

**Millennium/Empower  
Advance Training for Teva**

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**Reviewing Data**

## Review Multiple Channels

The screenshot shows the 'GEMFIBROZIL as shula/Shula\_Demo - Project' window. The main table lists channels with columns for SampleName, Vial, Injection, Sample Type, Date Acquired, Channel, Channel Description, Acq Method Set, # of Results Stored, and Channel ID. A context menu is open over the row for 'STD 2 ASSAY' (Vial 15, Injection 3), showing options like 'New Method', 'Review', 'Compare', 'Preview/Publisher Process...', 'Print...', 'Export...', 'Alter Sample', 'Run Samples', 'Copy To Project...', 'Lock Channel', 'Unlock Channel', 'View As', 'Copy', 'Paste', 'Hide Column', 'Show All Columns', 'Print Table', and 'Column Properties...'. The status bar at the bottom indicates '33 Selected'.

SampleName	Vial	Injection	Sample Type	Date Acquired	Channel	Channel Description	Acq Method Set	# of Results Stored	Channel ID
1 STD 1 CONTR ASS	22	1	Unknown	24/01/2004 0:54:06	SATIN	276nm	Gemfibrozil_STB_Assay_401007	2	338
2 248922203-1 INITIAL	21	1	Unknown	24/01/2004 0:37:23	SATIN	276nm	Gemfibrozil_STB_Assay_401007	2	337
3 248922203-1 INITIAL	20	1	Unknown	24/01/2004 0:20:39	SATIN	276nm	Gemfibrozil_STB_Assay_401007	2	337
4 248922103-2 INITIAL	19	1	Unknown	24/01/2004 0:03:57	SATIN	276nm	Gemfibrozil_STB_Assay_401007	2	337
5 248922103-1 INITIAL	18	1	Unknown	23/01/2004 23:47:14	SATIN	276nm	Gemfibrozil_STB_Assay_401007	2	338
6 248921003-2 INITIAL	17	1	Unknown	23/01/2004 23:30:32	SATIN	276nm	Gemfibrozil_STB_Assay_401007	2	338
7 248921003-1 INITIAL	16	1	Unknown	23/01/2004 23:13:47	SATIN	276nm	Gemfibrozil_STB_Assay_401007	2	336
8 STD 2 ASSAY	15	3	Stand	22:57:04	SATIN	276nm	Gemfibrozil_STB_Assay_401007	2	336
9 STD 2 ASSAY	15	2	Stand	22:40:22	SATIN	276nm	Gemfibrozil_STB_Assay_401007	2	335
10 STD 2 ASSAY	15	1	Stand	22:23:40	SATIN	276nm	Gemfibrozil_STB_Assay_401007	2	335
11 STD 1 ASSAY	14	3	Stand	22:06:57	SATIN	276nm	Gemfibrozil_STB_Assay_401007	2	335
12 STD 1 ASSAY	14	2	Stand	21:50:13	SATIN	276nm	Gemfibrozil_STB_Assay_401007	2	335
13 STD 1 ASSAY	14	1	Stand	21:33:31	SATIN	276nm	Gemfibrozil_STB_Assay_401007	2	334
14 DILUENT	13	1	Unknown	21:16:48	SATIN	276nm	Gemfibrozil_STB_Assay_401007	2	334
15 SST	1	1	Unknown	21:00:05	SATIN	276nm	Gemfibrozil_STB_Assay_401007	2	334
16 STD 2% IMP. 1 CONTR.	12	1	Unknown	20:43:23	SATIN	276nm	Gemfibrozil_STB_Assay_401007	2	333
17 DILUENT	11	2	Unknown	20:26:38	SATIN	276nm	Gemfibrozil_STB_Assay_401007	2	333
18 DILUENT	11	1	Unknown	20:09:54	SATIN	276nm	Gemfibrozil_STB_Assay_401007	2	333
19 248922203-2 INITIAL	10	1	Unknown	19:33:08	SATIN	276nm	Gemfibrozil_STB_Assay_401007	5	333
20 248922203-1 INITIAL	9	1	Unknown	18:56:22	SATIN	276nm	Gemfibrozil_STB_Assay_401007	5	332
21 248922103-1 INITIAL	8	1	Unknown	18:19:39	SATIN	276nm	Gemfibrozil_STB_Assay_401007	5	332
22 248922103-1 INITIAL	7	1	Unknown	17:42:53	SATIN	276nm	Gemfibrozil_STB_Assay_401007	5	332
23 248921003-2 INITIAL	6	1	Unknown	17:06:09	SATIN	276nm	Gemfibrozil_STB_Assay_401007	5	331
24 248921003-1 INITIAL	5	1	Unknown	16:29:25	SATIN	276nm	Gemfibrozil_STB_Assay_401007	5	329
25 STD 2% IMP. 1	4	2	Stand	16:12:40	SATIN	276nm	Gemfibrozil_STB_Assay_401007	3	328

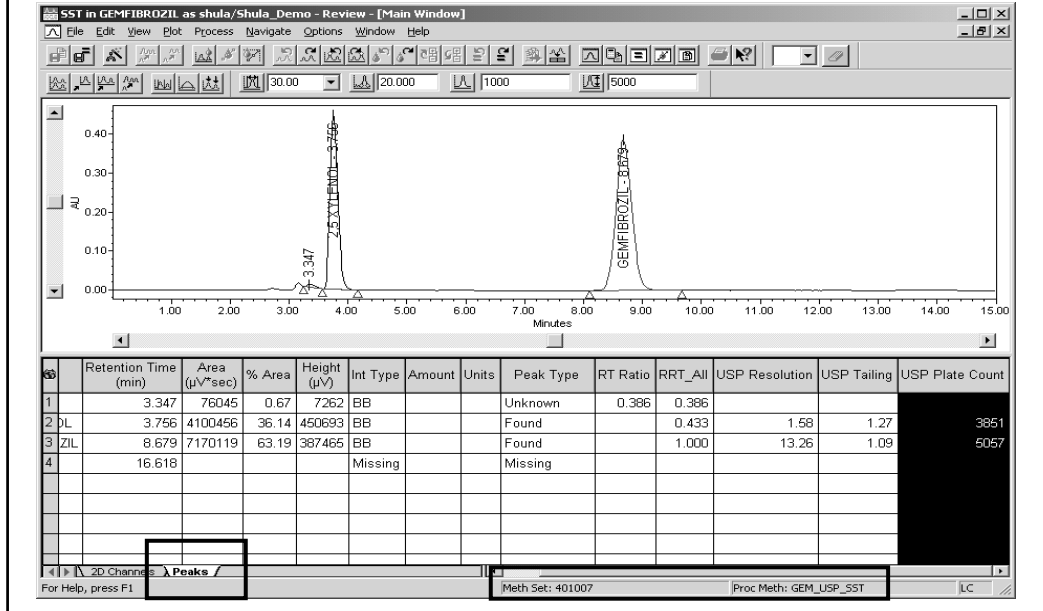
## The list of channels is in the 2D channels view

The screenshot shows the 'SST in GEMFIBROZIL as shula/Shula\_Demo - Review - [Main Window]' window. The top part displays a chromatogram plot with 'AU' on the y-axis (0.00 to 0.40) and 'Minutes' on the x-axis (1.00 to 15.00). Two peaks are visible at approximately 4.5 and 9.0 minutes. Below the plot is a table of channels in the '2D Channels' view. A red box highlights the '2D Channels' tab, and a red arrow points from the 'SST' row in the table to the corresponding peak in the chromatogram.

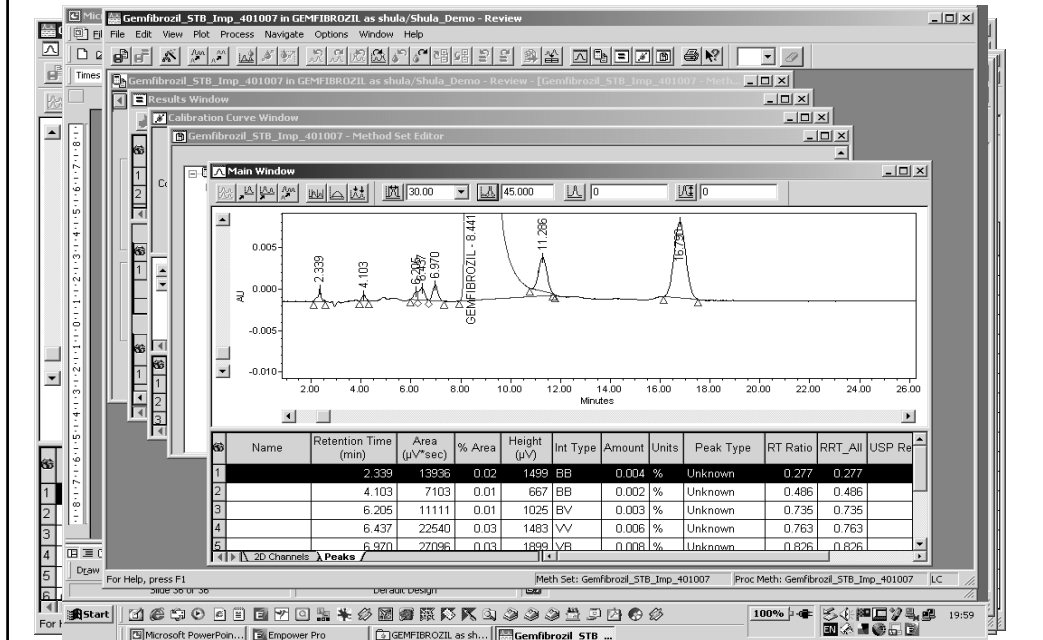
Visible	Vial	Injection	SampleName	Sample Type	Date Acquired	Acq Method Set
1	1	1	SST	Unknown	23/01/2004 13:16:03	Gemfibrozil_STB_Imp_401007
2	1	1	SST	Unknown	23/01/2004 13:40:27	Gemfibrozil_STB_Imp_401007
3	2	1	DILUENT	Unknown	23/01/2004 13:57:13	Gemfibrozil_STB_Imp_401007
4	2	1	DILUENT	Unknown	23/01/2004 14:13:56	Gemfibrozil_STB_Imp_401007
5	3	1	STD 2% IMP. 1	Standard	23/01/2004 15:05:40	Gemfibrozil_STB_Imp_401007
6	3	2	STD 2% IMP. 1	Standard	23/01/2004 15:22:25	Gemfibrozil_STB_Imp_401007
7	3	3	STD 2% IMP. 1	Standard	23/01/2004 15:39:11	Gemfibrozil_STB_Imp_401007
8	3	1	STD 2% IMP. 1	Standard	23/01/2004 15:55:56	Gemfibrozil_STB_Imp_401007



The results of the integration and calculation is in the "Peaks" view

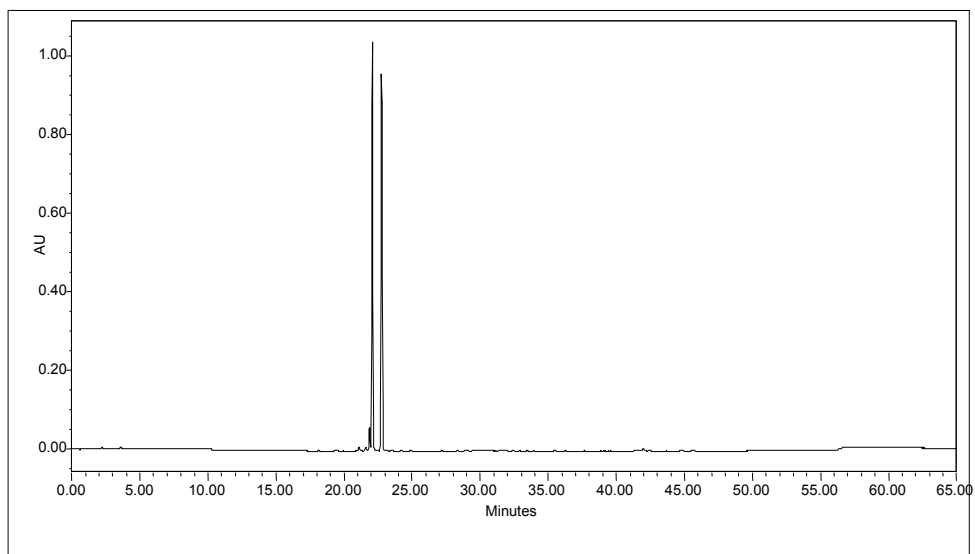


All Review's Windows

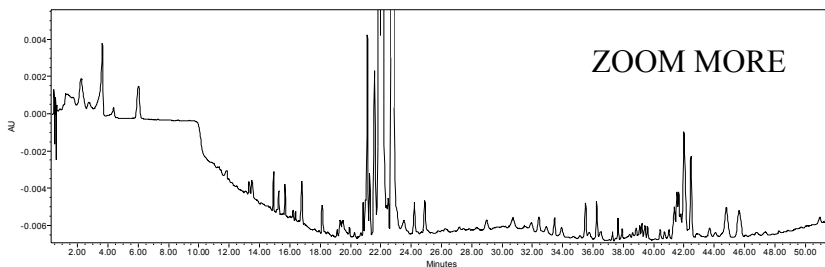
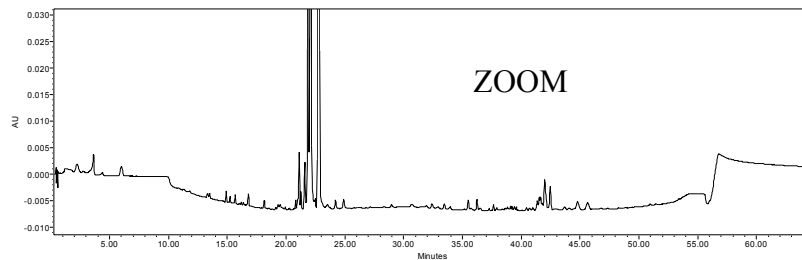


# Integrating Complex Chromatograms

**A Complex Chromatograms**



### Integrating Complex Chromatograms

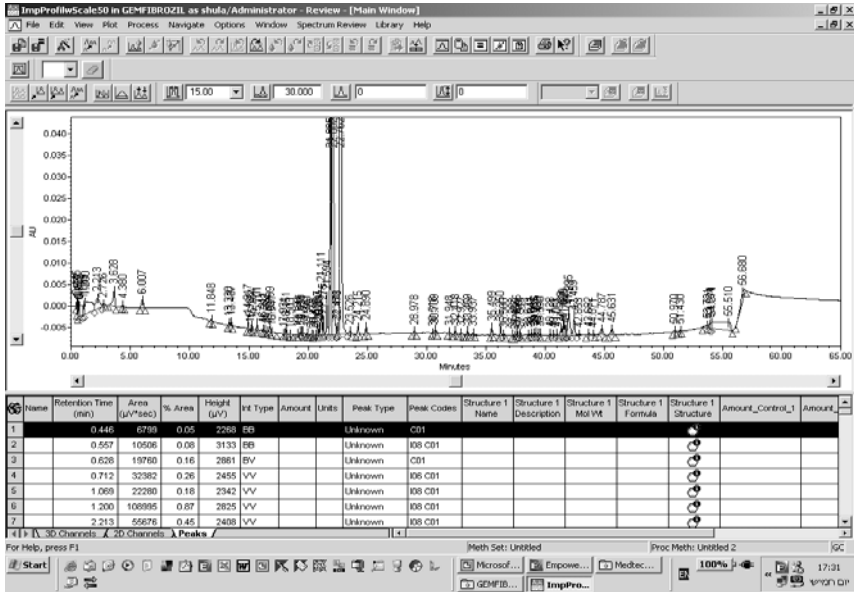


### Integrating Complex Chromatograms Scaling Options

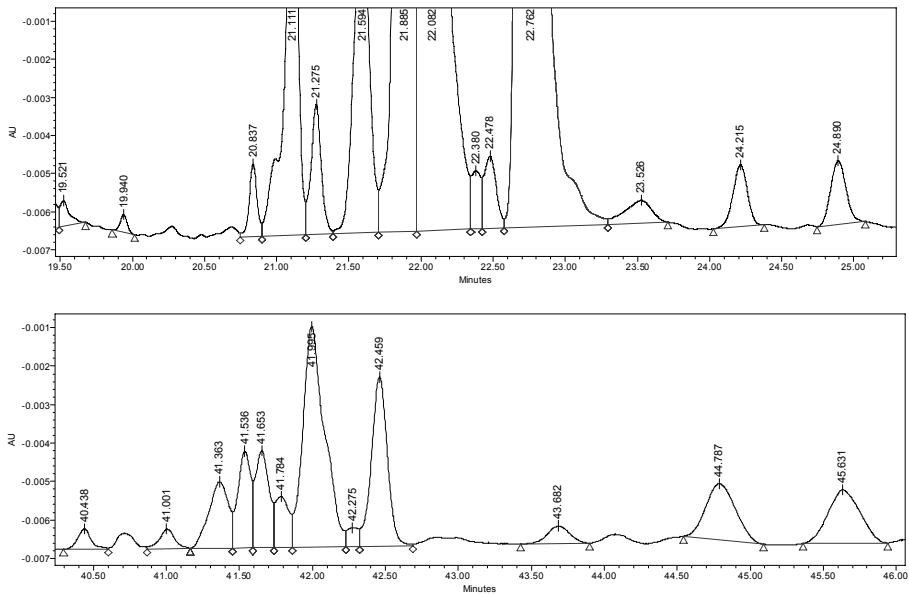
The screenshot shows the ImpPro software interface with the "Plot Properties" dialog box open. The dialog has several tabs: "Plot", "Chromatogram", "Structures", "Overlay", "Peak Labels", "Scaling", "Legend", "Fonts", "Colors", and "Name". The "Scaling" tab is active, showing options for "Scaling Type" (Autoscale, Highest Peak, 2nd Highest Peak, Scale to Baseline, % Highest Peak) and "Normalization" (Normalize X, Normalize Y, Min/Max, Use Fid X Range, All X Values). The "Scale to Baseline" option is selected. The "Scaling" section has fields for X-start, X-end, Y-start, and Y-end. The "Get Values from Plot" button is also visible. The background shows a chromatogram plot with a peak at 34.7881 minutes and a table of peak data.

Name	Retention Time (min)	Area (AU*min)	% Area
1	0.445	6799	0
2	0.557	10506	0.08
3	0.828	19760	0.16
4	0.712	32362	0.26
5	1.069	22200	0.18
6	1.200	108995	0.87
7	2.213	85676	0.45

## A Complex Chromatograms Where to begin?



## Integrating Complex Chromatograms Integration Events



**Integrating Complex Chromatograms**  
**Setting Up The Components in the Component Table**

Clear integration, then select manually the major peaks, go to The Processing Method view and use:  
**Options-> Fill from results**

Name	Retention Time (min)	Area (UV*sec)	% Area	Height (UV)	Int Type	RT Ratio	Points Across Peak	2nd Derivative Apex (min)
1	21.885	332086	3.01	55820	bv		487	
2	22.082	5171447	46.91	1038507	vb		580	
3	22.762	5521381	50.08	954856	tb		616	

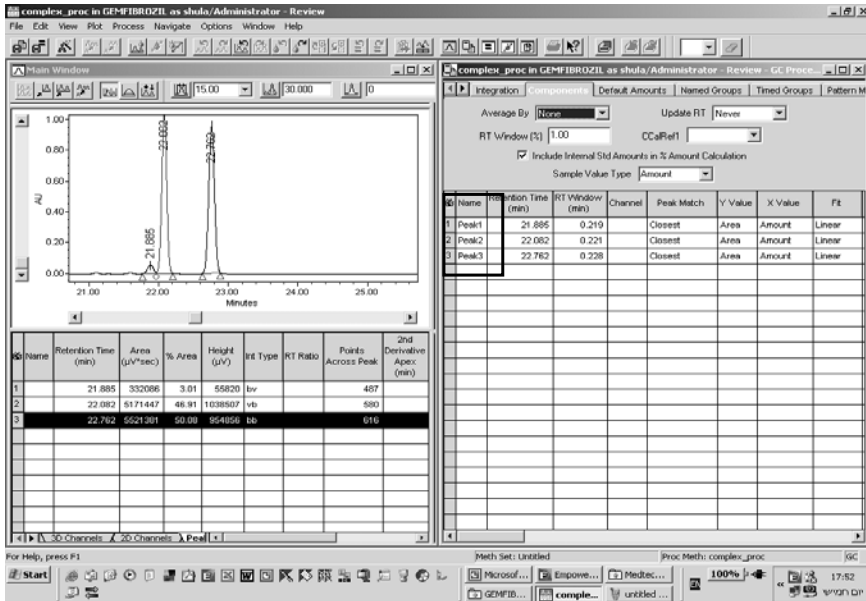
**Integrating Complex Chromatograms**  
**Setting Up The Components in the Component Table**

Fill from Results

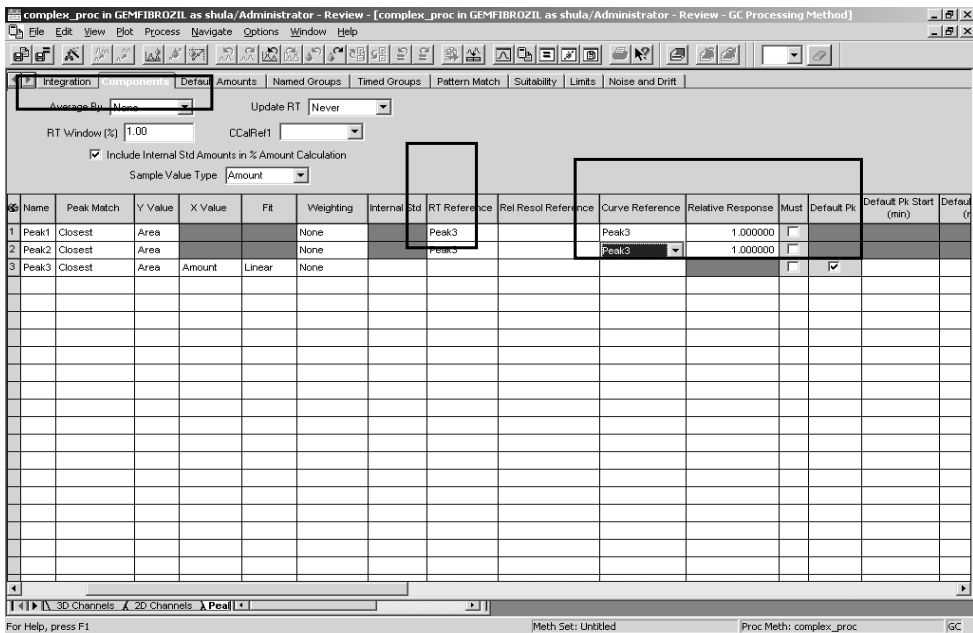
- Show Events
- Show Components
- Fill from Results
- Set Peak Width
- Set Threshold
- Set Minimum Area
- Set Minimum Height
- Method Set Options...
- Copy All Limits
- Copy Selected Limits
- Set GC Reference RT

Name	Retention Time (min)	Area (UV*sec)	% Area	Height (UV)	Int Type	RT Ratio	Points Across Peak	2nd Derivative Apex (min)
1	21.885	332086	3.01	55820	bv		487	
2	22.082	5171447	46.91	1038507	vb		580	
3	22.762	5521381	50.08	954856	tb		616	

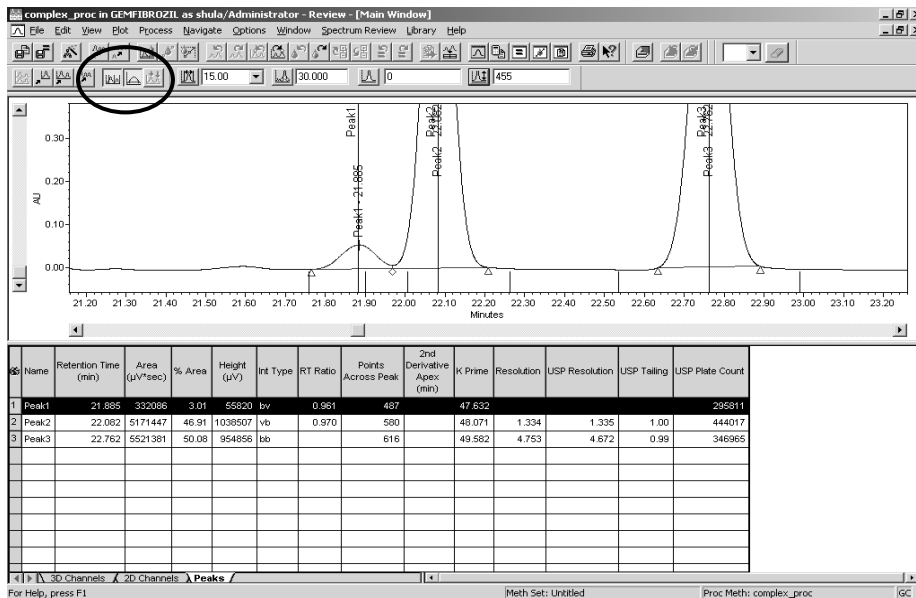
**Integrating Complex Chromatograms  
Setting Up The Components in the Component Table**



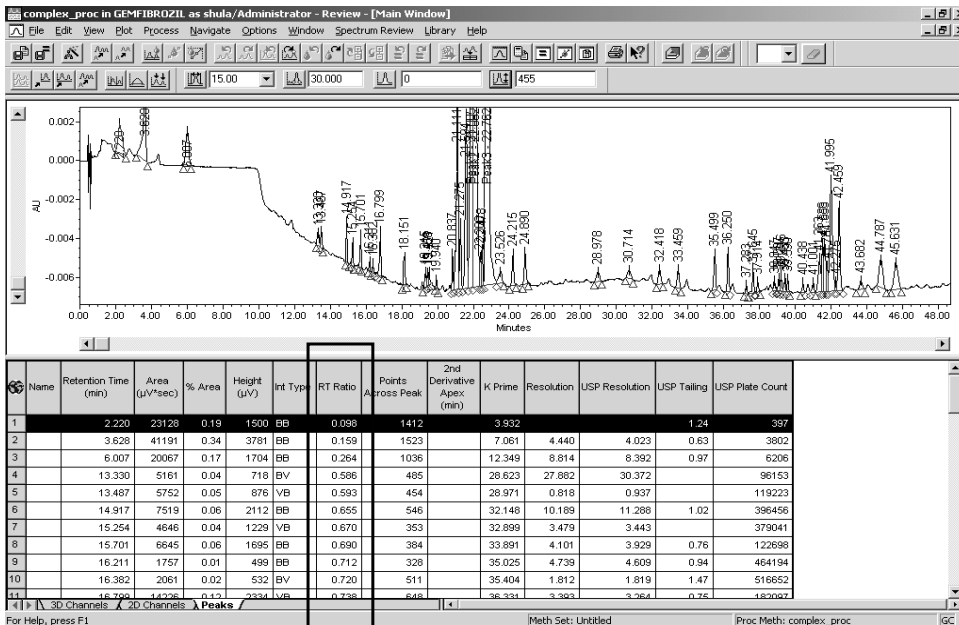
**Integrating Complex Chromatograms  
Setting Up The Components in the Component Table**



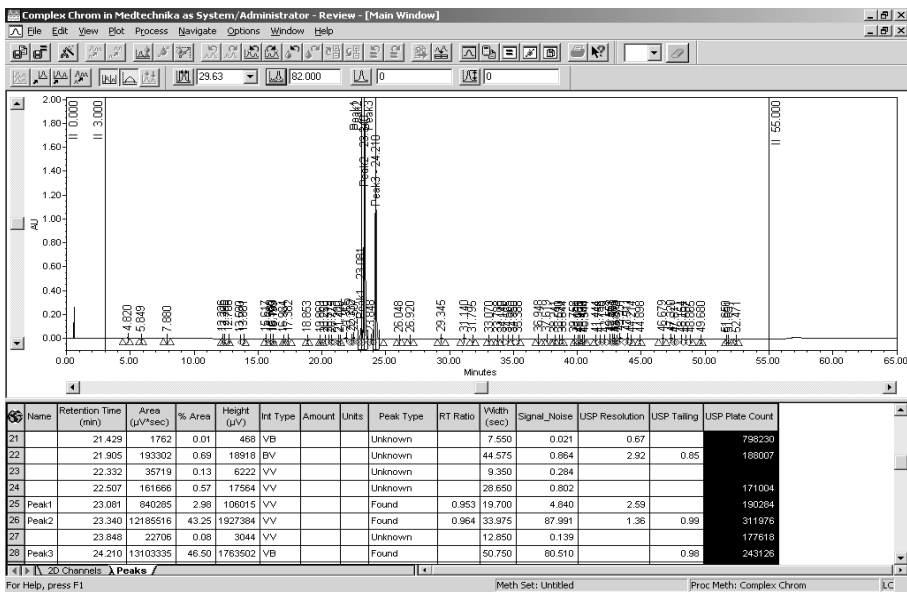
**Integrating Complex Chromatograms**  
 Use: *Show Components* and *Show Events*



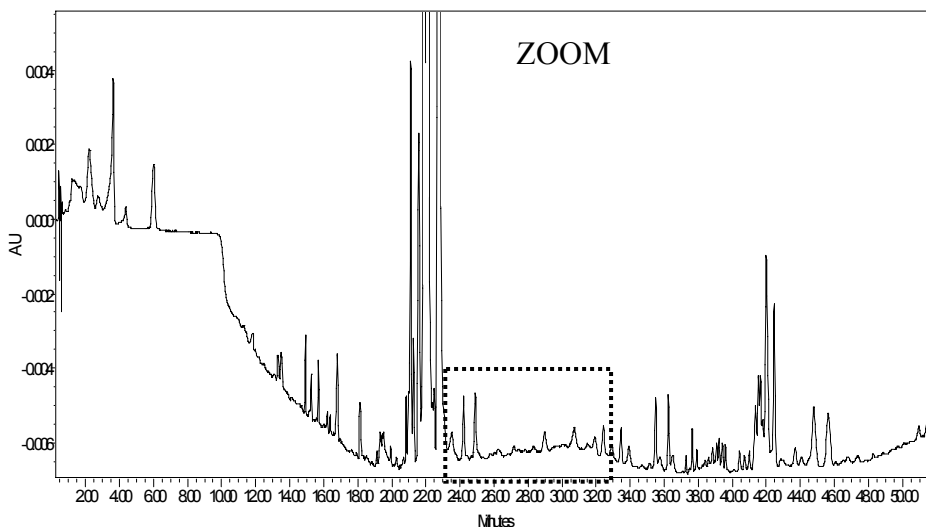
**Integrating Complex Chromatograms**



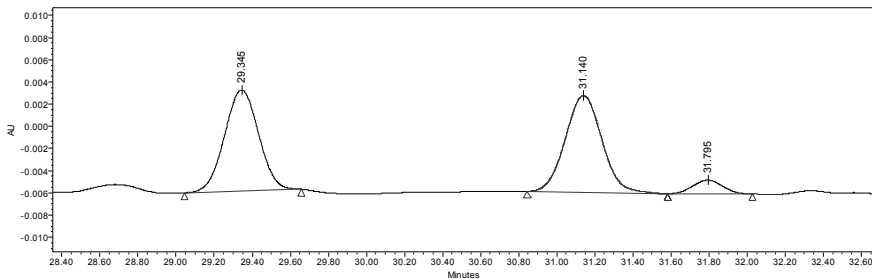
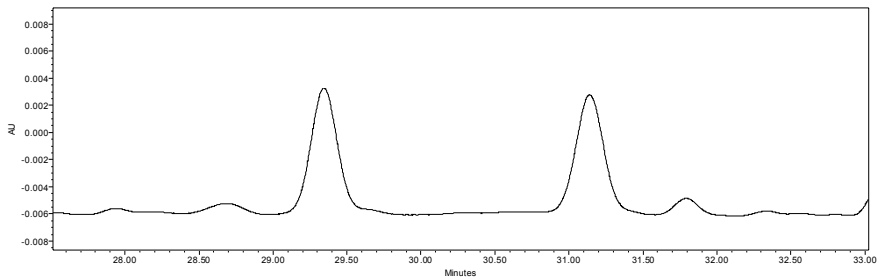
## Integrating Complex Chromatograms Inhibit Integration



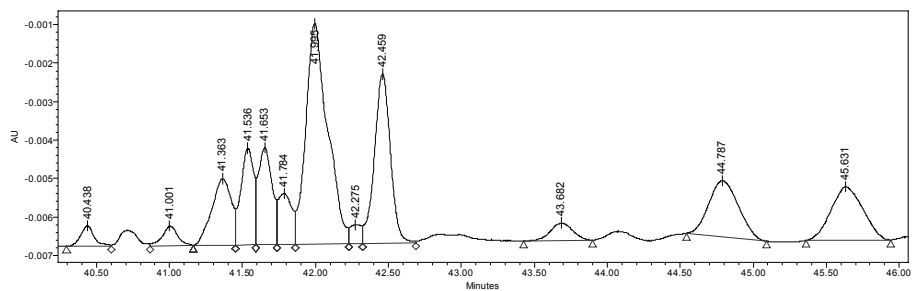
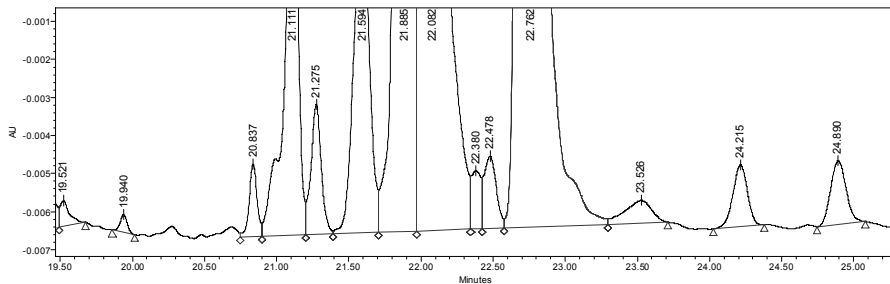
### Integrating Complex Chromatograms Select a small narrow peak and get Peak width and Threshold



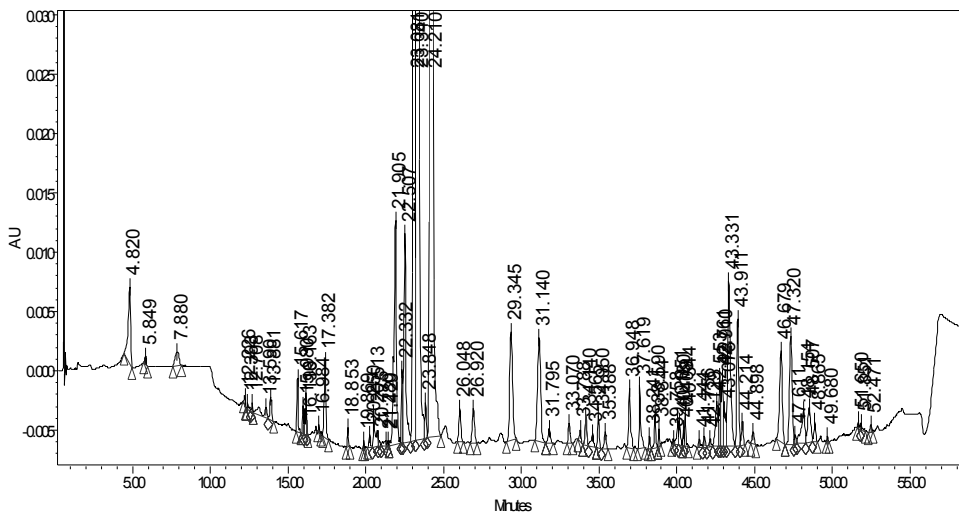
### Integrating Complex Chromatograms



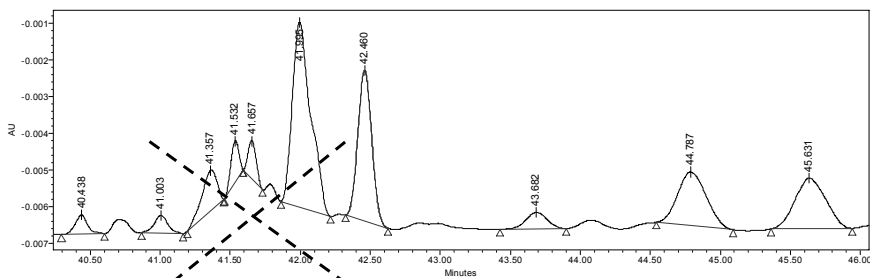
### Integrating Complex Chromatograms Integration Events



**Integrating Complex Chromatograms**  
**Just from Peak Width, Threshold & Inhibit Integration**

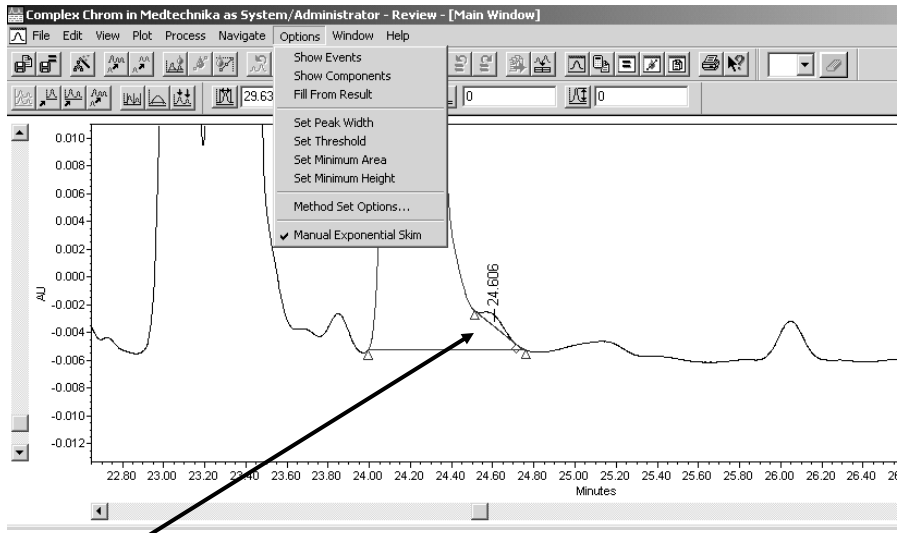


**Integrating Complex Chromatograms**  
**Valey to Valey**

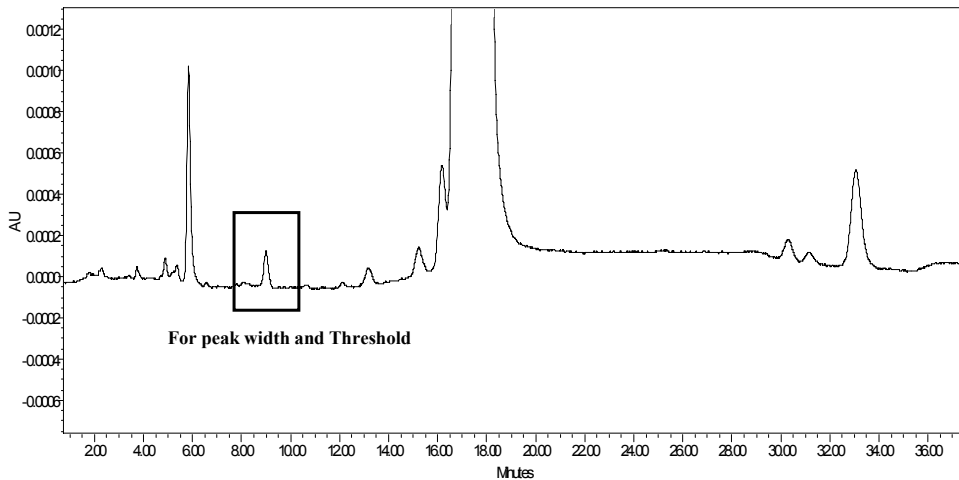


Name	Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int Type	RT Ratio	Points Across Peak	2nd Derivative Apex (min)	K Prime	Resolution	USP Resolution	USP Tailing	USP Plate Count
	40.438	3186	0.03	52	BB		732		88.861	6.299	6.123	1.07	1053671
	41.003	3323	0.03	48	BB		713		90.118	3.511	3.322	1.10	983926
	41.357	8317	0.07	110	BB		629		90.905	1.862	1.813	0.78	632089
	41.532	4537	0.04	113	BB		316		91.293	1.076	1.087	0.93	2317131
	41.657	4170	0.04	103	BB		326		91.570	1.097	1.140	1.08	2253306
	41.995	45868	0.39	505	BB		852		92.323	1.934	1.579	1.36	465268
	42.460	28492	0.25	411	BB		737		93.355	2.202	1.830	1.08	826035
	43.682	4933	0.04	46	BB		1132		96.070	5.217	5.172	0.97	368950

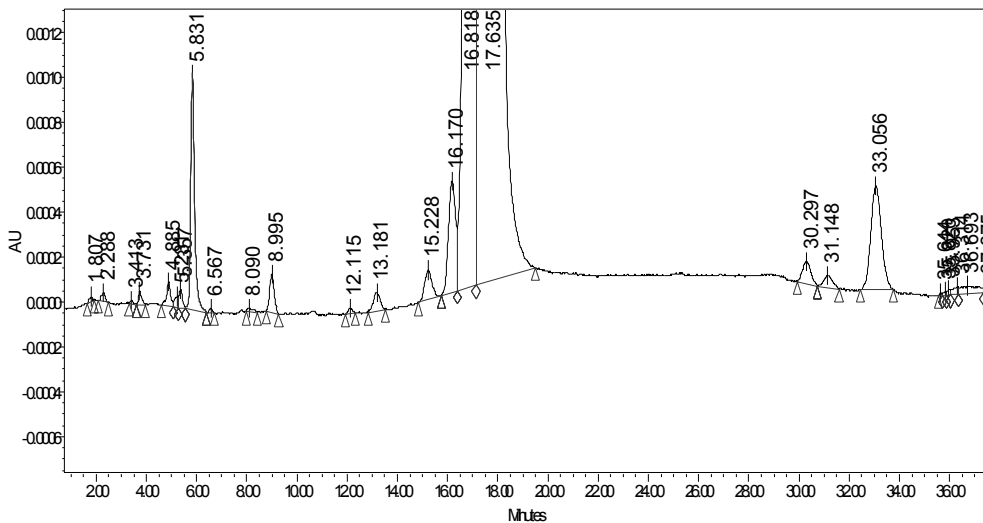
**Integrating Complex Chromatograms**  
**Manual Exponential Skim**



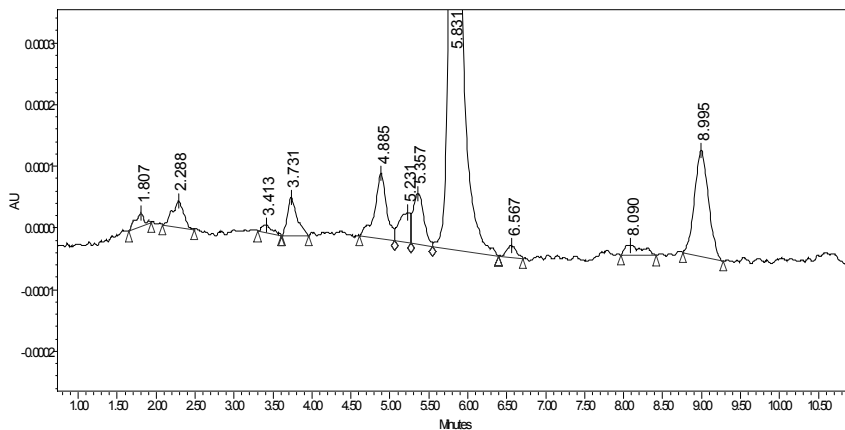
**Integrating Complex Chromatograms**  
**Height Rejection – How to do it right**



**Integrating Complex Chromatograms**  
**Height Rejection – How to do it right**



**Integrating Complex Chromatograms**  
**Height Rejection – How to do it right**



**A segment in the chromatogram:**  
**Some of them are not peaks**

## Height Rejection: $h > 3x$ baseline noise

Integration Components Default Amounts Named Groups Timed Groups Pattern Match **Suitability** Limits Noise and Drift

Calculate Suitability Results

Calculate Suitability Results for Unknown Peaks

System and Separation Efficiency

Void Volume Time (min) 1.800

US Pharmacopoeia  Japanese Pharmacopoeia

European Pharmacopoeia  All

Tangent Percent 61

Baseline Noise and Drift Measurements

Maximum Allowable Noise  Maximum Allowable Drift

% Run Time Over Which to Average 5.0

Baseline Start Time (min) 22.60 Baseline End Time (min) 23.60

## Baseline Start Time and Baseline End Time

Noise (maximum voltage change over a 30-second interval). Noise value is a number of 30-second intervals as determined by the % Run Time Over Which to Average parameter

The software determines the 30-second intervals by counting from the Baseline Start Time and the Baseline End Time toward the middle of the chromatogram.

If the averaged region is  $< 30$  seconds, noise is reported as a blank.

Condition	Setting
Total Run Time	10 minutes
% of Run Time to Average	5%
Average Time	0.5 minute (5% of 10 minutes)
Baseline Start	1 minute
Baseline End	9 minutes

**USP Noise**  
System Suitability

## Signal (Peak Height) to Noise



- Must use System Suitability and Custom Fields\*\*\*
  - ❑ Equation to use to calculate Signal/Noise:
  - ❑  $\text{Height}/(1000 * \text{Baseline Noise})$
  - ❑ (same injection for noise and peak for s/n calculation)
  
- Modifications to this calculation:
  - ❑ Using different sample to calculate signal to noise
  - ❑  $(\text{Height}/1000)/S0101.1.2487(\text{Baseline\_Noise})$  where S0101 is the Sample label, 1 is the injection number and 2487channel1 is the Channel Name)

\*\*\* - Covered in the custom fields section

## Detector Noise and Drift (ASTM)

Specify a time to calculate noise and drift results. For averaged noise/drift values, specify a segment width (in seconds) for the software to divide the data into segments and averages for each segment.

The noise and drift chromatogram results include: Total Values – Detector Noise, Peak to Peak Noise, and Detector Drift

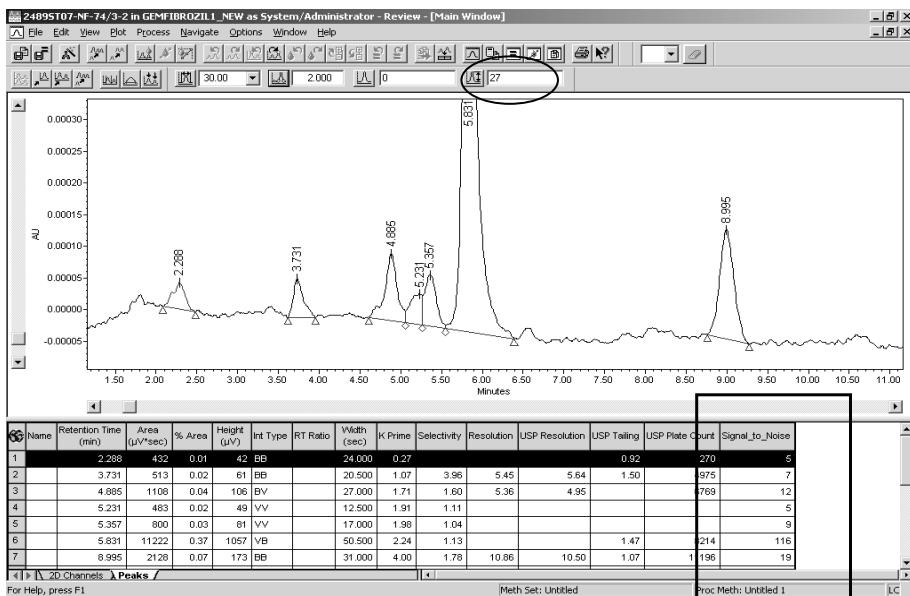
Average Values – Average Detector Noise, Average Peak to Peak Noise, and Average Detector Drift

A screenshot of a software interface window titled "Noise and Drift Parameters". The window has a menu bar with "Integration", "Components", "Default Amounts", "Named Groups", "Timed Groups", "Suitability", "Limits", and "Noise and Drift". The main area contains a checked checkbox labeled "Calculate Detector Noise and Drift". Below this are three input fields: "Start Time: (min)" with the value "5.000", "Stop Time: (min)" with the value "10.000", and "Segment Width: (sec)" with the value "60".

Only Detector Noise

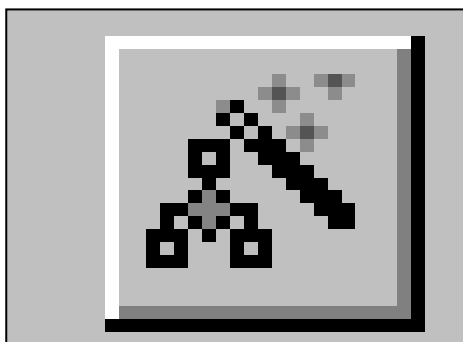


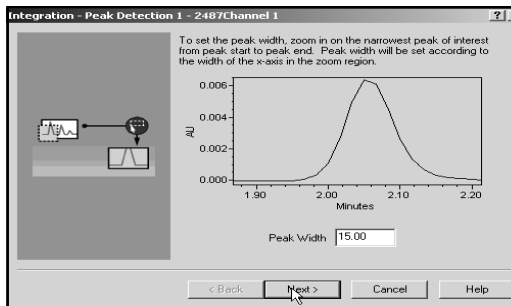
## Integrating Complex Chromatograms



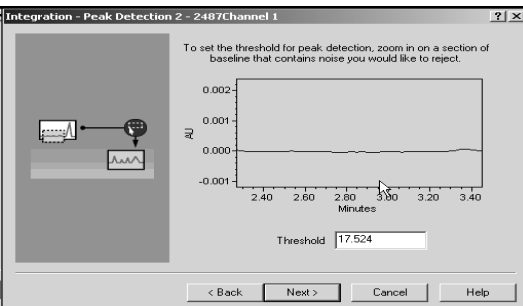
## Simple Processing Using the Processing Wizard

- Pre-enter the amounts in Run Samples or Alter Sample for the standard – even for Internal Standard
- Always select low concentration standards/unknowns when using the wizard to create a Processing Method
- Recommended to select an unknown to edit/test
- Save the processing method, batch process using the Sample Set, Injections or Channel View
- (If using the Injection/Channel view, make sure to select Standards before unknowns)

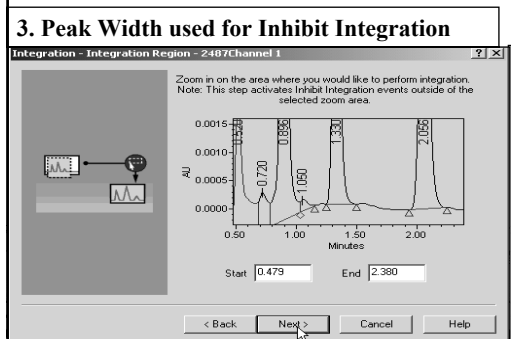




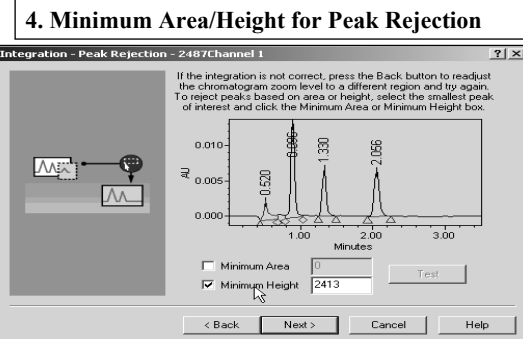
### 1. Peak Width used for Data Bunching



### 2. Threshold for Peak Detection



### 3. Peak Width used for Inhibit Integration



### 4. Minimum Area/Height for Peak Rejection

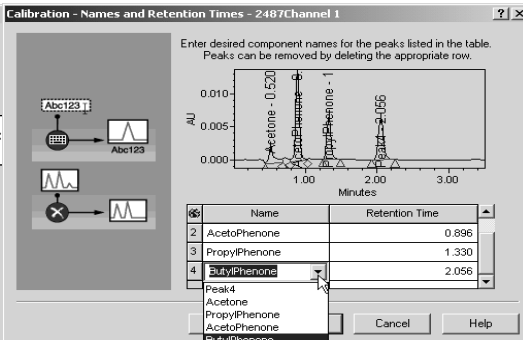
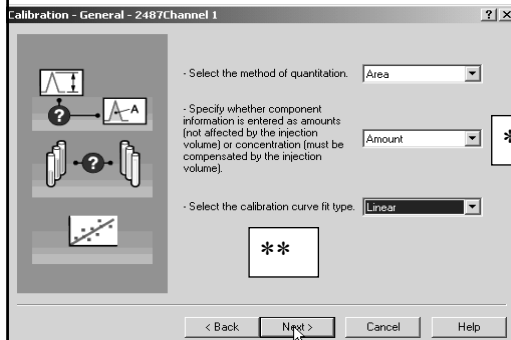
## 5. Calibration - General

\*Injection Volumes for concentration

\*\*Recommended for Linear - 3 standard concentrations, 3<sup>rd</sup> order - 5 concentrations, etc.

## 6. Component Names

If a standard was selected, the component names displayed in the drop down box is from the names entered in the Amounts table in Run Samples or Alter Sample



## 7. Default Amounts

Ignore unless using Single Point Calibration through Zero

Use Run Sample Amounts or Default Amounts from the Processing Method view

## 8. External or Internal Calibration

For internal standard, select the component desired (names will be displayed from the entries in step # 6 of the wizard).

**Calibration - Default Amounts - 2487Channel 1**

Enter an amount and the corresponding units for each component in the table. Note: The amounts entered here are default amounts and are superseded by amounts entered in the Run Samples Window or with the Alter Sample tool.

#	Name	Amount	Units
1	Acetone		
2	AcetoPhenone		
3	PropylPhenone		
4	ButylPhenone		

**Calibration - Internal Standards - 2487Channel 1**

Select type of calibration:

- External Standard Calibration
- Internal Standard Calibration

OR Internal Standard Calibration With:

- Single Internal Standard
- Multiple Internal Standards

Select Component To Use As Standard:

- AcetoPhenone
- Acetone
- AcetoPhenone
- PropylPhenone
- ButylPhenone

## 9. Method Name is the processing method

\*Regulated customers must enter in a comment

## 10. View the wizard results for the standard

Select the peaks tab and verify all desired peaks are identified.

**Processing Method Name - 2487Channel 1**

Method Name: Basic Processing Method

Default Comments:

Comments: \*

**Standard2 in M32\_40\_Training as System/Administrator - Review - [Main Window]**

File Edit View Plot Process Navigate Options Window Help

15.00 17.524 0 2413

0.012  
0.010  
0.008  
0.006  
0.004  
0.002  
0.000

Acetone-0.520  
AcetoPhenone-0.896  
PropylPhenone-1.330  
ButylPhenone-2.056

Minutes

#	Name	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)	Int Type	Amount	Units	Peak Type
1	Acetone	0.520	13899	11.88	2540	BV	10.000000	ug	Found
2	AcetoPhenone	0.896	46776	39.98	12607	VV	10.000000	ug	Int Std
3	PropylPhenone	1.330	24894	21.27	6413	BB	10.000000	ug	Found
4	ButylPhenone	2.056	31444	26.87	6347	BB	10.000000	ug	Found

For Help, press F1 Meth Set: Unlabeled Proc Meth:

### 11. Method Name is the processing method

\*Regulated customers must enter in a comment

### 12. View the wizard results for the standard

Select the **Peaks** tab and verify all desired peaks are identified.

The screenshot shows two windows from the software. The left window is titled 'Processing Method Name - 2487Channel 1'. It has a 'Method Name' field containing 'Basic Processing Method', a 'Default Comments' dropdown, and a 'Comments' text area with an asterisk '\*'. The right window is titled 'Standard2 in M32\_40\_Training as System/Administrator - Review - [Main Window]'. It displays a chromatogram with peaks labeled 'Acetone-0.520', 'AcetPhenone-0.896', 'PropylPhenone-1.330', and 'ButylPhenone-2.056'. Below the plot is a table with the following data:

Name	Retention Time (min)	Area (μV*sec)	% Area	Height (μV)	Int. Type	Amount	Units	Peak Type
1 Acetone	0.520	13899	11.88	2540	EV	10.000000	ug	Found
2 AcetPhenone	0.896	46776	39.98	12607	VV	10.000000	ug	Int Std
3 PropylPhenone	1.330	24894	21.27	6413	BB	10.000000	ug	Found
4 ButylPhenone	2.056	31444	26.87	6347	BB	10.000000	ug	Found

### 13. Verify peak Integration

Select a 2<sup>nd</sup> standard and click the Integrate button.

### 14. Verify peak identification

Select the Calibrate. Make sure all desired peaks are properly ID'ed.

The screenshot shows two windows. The left window is titled 'Standard1 in M32\_40\_training as System/Administrator - Review - [Main Window]'. It shows a chromatogram with peaks labeled 'Acetone-0.520', 'AcetPhenone-0.896', and 'PropylPhenone-1.330'. The 'Integrate' button is highlighted. The right window is titled 'Standard1 in M32\_40\_Training as System/Administrator - Review - [Main Window]'. It shows a chromatogram with peaks labeled 'Acetone-0.520', 'AcetPhenone-0.896', 'PropylPhenone-1.330', and 'ButylPhenone-2.000'. The 'Calibrate' button is highlighted. Below the plots are data tables. The left table has the following data:

Visible	Vial	Injection	SampleName	Sample Type	Date Acquired	Acq Method Set
<input checked="" type="checkbox"/>	4	1	Standard3	Standard	5/8/2001 12:08:52 PM	Custom_Fields_Terst
<input checked="" type="checkbox"/>	3	1	Standard2	Standard	5/8/2001 12:04:10 PM	Custom_Fields_Terst
<input checked="" type="checkbox"/>	2	1	Standard1	Standard	5/8/2001 11:59:33 AM	Custom_Fields_Terst
<input checked="" type="checkbox"/>	2	1	Standard1	Standard	5/8/2001 12:26:54 PM	Custom_Fields_Terst

The right table has the following data:

Visible	Vial	Injection	SampleName	Sample Type	Date Acquired	Acq Method Set
<input checked="" type="checkbox"/>	4	1	Standard3	Standard	5/8/2001 12:08:52 PM	Custom_Fields_Terst
<input checked="" type="checkbox"/>	3	1	Standard2	Standard	5/8/2001 12:04:10 PM	Custom_Fields_Terst
<input checked="" type="checkbox"/>	2	1	Standard1	Standard	5/8/2001 11:59:33 AM	Custom_Fields_Terst
<input checked="" type="checkbox"/>	2	1	Standard1	Standard	5/8/2001 12:26:54 PM	Custom_Fields_Terst

**15. Modify the acquisition Method Set to include the newly created processing method(s) and save it**

**16. Select data to batch Process**

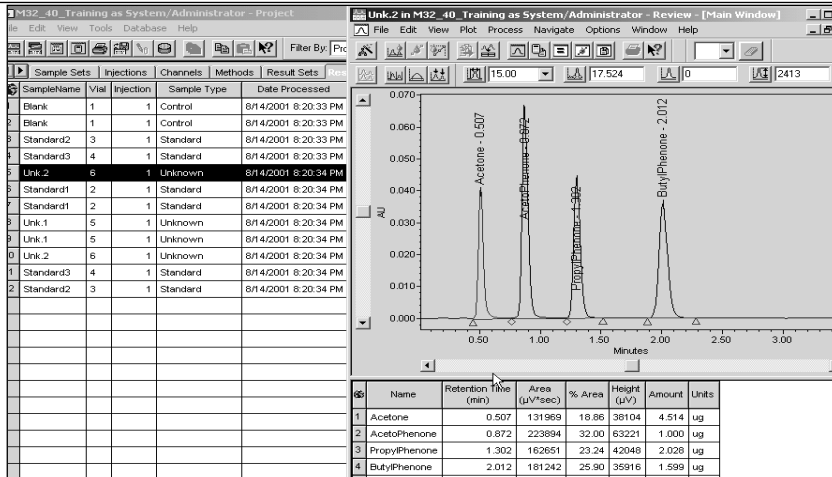
Highlight Sample Sets, Injections, or Channels and select process Standards before Sample & Controls

**17. Select the Processing Method/Method Set (Calibrate and Quantitate) & OK.**

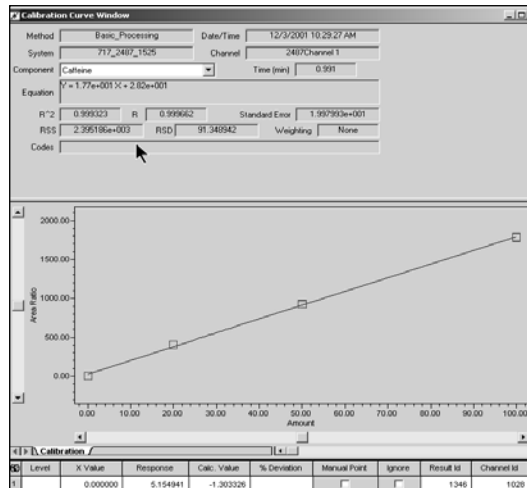
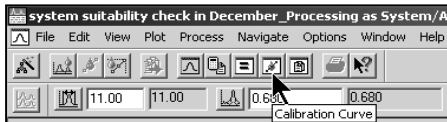
Use Clear Calibration to create a new calibration curve instead of adding points to an existing curve

## 18. Review the processed results

Highlight the Results Tab, select the desired samples and click the review icon



## 19. Select the Calibration Curve icon to view Calibration information



## Order of Calibration Processing

### Run Samples\*\*

**Calibrate & Report  
uses most current  
Cal ID – will change**

### Review\*\*

**Calibrate & Report  
uses most current  
Cal ID – will change**

**Batch Process – uses Cal  
ID at beginning of  
processing  
will not change**

\*\*If you calibrate in Review and save, the data processing in Run Samples will be affected\*\* if using the same processing method

## General Rules for Develop Peak Integration Parameters

- Take 2 chromatograms in to Review
  - ❑ Lowest concentration standard or sample for testing
  - ❑ High concentration standard of unknown
- Preferable to include a low concentration sample
  - ❑ Will have extraneous peaks needed to be rejected

Peak Integration – General Rules

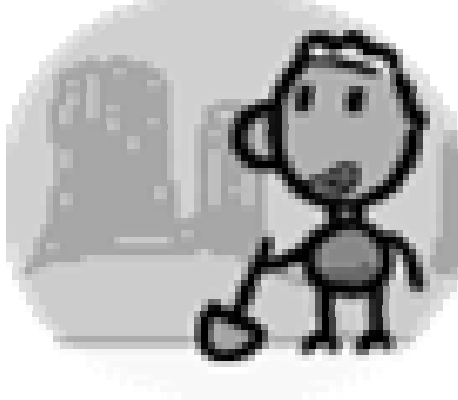
## Top 10 Calibration Problems

- 10) Processing different type channels for standards/sample (254 vs 280)  
(only works with interchannel processing procedure (MS option enabled – covered in 3D Processing section))
- 9) Not using clear calibration (points are added to the existing calibration curve, and not a new curve) – incorrect calibration values
- 8) Not enough data points for calibration curve  
(example: Quadratic curve with only 2 calibration points)
- 7) Point for unknown outside the calibration curve
- 6) “Peak Match” ie 2nd Peak selected when no 1st Peak in component table

## Top 10 Calibration Problems

- 5) Internal Standard Peak not found – no calibration curve for any components referencing the Internal Standard
- 4) Peak falls outside the processing methods component’s retention time window (use “Show Components”)
- 3) Using the same processing method in Review as in Run Samples during processing
- 2) Highlighting unknowns before standards when processing (standards must be selected before unknowns – unless using Label and Label Reference)
- 1) Component names entered in the Amounts button are miss-spelled in the component table for the processing method  
(M32 4.0 with standards will alleviate this problem)

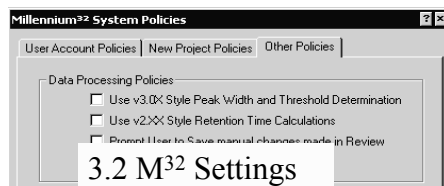
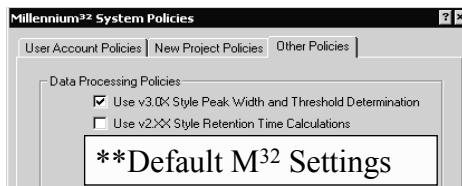
## The Secrets to Peak Detection



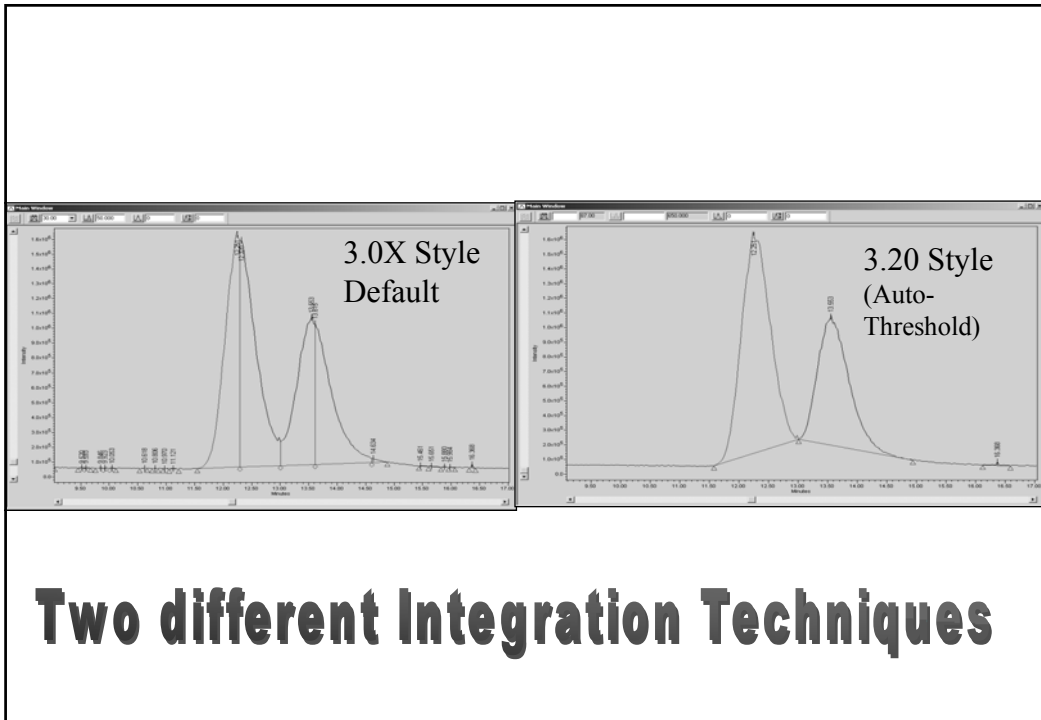
- 4 ways of Peak Detection
- Millennium 3.0 Integration and Peak Detection basics
- Millennium 3.2 Auto peak width & threshold calculations
- Apex Tracking and Inflection Points
- Integration Events

## Peak Detection 4 Ways

- 1) You determine **Peak Width** and **Threshold** settings\*\*
- 2) Auto calculation of Peak width and Threshold (3.2)
  - ❖ Enabled in System Policies
- 3) Peak Apex Tracking – Apex
  - ❖ Enabled in Registry, System Policies, Project Properties, and Processing Method
- 4) Peak Apex Tracking – Inflection Points



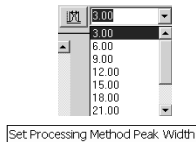
\* Recommended for regulated environments



# Two different Integration Techniques

## Basic of Peak Integration – Default Way

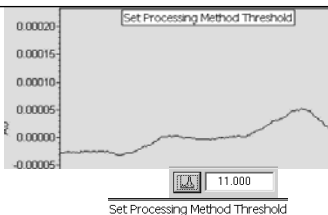
1) Select the smallest value in the peak width box



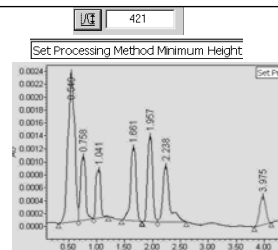
3) Click the Integrate icon



2) Box on the noise & click on the Threshold icon



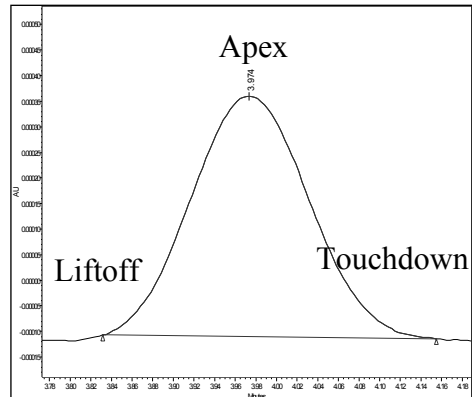
4) Click on smallest peak and click on Minimum Height (minimum area)



## Basic of Peak Integration

➤ For Integration to occur:

- ❑ Must have a Liftoff (start)
- ❑ Must have an Apex (top)
- ❑ Must have a Touchdown (Bottom)

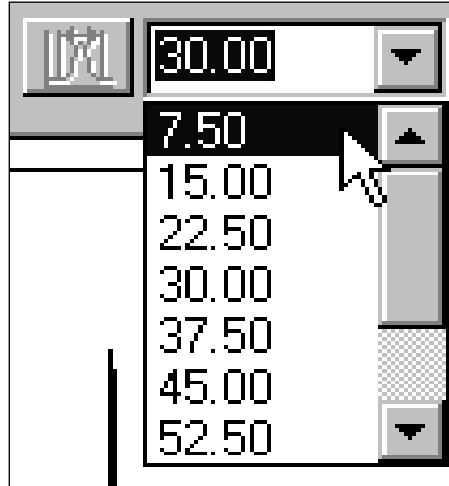


## *Global Peak Detection Parameters and Peak Detection Theory*

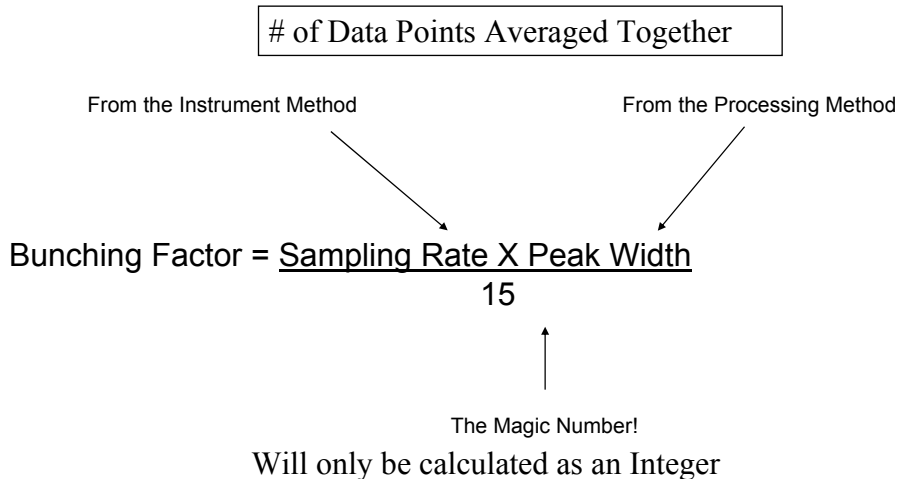
- Peak Detection:
- ❑ Peak Width - Bunching Factor
  - ❑ Threshold – slope determination
- Peak Integration - Rejection:
- ❑ Minimum Height (Peak Rejection)
  - ❑ Minimum Area (Peak Rejection)
- Understanding the basics of peak detection theory allows you to set these parameters based on the knowledge of how those settings will affect peak detection.

## Manually Calculating Peak Width - Threshold

- Step 1 – Turn off the Bunching factor
  - ❑ Select the lowest value listed in the drop down box
  - ❑ These values will be determined by the data rate selected in the Instrument method
  
- Bunched data points are used only to detect peaks; all raw data points are used for integration.



## *The Bunching Factor* *What is it?*



## The Meaning of Threshold

### Threshold & Peak Detection

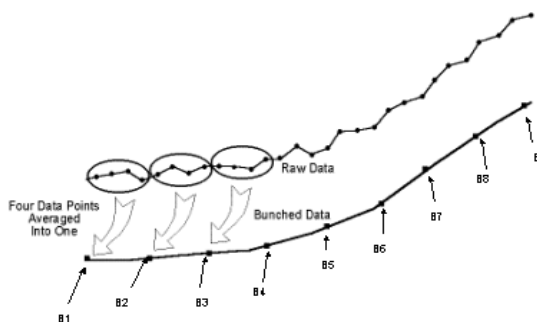
*Threshold* value separates baseline noise from peaks.

Peaks are detected by:

If the average of 2 slopes are  $\geq$  threshold value, **peak liftoff** is detected.

When the sign of the slope changes, the **peak apex** is detected.

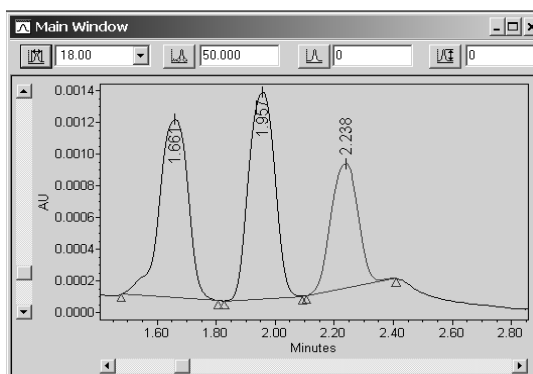
When two slopes are  $<$  threshold value, **peak touchdown** is detected.



Slopes are calculated between each bunched data point.

### 3.00 Style Peak Width and Threshold Determination

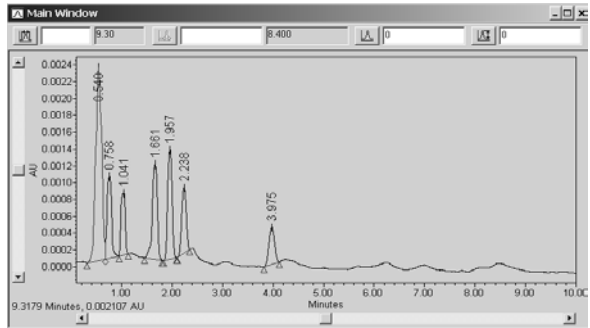
1. Threshold and Peak Width calculated manually
  - Manually integrate smallest peak
  - Click on Peak width
  - Manually box on largest baseline noise
2. Save the processing method
3. All injections processed will get the same peak and threshold values applied



**•Recommended for regulated environments – threshold and peak width set once for complete processing method**


## 3.20 Style Peak Width and Threshold Determination

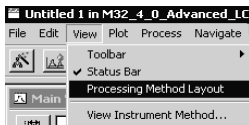
1. Threshold and Peak Width calculated automatically
  - Peak width with the greatest change in slope
  - Bunching factor used for all peaks in channel
2. Save the processing method
3. Each injection will have differing Peak Width and Threshold values



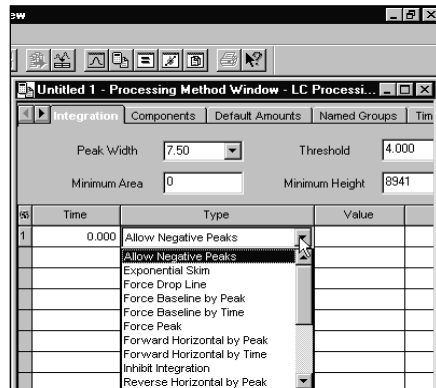
**•Not Recommended for regulated environments – threshold and peak width change for each channel in processing method**

## Processing Method - Integration Events 2 Ways to Select Processing Method

Select View – Processing Method layout or the  icon to access integration events



There are 18 different integration events that can be applied to specified time intervals in your chromatogram

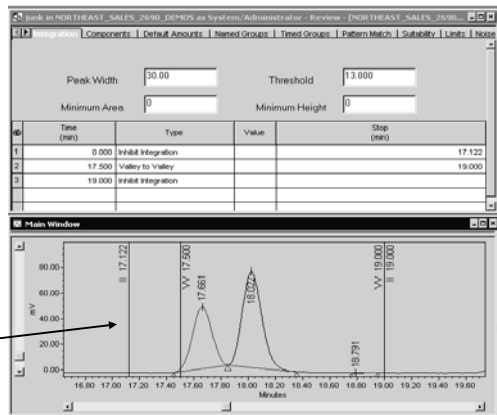


## Viewing Integration Events

To View the Integration Event Markers- click the



Select Options – Show Events



Event Marker Lines  
may be moved

## Most Common Integration Events

- Inhibit Integration
- Valley to valley
- Allow negative peaks
- Exponential Skim
- Force Baseline by peak



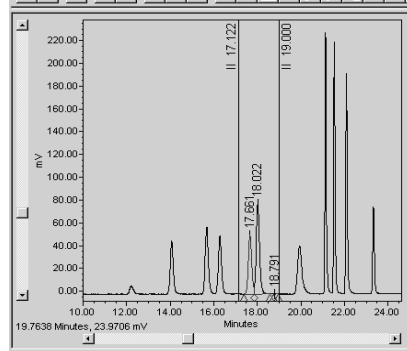
# Integration Inhibit

Integration Components Default Amounts Named Groups Timed Groups Pattern Match Suitability Limits

Peak Width  Threshold

Minimum Area  Minimum Height

#	Time (min)	Type	Stop (min)
1	0.000	Inhibit Integration	17.122
2	19.000	Inhibit Integration	



Enter a **start** and **stop** time for Integration Inhibit. Peaks during this time section will not be integrated or quantitated.

**Note:** If you want to inhibit after a time, just enter the Time and not the stop.

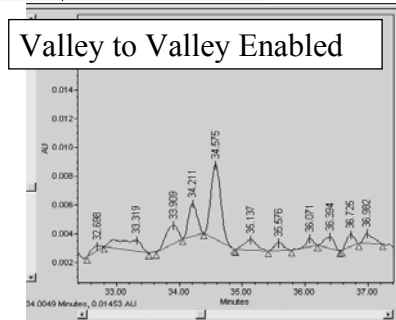
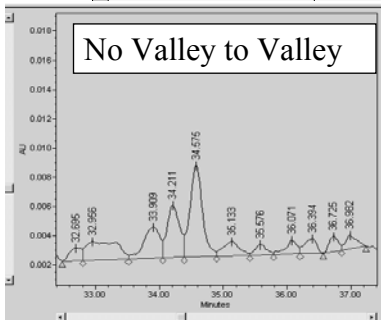
# Valley to Valley

Integration Components Default Amounts Named Groups Timed Groups Pattern Match Suitability Limits Noise ar

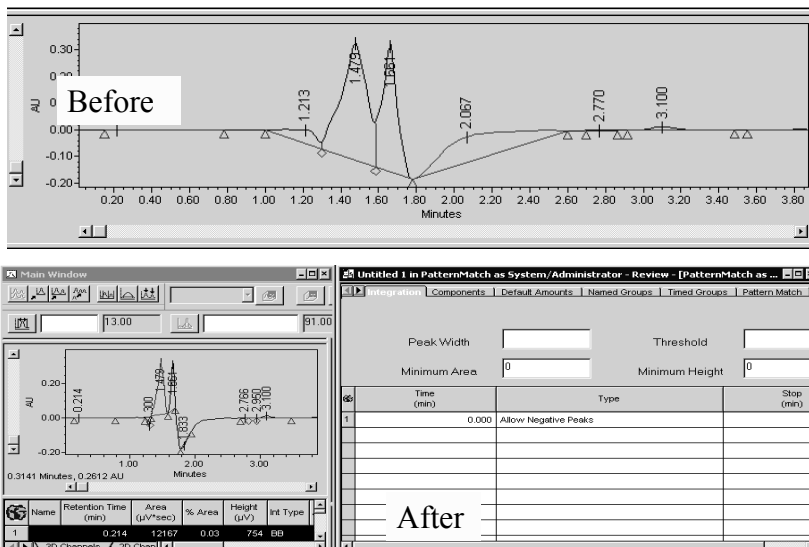
Peak Width  Threshold

Minimum Area  Minimum Height

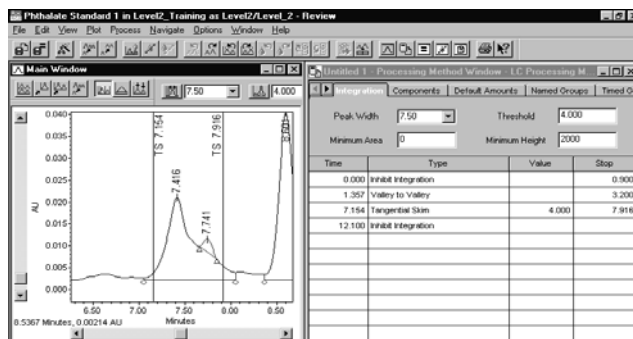
#	Time (min)	Type	Stop (min)
1	0.000	Valley to Valley	



## Negative Peaks Integrates Negative Dips



## Tangential Skim



Enter a **start** and **stop** time for Tangential skim. The Value is the ratio of the parent peak height to the rider peak height(s) at the valley. The default value for this is 4.

**Note:** If you want to skim the front peak, use a negative value.

## Use Options - Fill From Result



Integrated peaks will be filled down in the component table, by Peak 1, Peak 2, etc. : enter in component names

The screenshot shows the 'PQ Final Parameters in PatternMatch as System/Administrator - Review' window. The 'Options' menu is open, highlighting 'Fill From Result'. Below the menu, a table lists peak data:

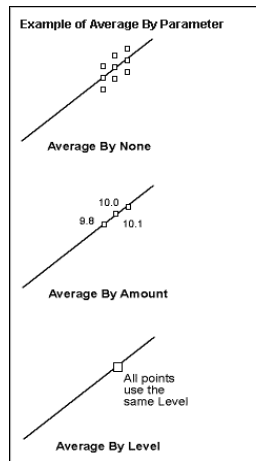
Peak	Name	Retention Time (min)	RT Window (min)	Channel	Peak Match	Y Value	X Value	Fit	Weighting	Internal Std	RT Reference
1	Peak5	0.216	0.011	486	Closest	Area	Amount	Linear	None		
2	Peak6	0.754	0.038	486	Closest	Area	Amount	Linear	None		
3	Peak7	1.212	0.061	486	Closest	Area	Amount	Linear	None		
4	Peak1	1.249	0.062		Closest	Area	Amount	Linear	None		

Component Names Must be spelled the same as was entered in Run Samples

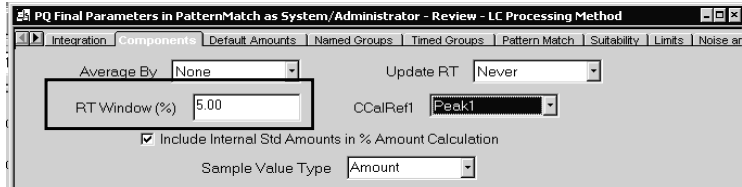
## Global Processing Method Parameters

The screenshot shows the 'PQ Final Parameters in PatternMatch as System/Administrator - Review - LC Processing Method' window. The 'Average By' dropdown is set to 'None'. Other parameters include 'RT Window (%)' at 5.00, 'Update RT' set to 'Never', and 'CcalRef1' set to 'Peak1'.

**Average By:** How to calculated points on the calibration curve if multiple injections of the same relative concentrations are used



## Global Processing Method Parameters



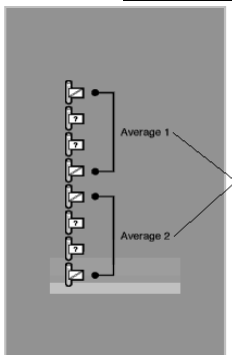
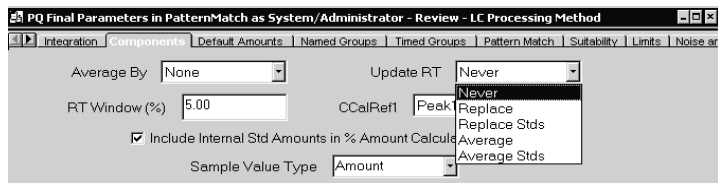
**RT Window (%):** Retention time window for each peak as a percentage of the component's retention time. Default: 5%.

**CCalRef1:** A reference peak for a custom calculation.

**Include Internal Standard Amounts in % Amount Calculation:** Specifies if the Internal Standard Amounts are to be included when calculating %Amount.

**Sample Value Type:** Specifies if the values entered for calibration in the Component Editor are concentrations or amounts.

## Global Processing Method Parameters



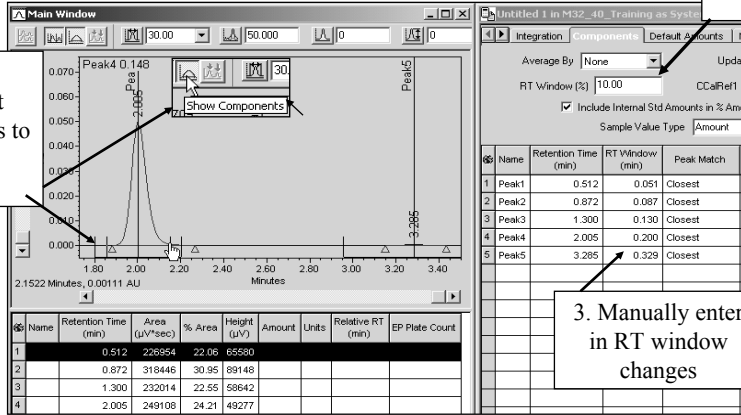
**Update RT:** Whether retention time will be updated in the calibration curve.

## Retention Time Window

The time window that a peak is still identified as that component.  
Retention Time Window can be set 3 ways:

1. Global in % of RT (default is 5%)

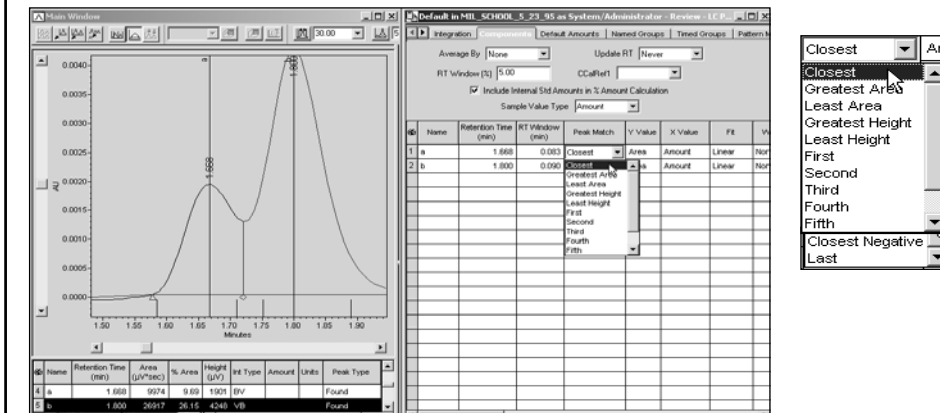
2. Adjust Component start/end times to change RT Window



3. Manually enter in RT window changes

## Peak Match

Identification of peaks when multiple peaks are within the same Component retention Time Window



## External Standard Calculation

### External Standard

$$RF_{(X_i)} = \frac{\text{Conc. (X}_i\text{)std}}{\text{Response (X}_i\text{)std}}$$

### Unknown Concentration

$$(X_i) = RF_{(X_i)} \times \text{Response (X}_i)$$

## X value (Axis)

Amount - (Actual Amount in a vial)  
Concentration – (Injection Volume \* Amount in the Vial)

The screenshot shows a software window titled "Soft Drink C in Level\_1 as Level\_1/Level1 - Review - [Soft Drink PM - Processing]". The interface includes a menu bar (File, Edit, View, Plot, Process, Navigate, Options, Window, Help) and a toolbar. Below the toolbar are tabs for "Integration", "Components", "Default Amounts", "Named Groups", "Timed Groups", and "Suitability". The "Integration" tab is active, showing settings for "Average By" (None), "Update RT" (Never), "RT Window [s]" (5.00), and "CCalRef1". A checkbox for "Include Internal Std Amounts in % Amount Calculation" is checked, and "Sample Value Type" is set to "Amount".

Name	Retention Time	RT Window	Peak Match	Y Value	X Value	Fit
1 Caffeine	2.474	0.124	Closest	Area	Amount	Linear
2 Aspartame	4.889	0.244	Closest	Area	Amount	Linear
3 Benzoate	8.146	0.407	Closest	Area	Amount	Linear
4 Sorbate	10.297	0.515	Closest	Area	Amount	Linear

A dropdown menu is open over the "X Value" column for the "Sorbate" row, showing options for "Amount" and "Concentration".

# Y Axis value - Response

Peak Area  
Peak Height

Any Peak Related Calculation (custom field)

Custom Calculations in CUSTOM\_FIELD51\_215 as System/Administrator - Review - LC Processing

Integration Components Default Amounts Named Groups Timed Groups Pattern Match S

Average By: None Update RT: Never

RT Window (%): 12.00 CCalRef1: [dropdown]

Include Internal Std Amounts in % Amount Calculation

Sample Value Type: Amount

#	Name	Retention Time (min)	RT Window (min)	Peak Match	Y Value	X Value	Fit	Weighting
1	Void	1.232	0.148	Greatest Area	Area	Amount	Linear	None
2	1	2.065	0.248	Greatest Area	Area	Amount	Linear	None
3	2	2.998	0.360	Greatest Area	% Area	Amount	Linear	None
4	3	5.007	0.601	Greatest Area	Height	Amount	Linear	None

Dropdown menu options: Area, Amount, Linear, % Area, Height, % Height, Active\_Amount\_Total\_Amount, Amount\_Amount, Area\_Area\_Total\_Area, Per\_Cent\_Active\_Amount, Per\_Cent\_Label\_Claim, Response\_Factor

## Fit Type

Select the calibration curve Fit Type - regulated Industries recommend only Linear or Linear through Zero

Integration Components Default Amounts Named Groups Timed Groups Suitability Limits Noise

Average By: None Update RT: Never

RT Window (%): 5.00 CCalRef1: [dropdown]

Include Internal Std Amounts in % Amount Calculation

Sample Value Type: Amount

#	Name	Retention Time (min)	RT Window (min)	Peak Match	Y Value	X Value	Fit	Weighting
1	Acetone	1.249	0.062	Closest	Area	Amount	Linear	None
2	Acetophenone	2.132	0.107	Closest	Area	Amount	Fourth	None
3	Propiophenone	3.110	0.156	Closest	Area	Amount	Fifth	None
4	Butyrophenone	4.781	0.239	Closest	Area	Amount	Linear thru Zero	None

Fit Type	# required	# Recomm
Linear	2	3
Linear through 0	1	2
Response Factor	1	1
Inverse Linear	2	3
Log-Log Linear	2	3
Point To Point	2	2
Cubic spline	4	6
Quadratic	3	5
Quadratic Thru 0	2	4
Cubic	4	5
Cubic Thru 0	3	4
Fourth	5	6
Fourth Thru 0	4	5
Fifth	6	7
Fifth Thru 0	5	6

Dropdown menu options: Linear, Linear thru Zero, Quadratic thru Zero, Cubic thru Zero, Fourth thru Zero, Fifth thru Zero, Inverse Linear, Log-Log Linear, Response Factor

## Weighting Factor

Weighting places more emphasis on the calibration curve points that have the (smallest deviation - weight).

None No Weighting

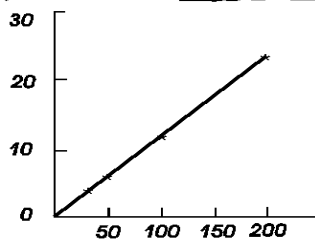
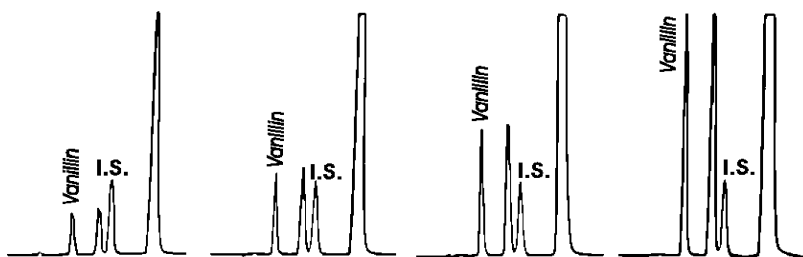
X-  $X^2$ ,  $\log x$ ,  $\ln X$  : fit to the points at the high end of the curve (X value).

$1/x$  and  $1/x^2$ : fit to the points at the low end of the curve (X value)

$1/y$  and  $1/y^2$ : fit to the points at the low end of the curve (Y value).

Integration Components								
Average By		None		Update RT		Never		
RT Window (%)		5.00		CCalRef1				
<input checked="" type="checkbox"/> Include Internal Std Amounts in % Amount Calculation								
Sample Value Type		Amount						
#	Name	Retention Time (min)	RT Window (min)	Peak Match	Y Value	X Value	Fit	Weighting
1	Acetone	1.249	0.062	Closest	Area	Amount	Linear	None
2	Acetophenone	2.132	0.107	Closest	Area	Amount	Linear	None
3	Propiophenone	3.110	0.156	Closest	Area	Amount	Linear	X
4	Butyophenone	4.781	0.239	Closest	Area	Amount	Linear	1/X
								X^X
								1(X^X)
								Ln X
								Log X
								1/Y
								1(Y^Y)

## Internal Standards

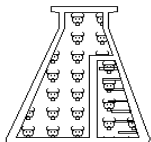


### Internal Standard

#	Name	Retention Time (min)	RT Window (min)	Internal Std	P
1	Barbital	1.037	0.052		Clc
2	Phenobarbital	1.664	0.083	Barbital	Clc
3	Butabarbital	1.959	0.090	Barbital	Clc
4	Amobarbital	2.237	0.112	Barbital	Clc
5	Secobarbital	3.976	0.199		Clc
				Barbital	
				Phenobarbital	
				Butabarbital	
				Secobarbital	

## Internal Standard

### Stock Standards



Stock

#	Name	Retention Time (min)	RT Window (min)	Internal Std	P
1	Barbital	1.037	0.052		Clc
2	Phenobarbital	1.664	0.083	Barbital	Clc
3	Butabarbital	1.959	0.098	Barbital	Clc
4	Amobarbital	2.237	0.112	Barbital	Clc
5	Secobarbital	3.976	0.199		
				Barbital	
				Phenobarbital	
				Butabarbital	
				Secobarbital	



Internal Standard

### Working Standards



0



1



2

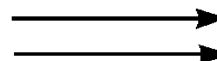


3

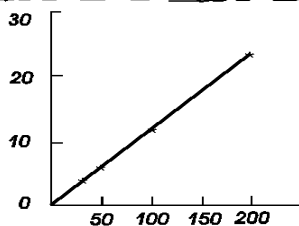
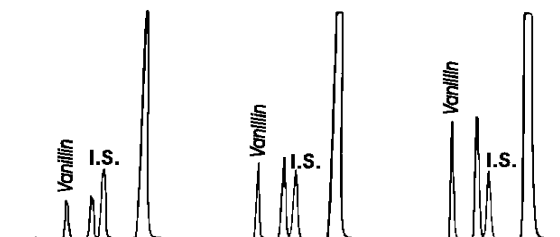


4

Increasing Solute Concentration  
Constant Internal Standard Concentration



## Internal Standards



### Internal Standard

$$RF_x = \frac{\text{Response (I.S.)}_{std}}{\text{Response (X}_i\text{)}_{std}} \times \text{Conc. (X}_i\text{)}_{std}$$

### Unknown

$$(X_i) = RF_x \times \frac{\text{Response (X}_i\text{)}}{\text{Response (I.S.)}}$$

## Retention Time Reference

	Name	Retention Time (min)	RT Reference
1	Barbital	1.037	
2	Amobarbital	2.237	Barbital
3	Butobarbital	1.959	Barbital
4	Phenobarbital	1.664	Barbital
5	Secobarbital	3.976	Amobarbital Phenobarbital Secobarbital

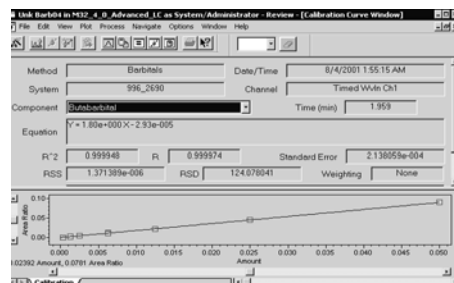
**RT Reference:** Adjust the component's retention time for the calibration curve based upon the selected RT Reference component retention time (changes each sample)

The adjusted retention time becomes the time for the component in the chromatogram (the retention times documented in the processing method are unchanged).

- Use this for shifting chromatography
- A RT Reference peak also automates two additional calculations - RT Ratio and RRT

## Default Peak

	Name	Retention Time (min)	Default Pk	Default Pk Start (min)	Default Pk End (min)
1	Barbital	1.037	<input type="checkbox"/>		
2	Phenobarbital	1.664	<input type="checkbox"/>		
3	Butobarbital	1.959	<input type="checkbox"/>		
4	Amobarbital	2.237	<input type="checkbox"/>		
5	Secobarbital	3.976	<input checked="" type="checkbox"/>	2.900	5.000

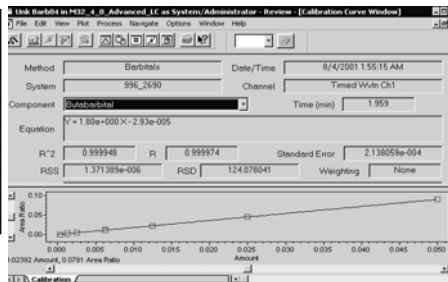


Uses the calibration curve of the listed component to calculate the amount of unknowns that are detected between the Start and End time listed.

## Curve Reference

Use designated component's calibration curve to quantitate (calculate amounts) for all other known components

	Name	Retention Time (min)	RT Window (min)	Curve Reference	Relative Response
1	Barbital	1.037	0.052		
2	Phenobarbital	1.664	0.083	Barbital	1.000000
3	Butabarbital	1.959	0.098	Barbital	0.500000
4	Amobarbital	2.237	0.112	Barbital	0.500000
5	Secobarbital	3.976	0.199	Barbital	0.750000
				Barbital	
				Phenobarbital	
				Butabarbital	
				Secobarbital	



## Relative Resolution Reference

Relative Resolution Reference only appears when System Suitability option is enabled.

Specifies the Reference peak used to calculate Relative Resolution equation for System Suitability testing to measure resolution between named peaks in the Components table and the specified reference peak.

	Name	Retention Time (min)	RT Window (min)	Rel Resol Reference
1	Barbital	1.037	0.052	
2	Phenobarbital	1.664	0.083	Barbital
3	Butabarbital	1.959	0.098	Barbital
4	Amobarbital	2.237	0.112	Barbital
5	Secobarbital	3.976	0.199	Barbital
				Phenobarbital
				Amobarbital
				Secobarbital

Used for calculation Relative Resolution – as part of system suitability

## Named Groups

1. Enter in a Name for the Group peak
  2. Highlight the Peaks and drag them to the named group tree
  3. Select the retention ID
  4. Select how the amounts are calculated: Sum or User Entered (User Entered = Run samples)
- The Group peak will be displayed in the Component Table as a group component

Name	Reported RT	Source of X Value
Barbital	First Peak	User Entered
	First Peak	Sum Peaks
	First Peak	
	Largest Area	
	Largest Height	

Name	Retention Time (min)
Barbital	1.037
Phenobarbital	1.664
Ethobarbital	1.959
Amobarbital	2.237
Secobarbital	3.976
Barbital	

## Timed Groups

Name	Start (min)	Stop (min)	Source of X Value	Exclude Known Peaks
Impurities	0.000	10.000	Sum Peaks	<input checked="" type="checkbox"/>

Multiple Components can be grouped according to specific times as a single component. Millennium<sup>32</sup> sums the areas and heights of the peaks within the specific time period.

Option: Exclude known peaks – for example, impurity profiles

## System Suitability

1. Enabled Option
2. Must enter Void Volume time

Baseline Noise – Baseline and Start must be > 30 seconds each side

## System Suitability Limits

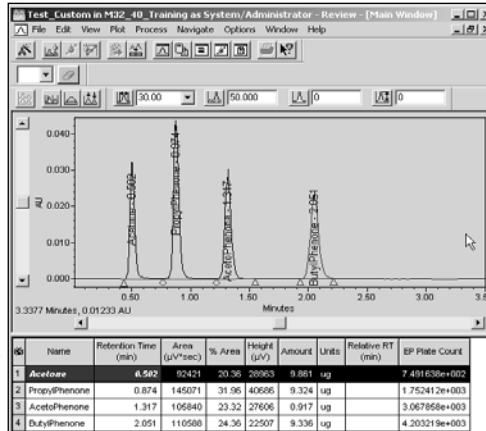
Set Target and when to calculate limits - limits will be flagged in the Peaks Table

Suitability Components									
Name	Calculate Suit Results	Flag Outside Limits							
1 Acetone	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>							
2 PropylPhenone	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>							
3 AcetoPhenone	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>							
4 ButylPhenone	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>							
Suitability Limits									
Field Name	Target	Error %	Lower Error Limit (LCL)	Upper Error Limit (UCL)	Warning %	Lower Warning Limit	Upper Warning Limit	Check Limits	Ignore Blank Values
1 Retention Time	2.051	4.00	1.969	2.133	2.00	2.010	2.092	During Quantitation	<input checked="" type="checkbox"/>

## System Suitability Limits

Set Target and when to calculate limits - limits will be flagged in the Peaks Table

Flagged Peak result outside of Retention Time System Suitability Limit



## Applications of Detector Noise

### Signal to Peak to Peak Noise

Example: Uses the detector noise section and custom fields to calculate ZQ detector noise specification

Height/Average Peak to Peak Noise

