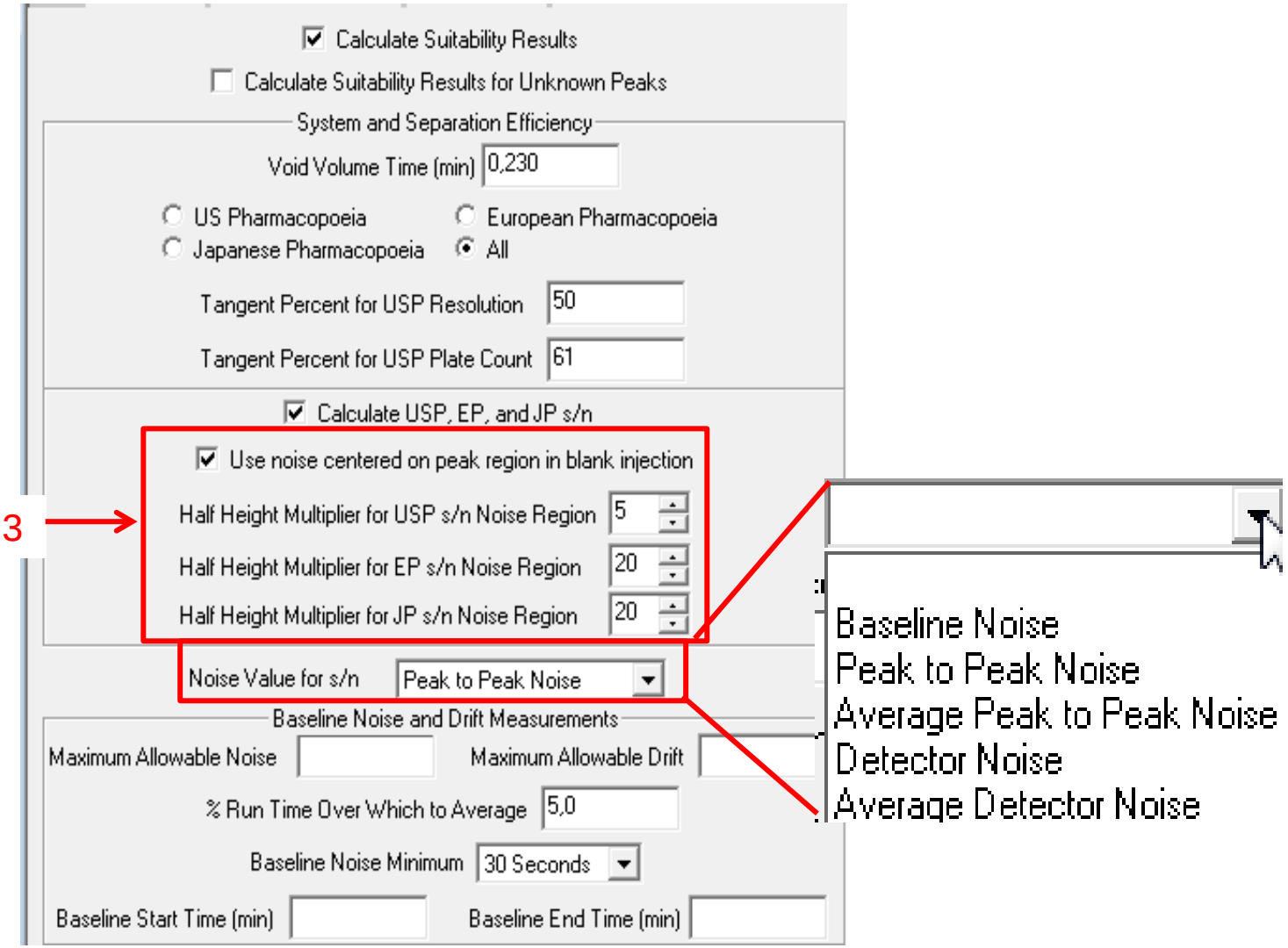
New in Empower3



Use Noise Centered on Peak Region in Blank Injection

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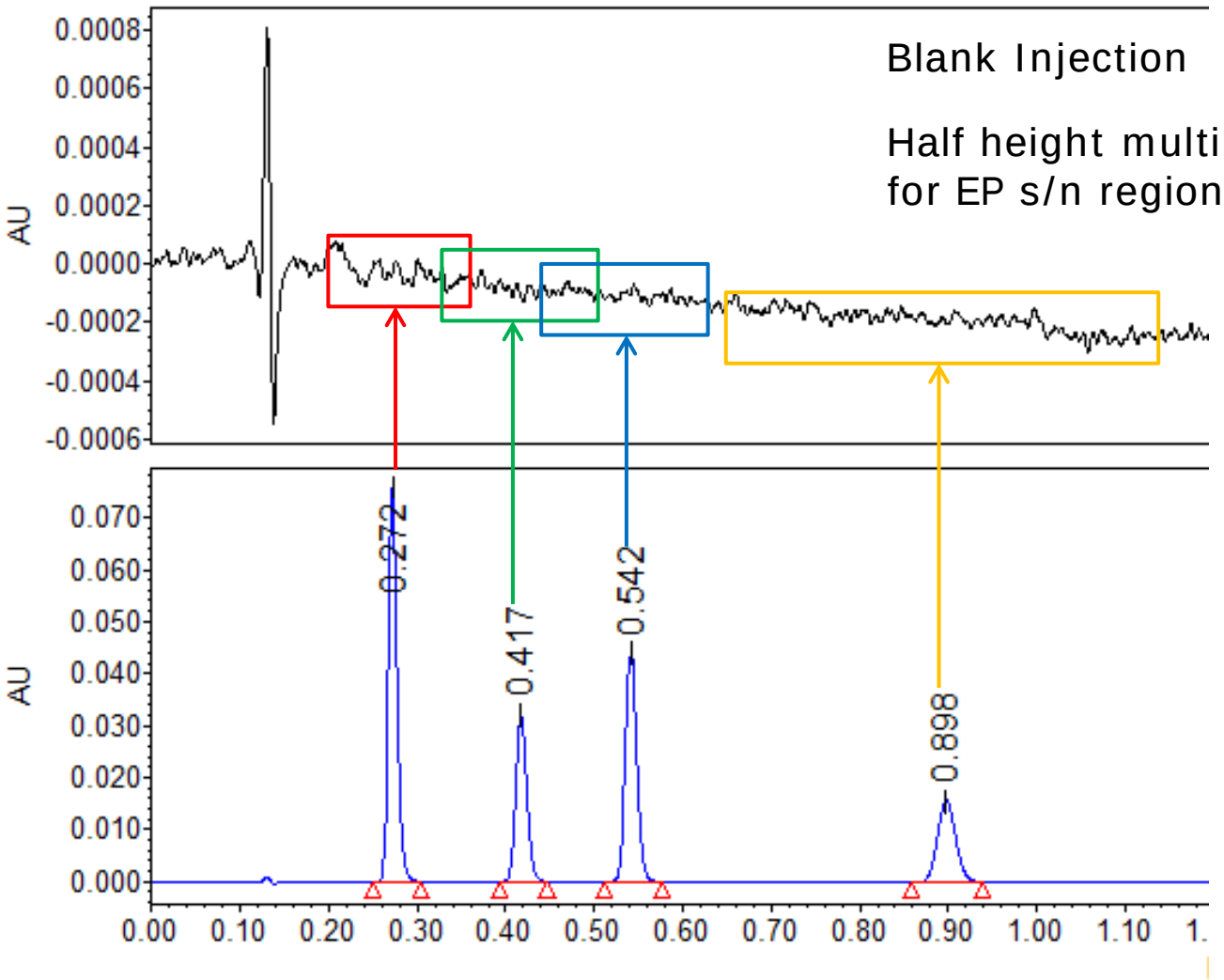

Choosing the Blank Injection

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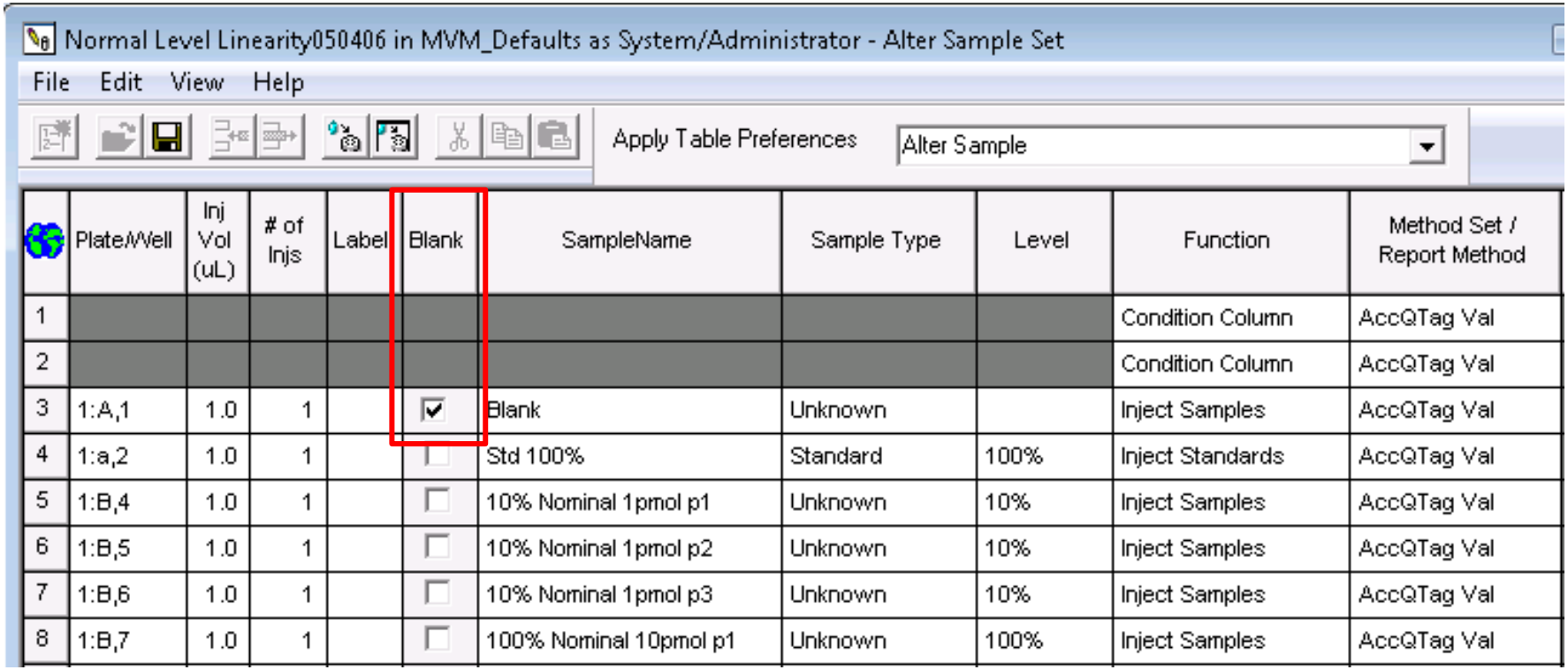
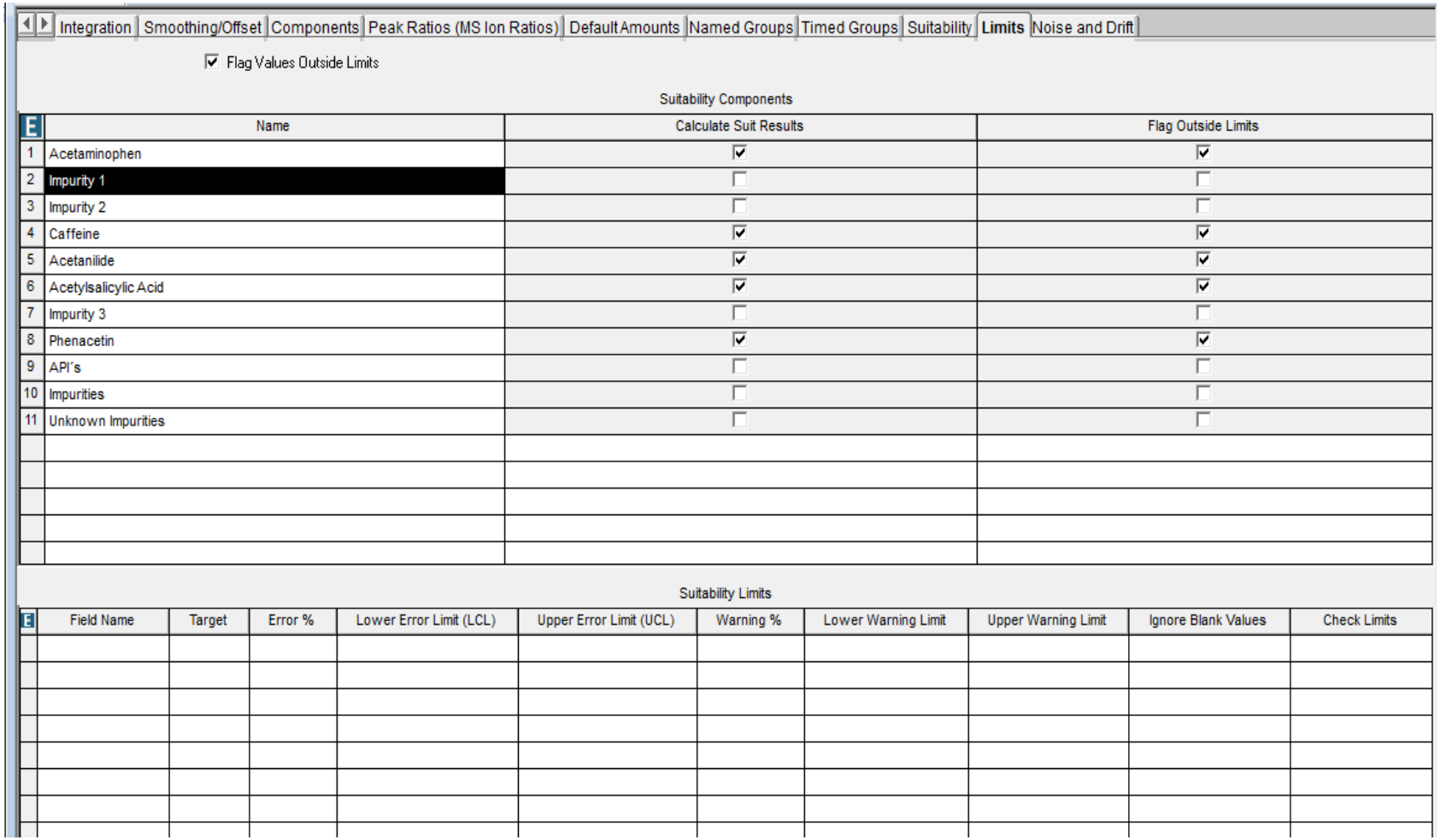


	Plate/Well	Inj Vol (uL)	# of Injs	Label	Blank	SampleName	Sample Type	Level	Function	Method Set / Report Method
1									Condition Column	AccQTag Val
2									Condition Column	AccQTag Val
3	1:A,1	1.0	1		☑	Blank	Unknown		Inject Samples	AccQTag Val
4	1:a,2	1.0	1		☐	Std 100%	Standard	100%	Inject Standards	AccQTag Val
5	1:B,4	1.0	1		☐	10% Nominal 1pmol p1	Unknown	10%	Inject Samples	AccQTag Val
6	1:B,5	1.0	1		☐	10% Nominal 1pmol p2	Unknown	10%	Inject Samples	AccQTag Val
7	1:B,6	1.0	1		☐	10% Nominal 1pmol p3	Unknown	10%	Inject Samples	AccQTag Val
8	1:B,7	1.0	1		☐	100% Nominal 10pmol p1	Unknown	100%	Inject Samples	AccQTag Val



Integration | Smoothing/Offset | Components | Peak Ratios (MS Ion Ratios) | Default Amounts | Named Groups | Timed Groups | Suitability | **Limits** | Noise and Drift

☑ Flag Values Outside Limits

Suitability Components

	Name	Calculate Suit Results	Flag Outside Limits
1	Acetaminophen	☑	☑
2	Impurity 1	☐	☐
3	Impurity 2	☐	☐
4	Caffeine	☑	☑
5	Acetanilide	☑	☑
6	Acetylsalicylic Acid	☑	☑
7	Impurity 3	☐	☐
8	Phenacetin	☑	☑
9	API's	☐	☐
10	Impurities	☐	☐
11	Unknown Impurities	☐	☐

Suitability Limits

	Field Name	Target	Error %	Lower Error Limit (LCL)	Upper Error Limit (UCL)	Warning %	Lower Warning Limit	Upper Warning Limit	Ignore Blank Values	Check Limits

Setting System Suitability Limits

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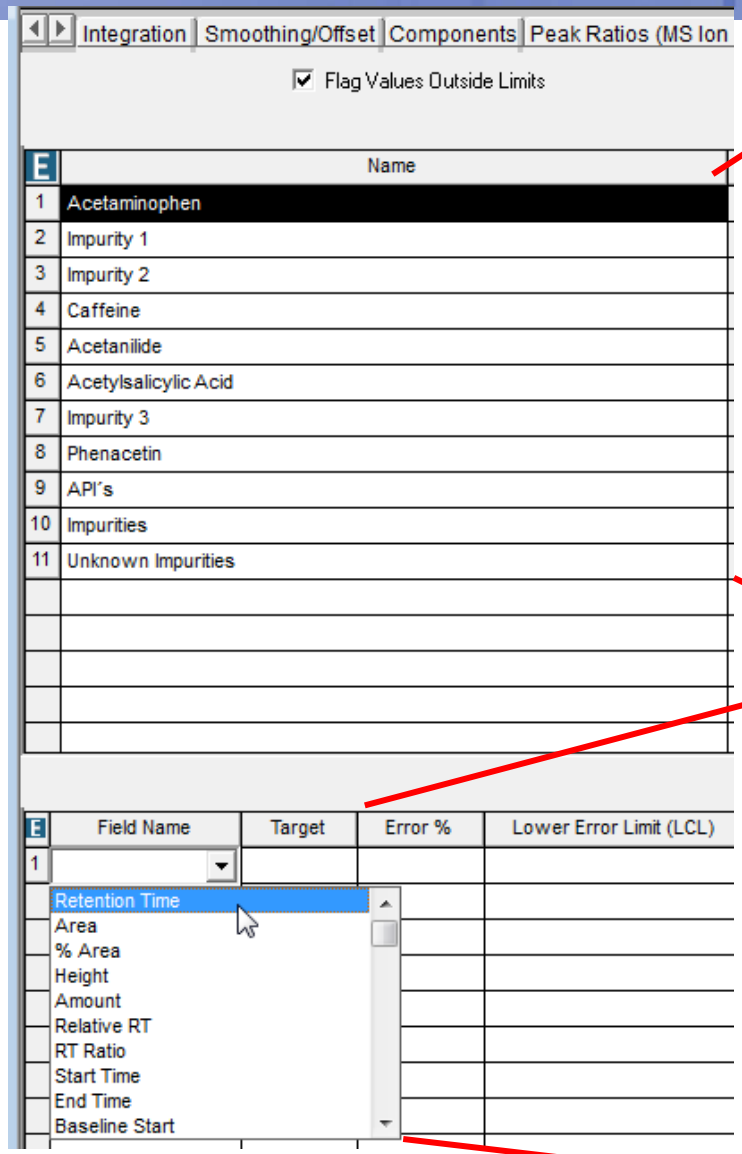

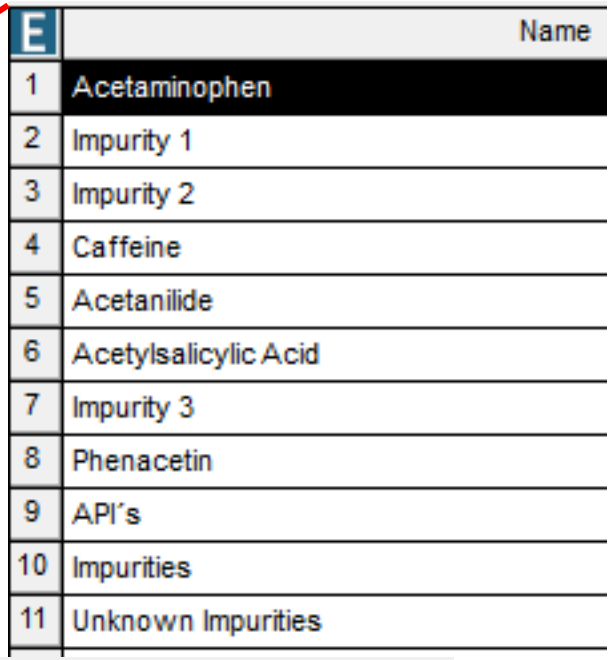

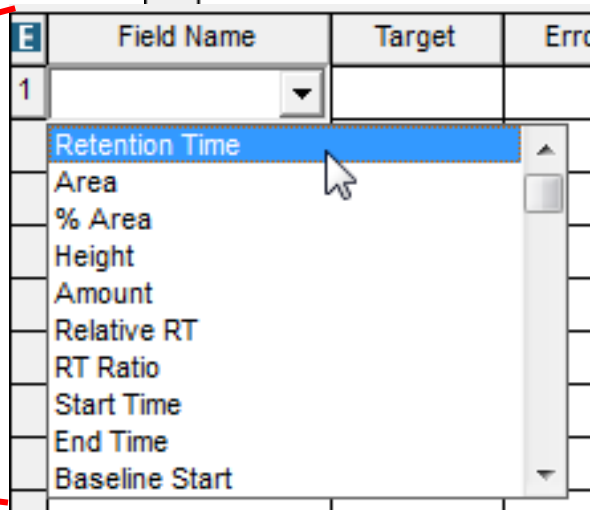

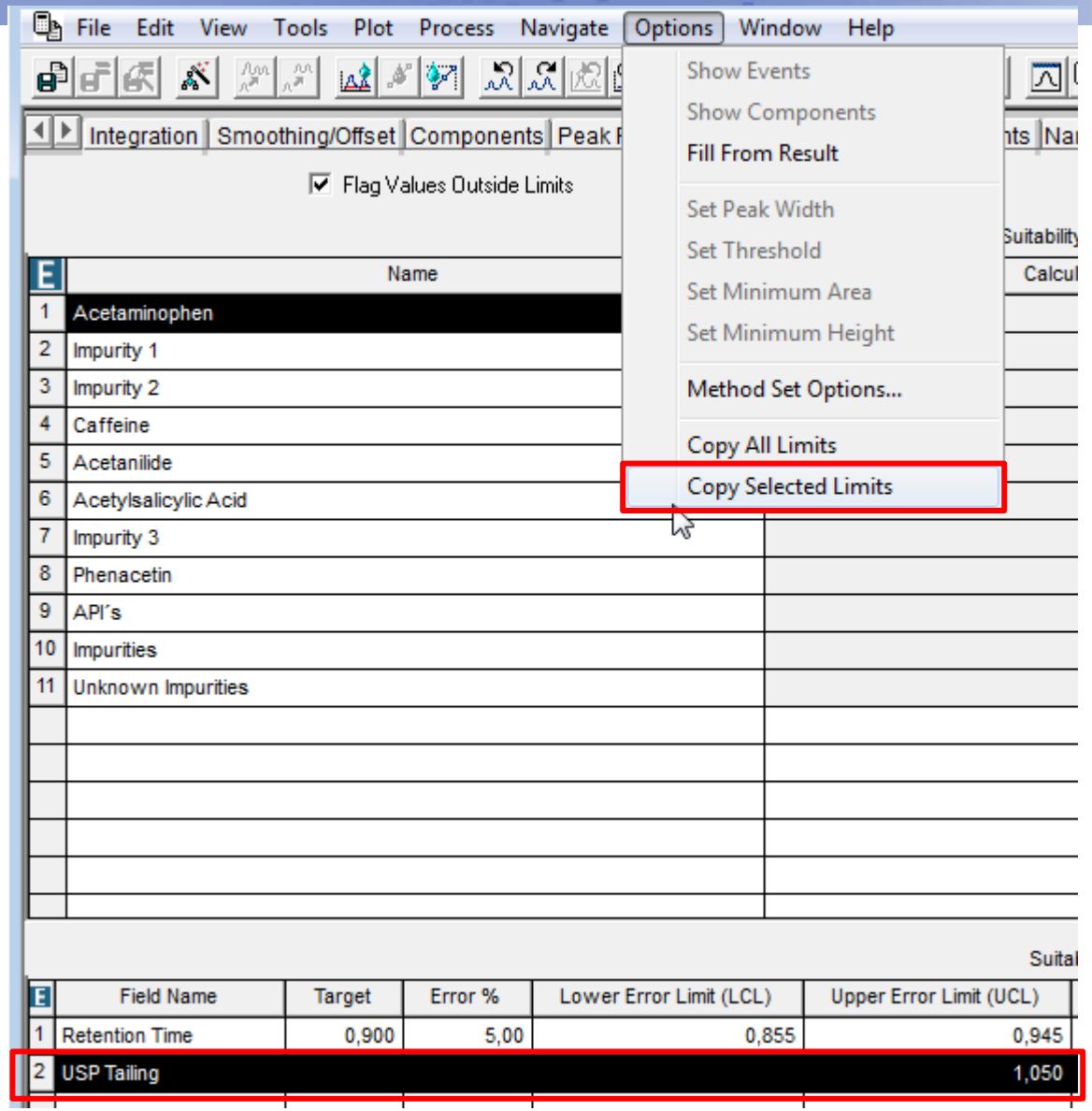

Noise & Drift Calculations

Empower Noise and Drift Calculations

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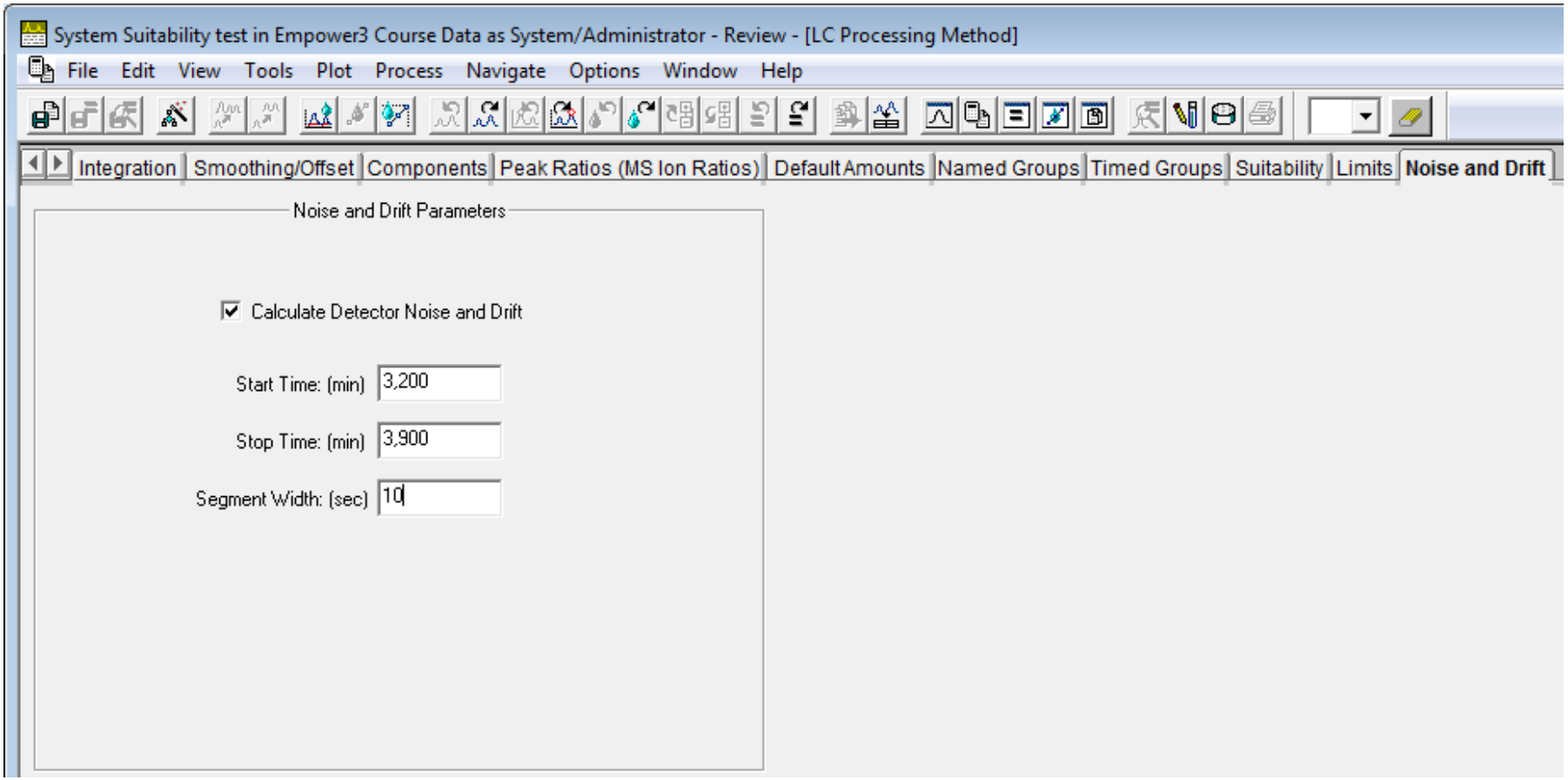
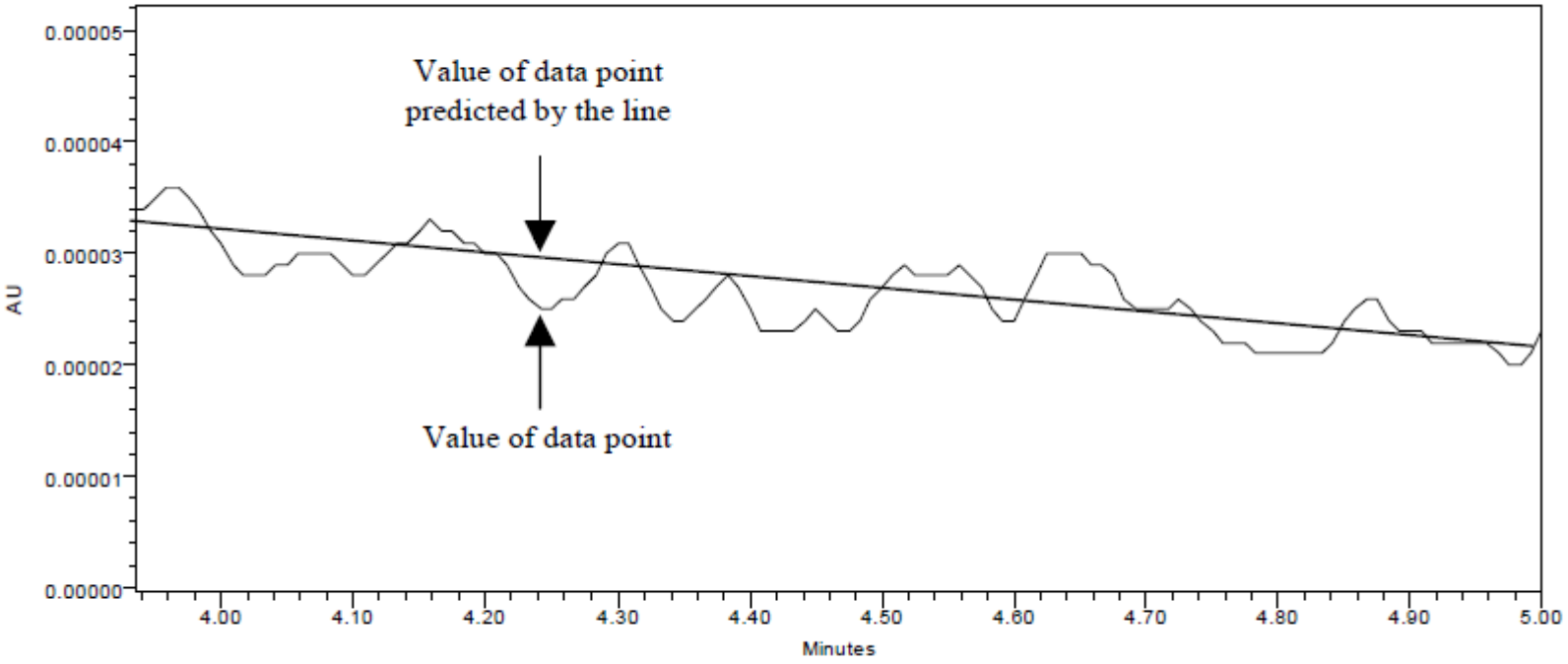
- There are 8 different calculations that can be performed:
- Detector Noise
- Peak-to-Peak Noise
- Detector Drift
- Average Detector Noise
- Average Peak-to-Peak Noise
- Average Drift
- Baseline Noise
- Baseline Drift

Enabling



Visual Representation of the Least-squares line



Detector Drift

- Detector drift is the slope of the least-squares line. Drift is expressed in detector units per hour.
  - For example, the drift calculation for a UV detector would be expressed in absorbance units (AU) per hour. Average Drift is calculated by dividing the data into segments (specified in the processing method) and averaging the values for each segments.

Detector Noise

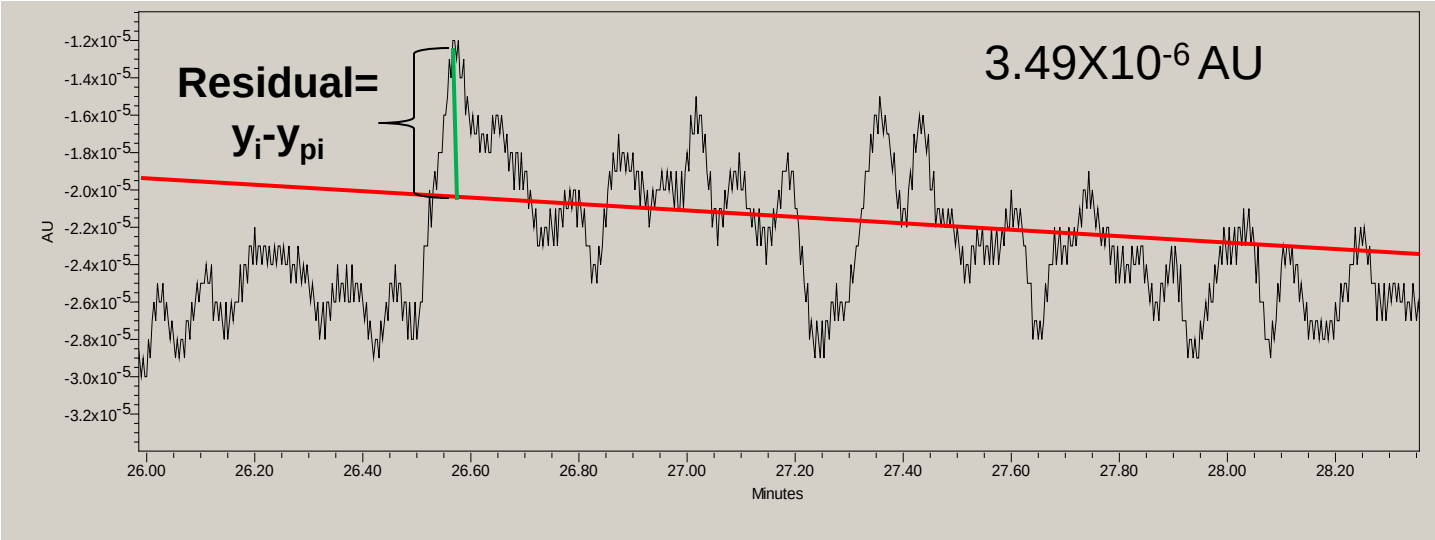
- The root mean square (RMS) noise of the data is calculated using the least-squares line. The formula for Detector Noise is:

$$\sqrt{\frac{\sum (y_i - y_{pi})^2}{n-2}}$$

- Where y_i = the y value of the data point

  y_{pi} = the y value of the data point predicted by the line

  n = the number of datapoints



Average Detector Noise

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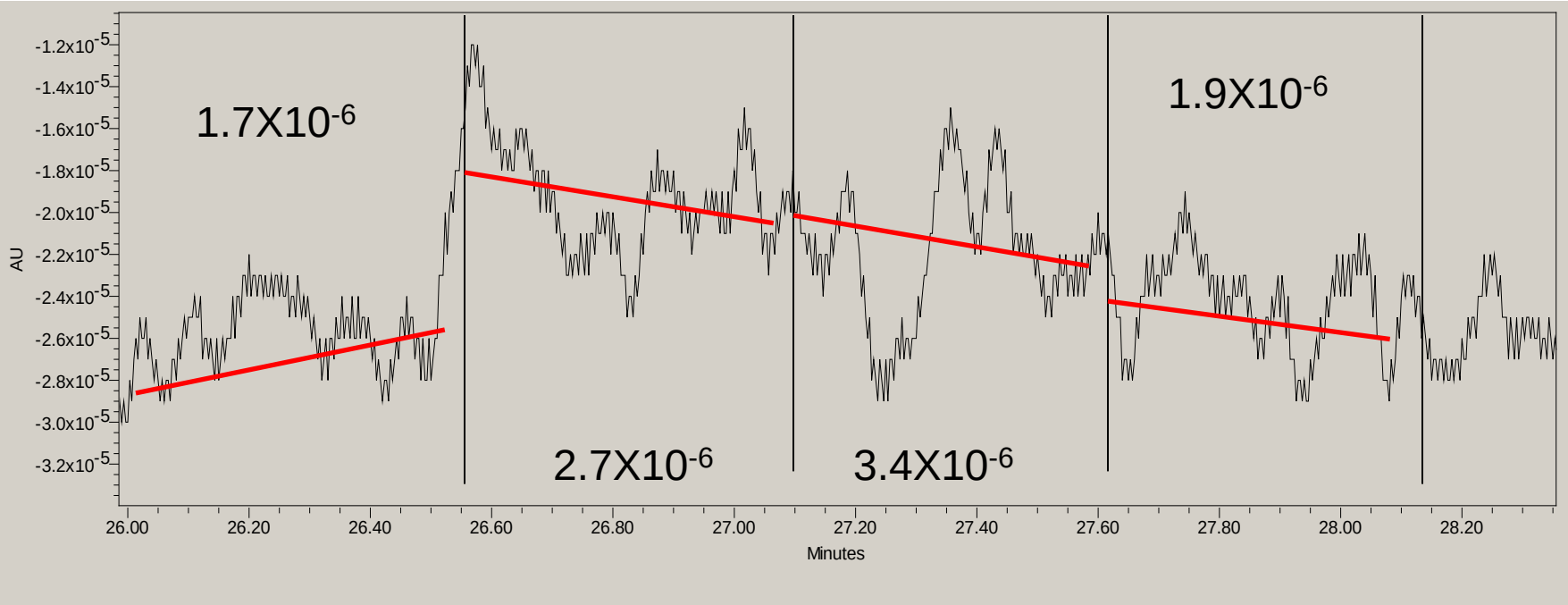
$2.43X10^{-6}$ AU

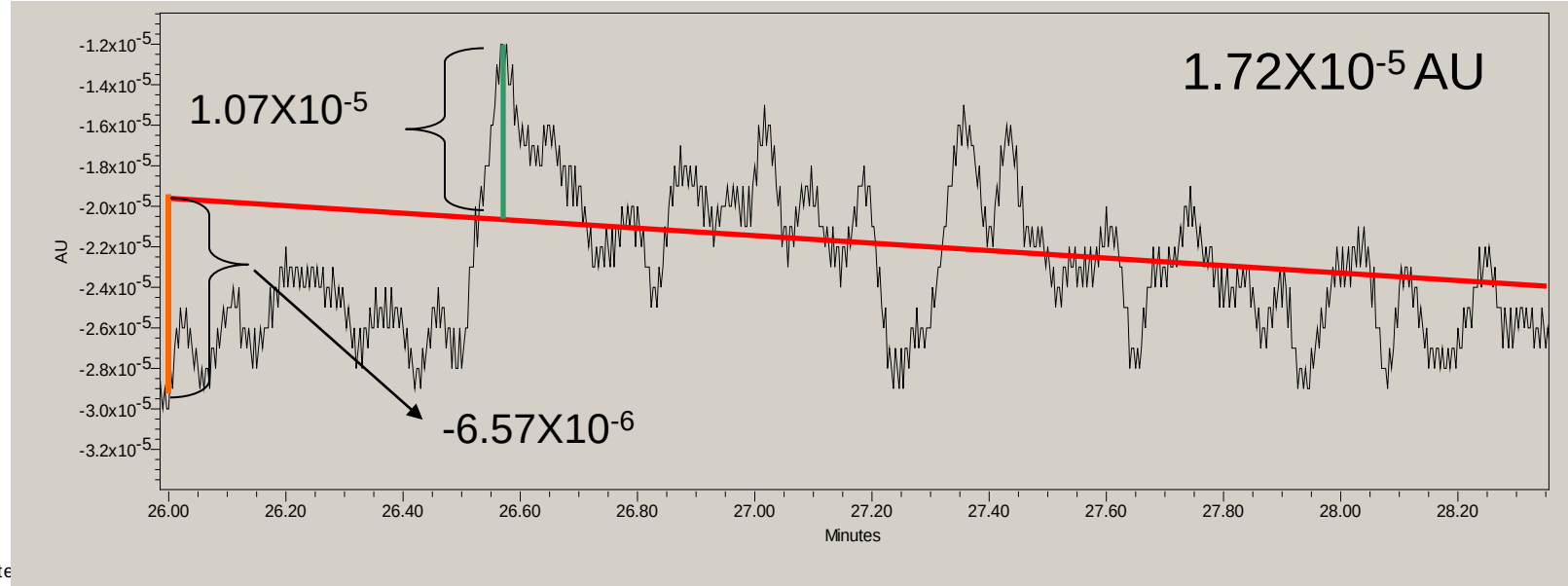


Peak to Peak Noise

- Peak to Peak Noise is defined to be the algebraic difference of the maximum and minimum residuals between each data point and the least-square line. The “residual” is determined by subtracting the y value of the data point predicted by the line from the y value of the data point.
- The formula for Peak to Peak Noise is:

  Peak to Peak Noise = Max residual – Min. residual

- Where Residual = $y_i - y_{pi}$



Average Peak to Peak Noise?

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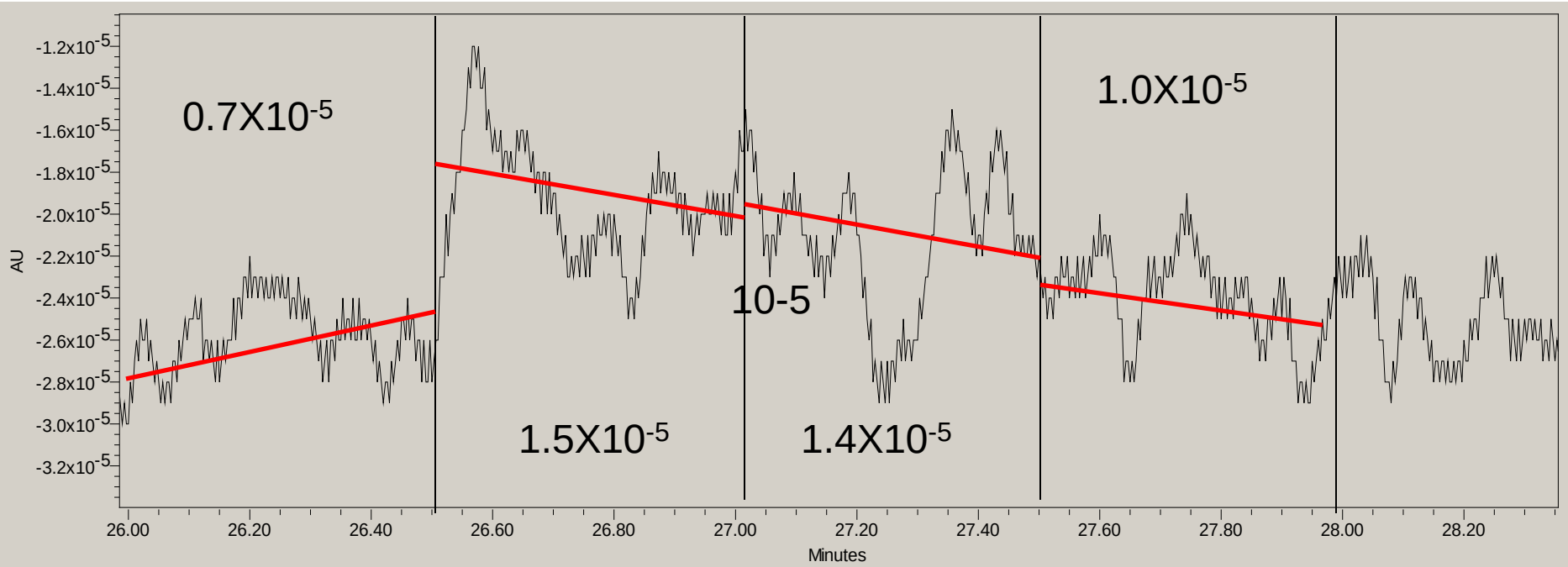
$1.15X10^{-5}$ AU



Baseline Noise and Drift Calculations

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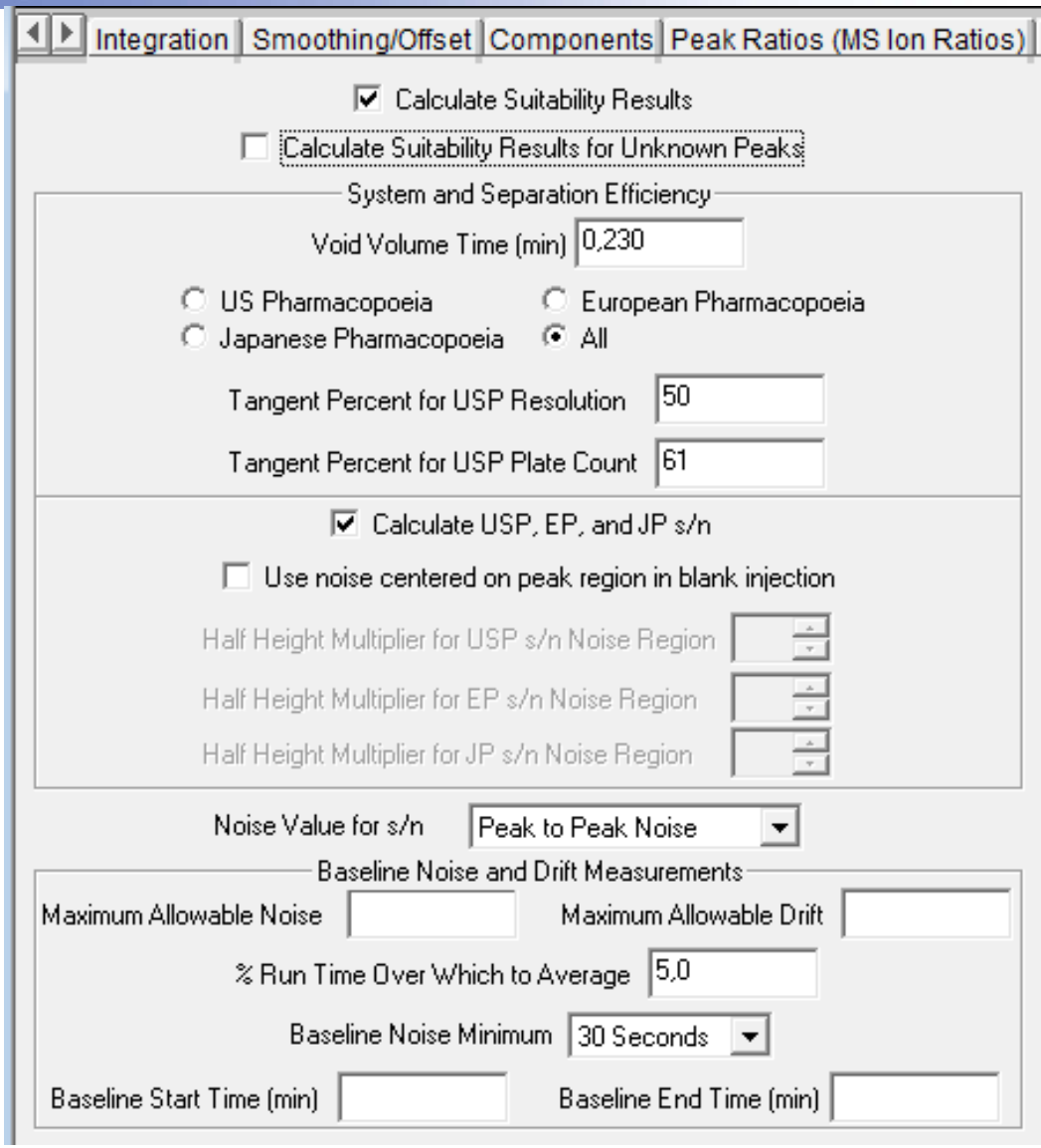
- Baseline Noise setup in System Suitability
  - Peak to Peak Calculation
  - 30 second segments (not adjustable)
  - Set time range and percent of run time.



Baseline Noise and Drift Measurements

Maximum Allowable Noise

Maximum Allowable Drift

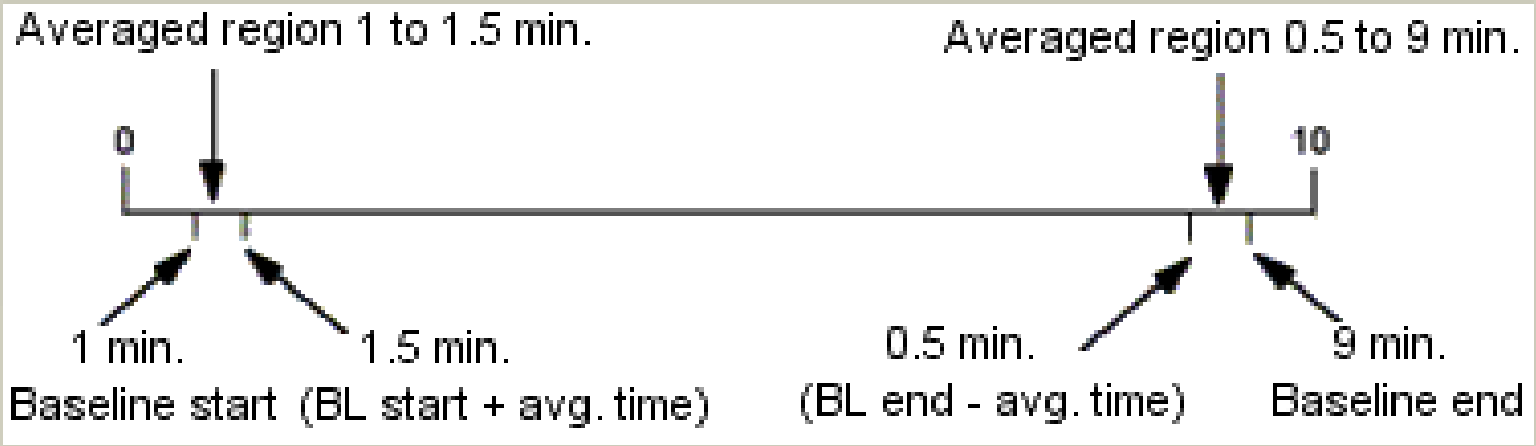
% Run Time Over Which to Average: 5.0

Baseline Noise Minimum: 30 Seconds

Baseline Start Time (min): 1.0

Baseline End Time (min): 9.0

Averaging Baseline Noise Example



Viewing the Calculated Results

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Chromatogram Result

Detector Noise (Plot Units)	Detector Drift (Plot Units/hour)	Peak to Peak Noise (Plot Units)	Average Detector Noise (Plot Units)	Average Detector Drift (Plot Units/hour)	Average Peak to Peak Noise (Plot Units)	Baseline Drift (mV)	Baseline Noise (mV)
0,007074	-0,887558	0,048889	0,002777	-2,922607	0,012021	-0,011	33,406

Managing Manually Integrated Results in a Result Set

Managing Manually Integrated Results in a Result Set, Samples and Controls

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Process Sample Set

- Uses Sample Set Information (bracketing)
- Use Acquisition Method Set (recommended)

Review Result Set

- Manual changes to integration
- Click **Quantitate** only
- DO NOT change standards or click Calibrate
- Save Result

Print Result Set

- Will ensure that only latest results will be printed

Managing Manually Integrated Results in a Result Set, Samples and Controls

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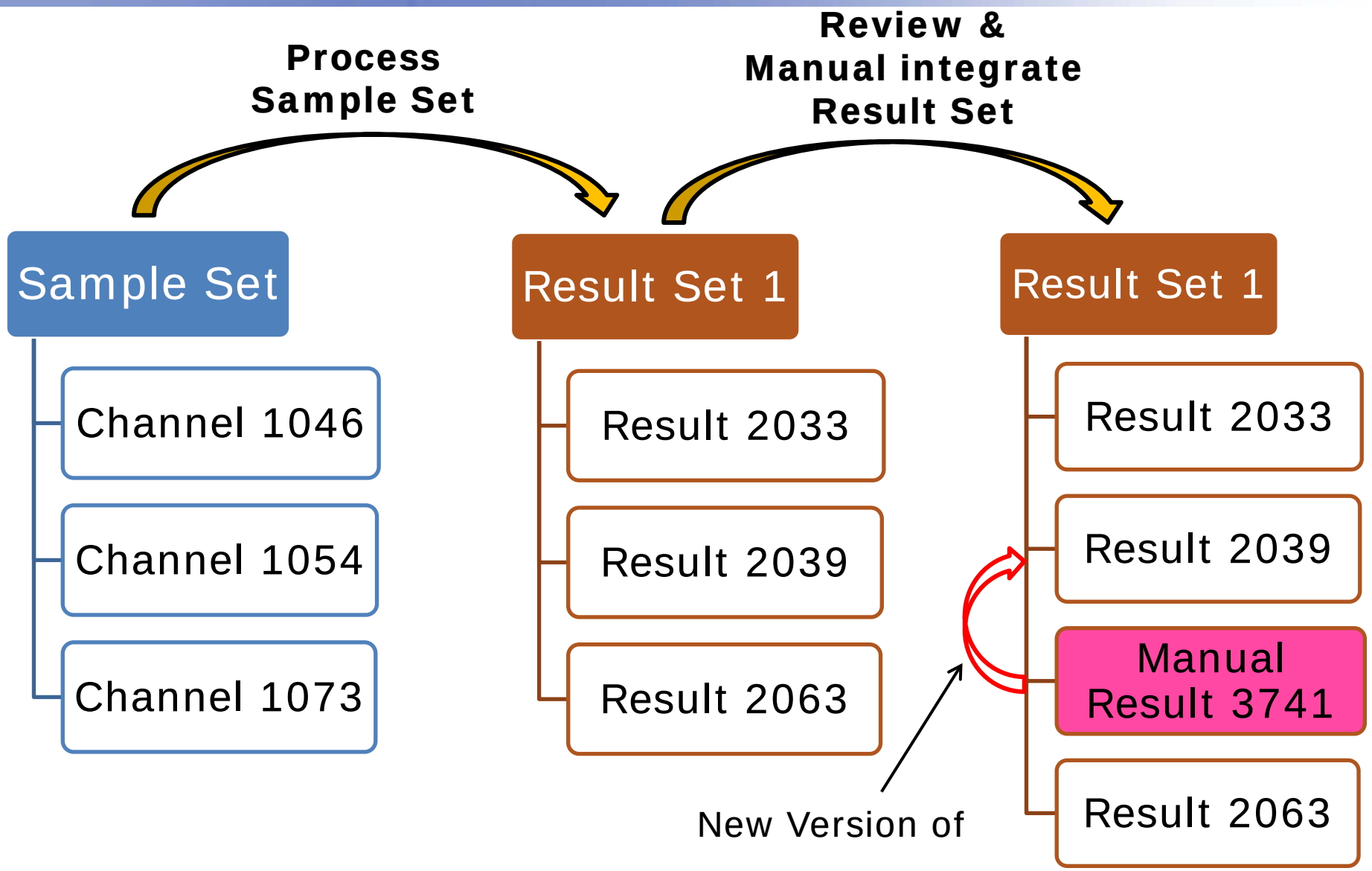

Managing Manually Integrated Results in a Result Set, Standards (& Samples/Controls)

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Process Sample Set

- Uses Sample Set Information (bracketing)
- Use Acquisition Method Set (recommended)

Review Result Set

- Manual changes to integration only
- DO NOT **Calibrate** or change proc. method
- Save Result

Reprocess Result Set

- Select **Use Existing Integration**
- **Calibrate and Quantitate**

Print Result Set

- Will ensure that only latest results will be printed

Managing Manually Integrated Results in a Result Set, Standards (& Samples/Controls)

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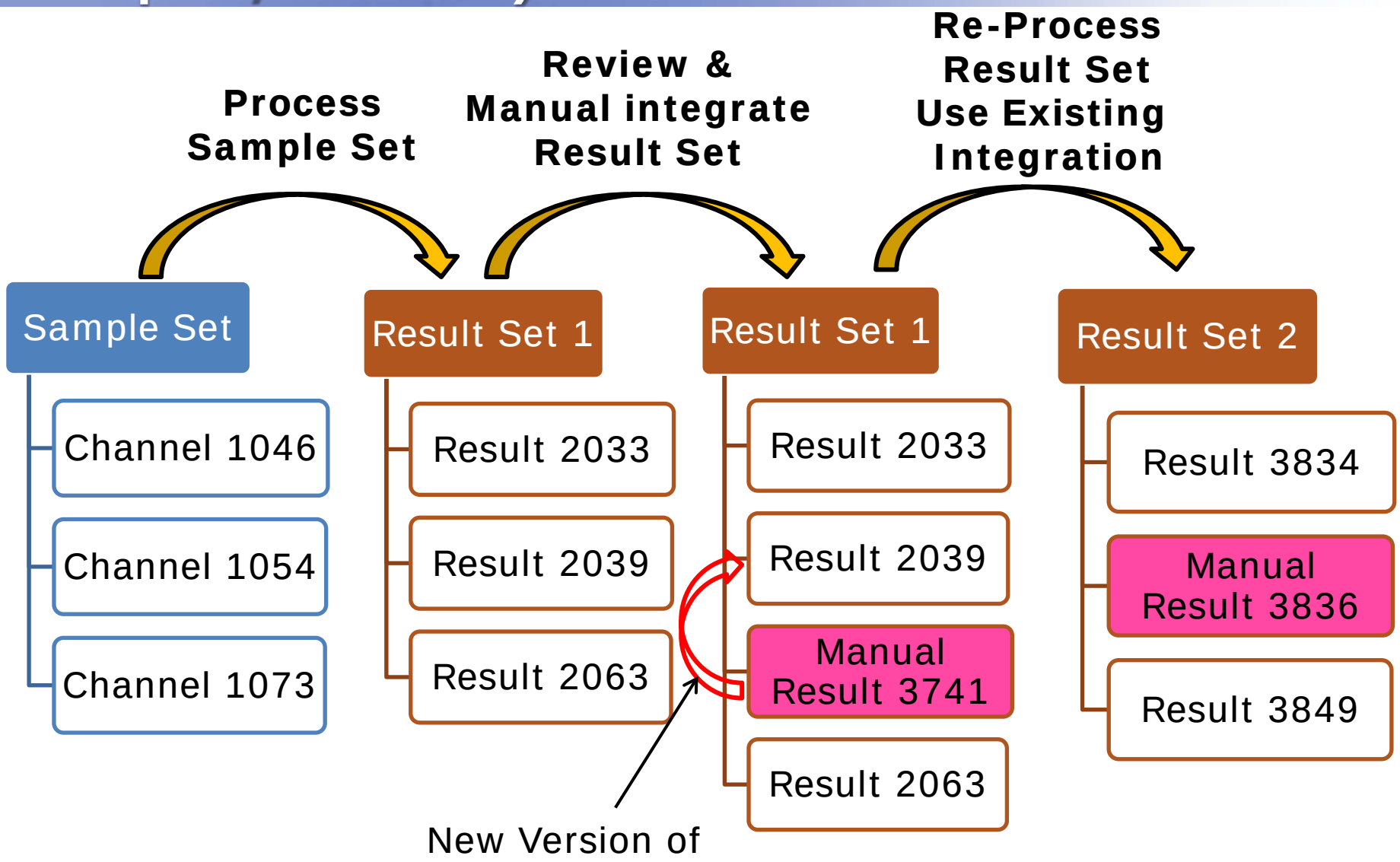

What happens if you do NOT follow this procedure

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Sample set → Process → Result set

	Plate/Well	Inj Vol (uL)	# of Injs	Label	SampleName	Sample Type
1	1:A,1	1.0	2		Softdrink Std1	Standard
2	1:A,2	1.0	2		Softdrink Std2	Standard
3	1:A,3	1.0	2		Softdrink Std3	Standard
4	1:A,4	1.0	2		Softdrink Std4	Standard
5	1:A,5	1.0	2		Softdrink Std5	Standard
6	1:A,6	1.0	2		Softdrink Sample A	Unknown
7	1:A,7	1.0	2		Softdrink Sample B	Unknown
8	1:A,8	1.0	2		Softdrink Sample C	Unknown
9	1:B,1	1.0	2		Softdrink Sample D	Unknown
10	1:B,2	1.0	2		Energy Drink A	Unknown
11	1:B,3	1.0	2		Energy Drink B	Unknown
12	1:B,4	1.0	2		Energy Drink C	Unknown
13	1:B,5	1.0	2		Energy Drink D	Unknown
14	1:B,6	1.0	2		Energy Drink E	Unknown

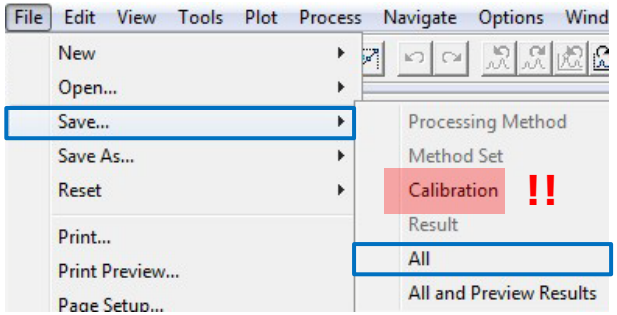
SampleName	Vial	Injection	Sample Type	Calibration Id
Softdrink Std1	1:A,1	1	Standard	7737
Softdrink Std1	1:A,1	2	Standard	7737
Softdrink Std2	1:A,2	1	Standard	7737
Softdrink Std2	1:A,2	2	Standard	7737
Softdrink Std3	1:A,3	1	Standard	7737
Softdrink Std3	1:A,3	2	Standard	7737
Softdrink Std4	1:A,4	1	Standard	7737
Softdrink Std4	1:A,4	2	Standard	7737
Softdrink Std5	1:A,5	1	Standard	7737
Softdrink Std5	1:A,5	2	Standard	7737
Softdrink Sample A	1:A,6	1	Unknown	7737
Softdrink Sample A	1:A,6	2	Unknown	7737
Softdrink Sample B	1:A,7	1	Unknown	7737
Softdrink Sample B	1:A,7	2	Unknown	7737
Softdrink Sample C	1:A,8	1	Unknown	7737
Softdrink Sample C	1:A,8	2	Unknown	7737
Softdrink Sample D	1:B,1	1	Unknown	7737
Softdrink Sample D	1:B,1	2	Unknown	7737

Change std

Change sample

Modified result set

SampleName	Vial	Injection	Sample Type	Calibration Id
Softdrink Std1	1:A,1	1	Standard	7737
Softdrink Std1	1:A,1	2	Standard	7737
Softdrink Std2	1:A,2	1	Standard	7737
Softdrink Std2	1:A,2	2	Standard	7737
Softdrink Std3	1:A,3	1	Standard	7737
Softdrink Std3	1:A,3	2	Standard	7737
Softdrink Std4	1:A,4	1	Standard	7737
Softdrink Std4	1:A,4	2	Standard	7737
Softdrink Std5	1:A,5	1	Standard	7737
Softdrink Std5	1:A,5	2	Standard	7737
Softdrink Sample A	1:A,6	1	Unknown	7737
Softdrink Sample A	1:A,6	1	Unknown	7779
Softdrink Sample A	1:A,6	2	Unknown	7737
Softdrink Sample B	1:A,7	1	Unknown	7737
Softdrink Sample B	1:A,7	2	Unknown	7737
Softdrink Sample C	1:A,8	1	Unknown	7737
Softdrink Sample C	1:A,8	2	Unknown	7737
Softdrink Sample D	1:B,1	1	Unknown	7737
Softdrink Sample D	1:B,1	2	Unknown	7737



Questions

Exercise 2

- System Suitability Calculations
- Noise and Drift Calculations
- Process Sample Set
- Review and Adjust Results