

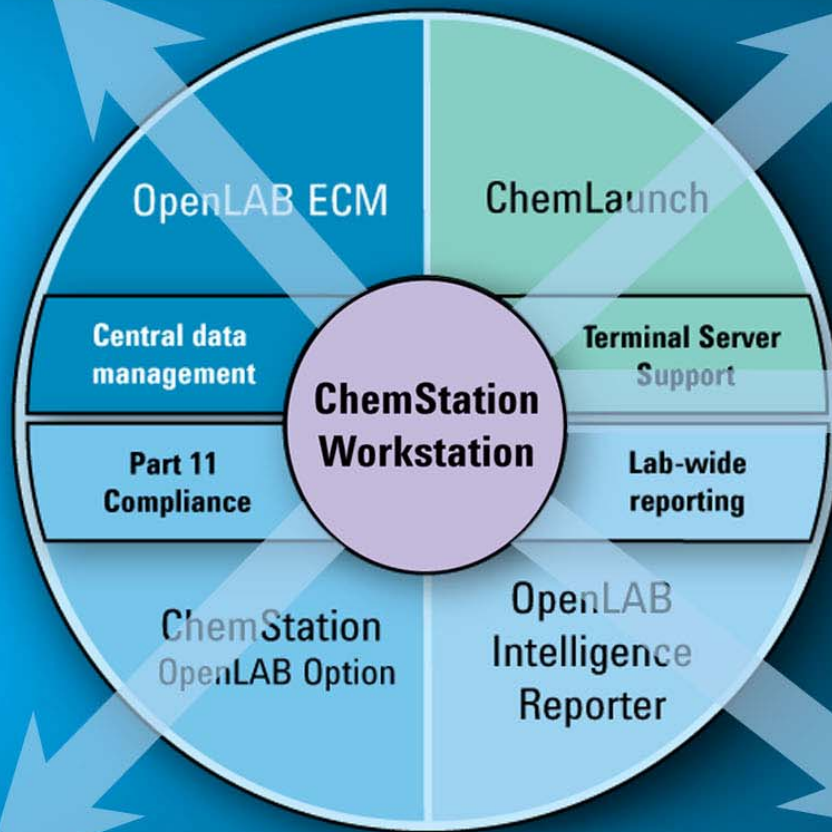
# ChemStation E-Seminars

**ChemStation Data Storage Concept.**

October 15, 2008

**Integrating my results.**

December 10, 2008



**How to set up  
a calibration table  
in ChemStation.**

January 14, 2009

**ChemStation Navigation Table –  
How to improve Review and Reprocess.**

November 12, 2008

**Various Reporting in Chemstation.**

February 04, 2009

# ChemStation E-Seminar Agenda

Schedule	Short description	Agilent Speaker
15. Oct. 2008	ChemStation Data Storage Concept	Steven Brown Ortrud Emde
12. Nov. 2008	ChemStation Navigation Table How to improve Review and Reprocess	Steven Brown Ortrud Emde
10. Dec. 2008	Integrating My Results in ChemStation	Steven Brown Ortrud Emde
14. Jan. 2009	How to set up a calibration table in ChemStation	Steven Brown
04. Feb. 2009	Various Reporting in Chemstation	Steven Brown Ortrud Emde
18. Feb. 2009	Updated Compliance 21 CFR Part 11 solution with Agilent ChemStation & OpenLAB ECM	Steven Brown Ortrud Emde

# Seminar 3: Integrating my Results in ChemStation

## Topics

**The Integration Task**

**General Integration Items and the use of auto integration**

**Initial Integration Events**

**Timed Events**

**Manual Integration events**

**Manual integration events and where to save them**  
**ChemStation Rev. A / Rev. B.02.01 – B.03.02 / B.04.01**

# The Integration Task

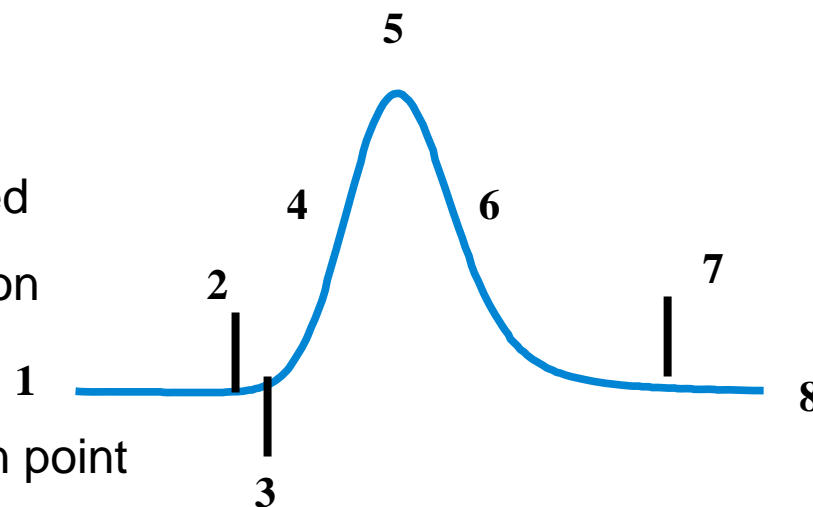
# Integration Process

**The Integration Process consists of the following:**

- construct initial baseline
- peak recognition – start and end time
- find the apex of the peak
- baseline allocation (construction)
- peak area measurement

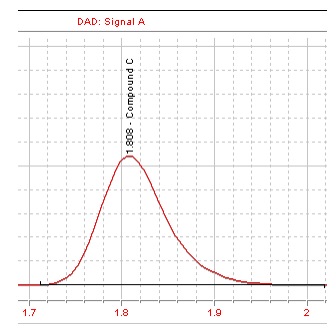
# Start, End and Apex of a Peak

- 1 Slope and Curvature within limit
- 2 Slope and curvature above limit: peak?
- 3 Slope remains above limit: peak recognized
- 4 Curvature becomes negative: front inflection
- 5 First Derivate zero: Apex
- 6 Curvature becomes positive: rear inflection point
- 7 Slope and curvature within limit: end of peak?
- 8 Slope and curvature within limit: end of peak



# To Integrate a Chromatogram, the Integrator:

- 1) Defines the initial baseline.
- 2) Continuously tracks and updates the baseline.
- 3) Identifies the start time for a peak and marks this point with a vertical tick mark.
- 4) Finds the apex of each peak, creates a parabolic fit for the peak top, and stores the retention time.
- 5) Identifies the end time for the peak, and marks this point with a vertical tick mark.
- 6) Constructs a baseline.
- 7) Calculates the area, height, and peak width for each peak.







# Integrator UI

Define integration parameters just by selecting and clicking on the chromatogram

Integration events for all signals

The screenshot displays the Agilent Integrator software interface. At the top, there are tabs for Integration, Calibration, Signal, Purify, and Spectrum. Below these are various tool icons and a 'Report: Short' dropdown. The main window shows a chromatogram with a y-axis labeled 'mAU' ranging from 0 to 140 and an x-axis from 0 to 5. Four peaks are visible, with retention times 0.579, 1.315, 1.779, and 6.947. A context menu is open over the 0.579 peak, listing integration methods: Baseline at Valleys, Baseline Hold, Tail Tangent Skim, Tangent Skim Mode, Area Sum, Integration (highlighted), and Slope Sensitivity. On the left, there are sections for 'Manual Events' and 'For All Signals'. The 'Integration Events' table is shown below, and an 'Events Table' is also present. A table at the bottom right provides peak data.

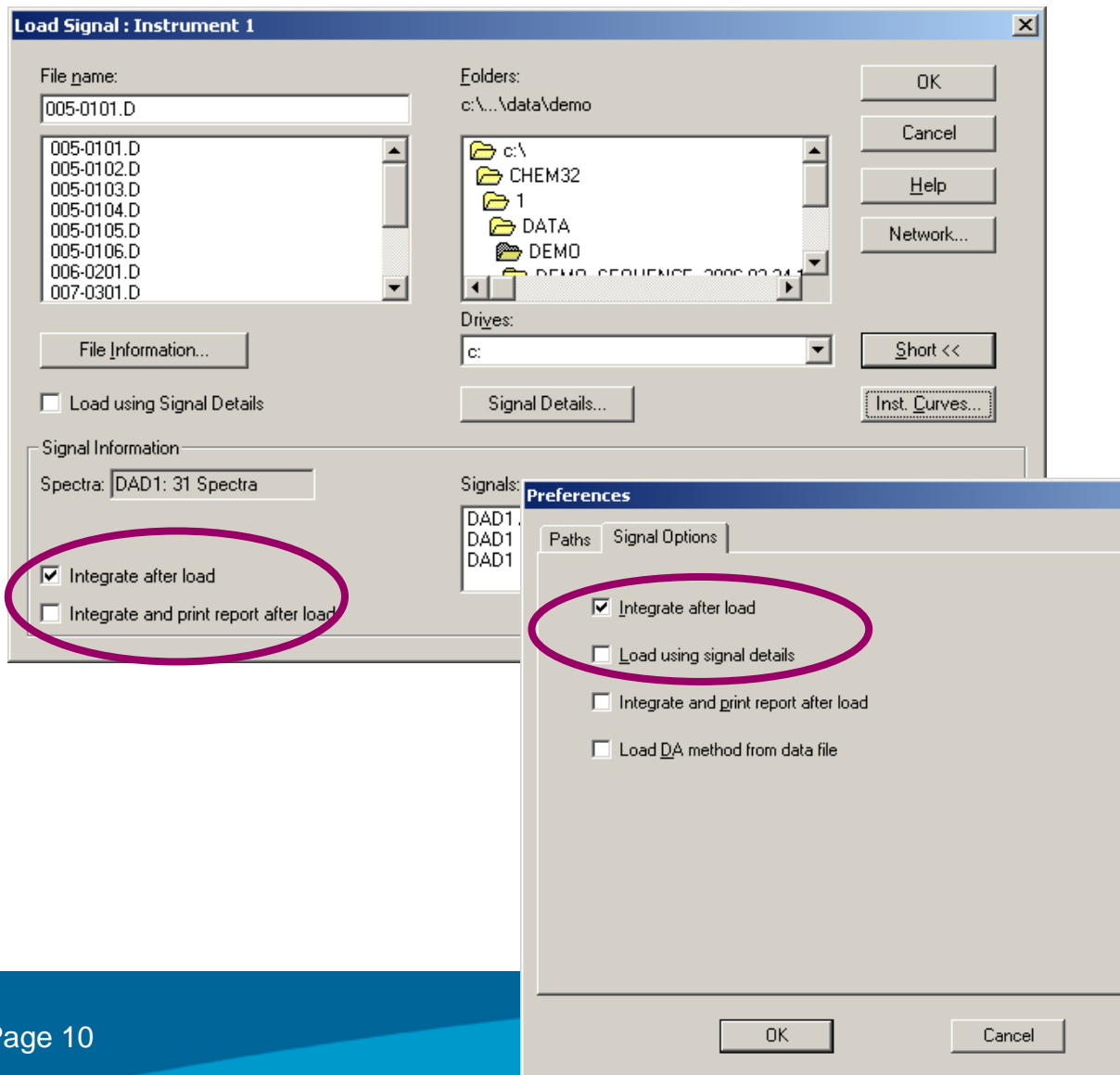
Integration Events	Value
Tangent Skim Mode	Standard
Tail Peak Skim Height Ratio	0.00
Front Peak Skim Height Ratio	0.00
Skim Valley Ratio	20.00

Time	Integration Events	Value
Initial	Slope Sensitivity	5
Initial	Peak Width	0.05
Initial	Area Reject	5
Initial	Height Reject	1
Initial	Shoulders	OFF
0.579	Integration	OFF

#	Time	Area	Height	Width	Area%	Symmetry
1	1.315	311.1	147.5	0.0336	22.297	0.83
2	1.779	282.4	105.8	0.0411	20.238	0.84
3	3.595	335.4	70.3	0.0746	24.035	0.849
4	6.947	466.5	50	0.1449	33.430	0.871

There are two sets of detector specific integration events:  
*initial events and time-based*

# When a Signal is Loaded, Integration May Occur Automatically



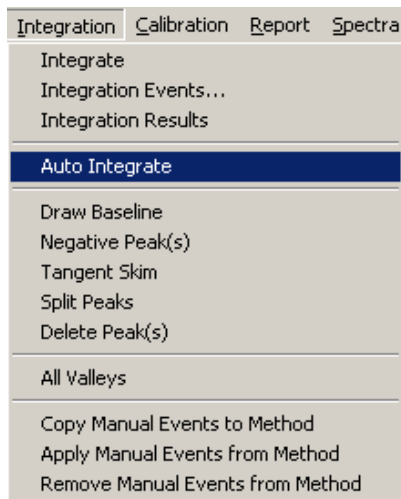
## Integrate by:

- Selecting Integrate after load in *Load Signal* dialog box or *Preferences* dialog box.
- Selecting *Integrate* or *Auto Integrate* from the menu.
- Selecting the Integration or Auto Integration Tool.
- Running a method where the Run Time Checklist includes Data Analysis.

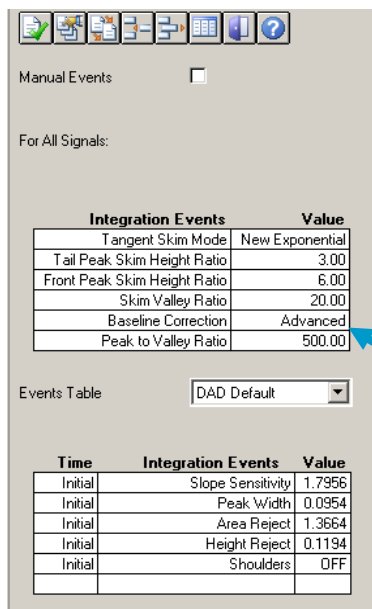
# Auto Integrate



Can be a good starting point for integration events.




- Examines beginning and end regions to estimate noise.
- Assigns initial Slope Sensitivity and Height Reject.
- Assigns temporary Peak Width value for first pass integration.
- Sets Area Reject to zero.



- Performs trial integration, may be repeated several times.
- Calculates Peak Width based on early eluting peaks.
- Refines Slope Sensitivity and Height Reject.
- Computes Area Reject as 90% area of most symmetrical peak.

Autointegrates based on your settings in **For All Signals**.

# Set Up Integration



Integration Calibration Signal

Report: Perf. + Noise

1) DAD1 A, Sig=...MO\005-0101.D

Manual Events

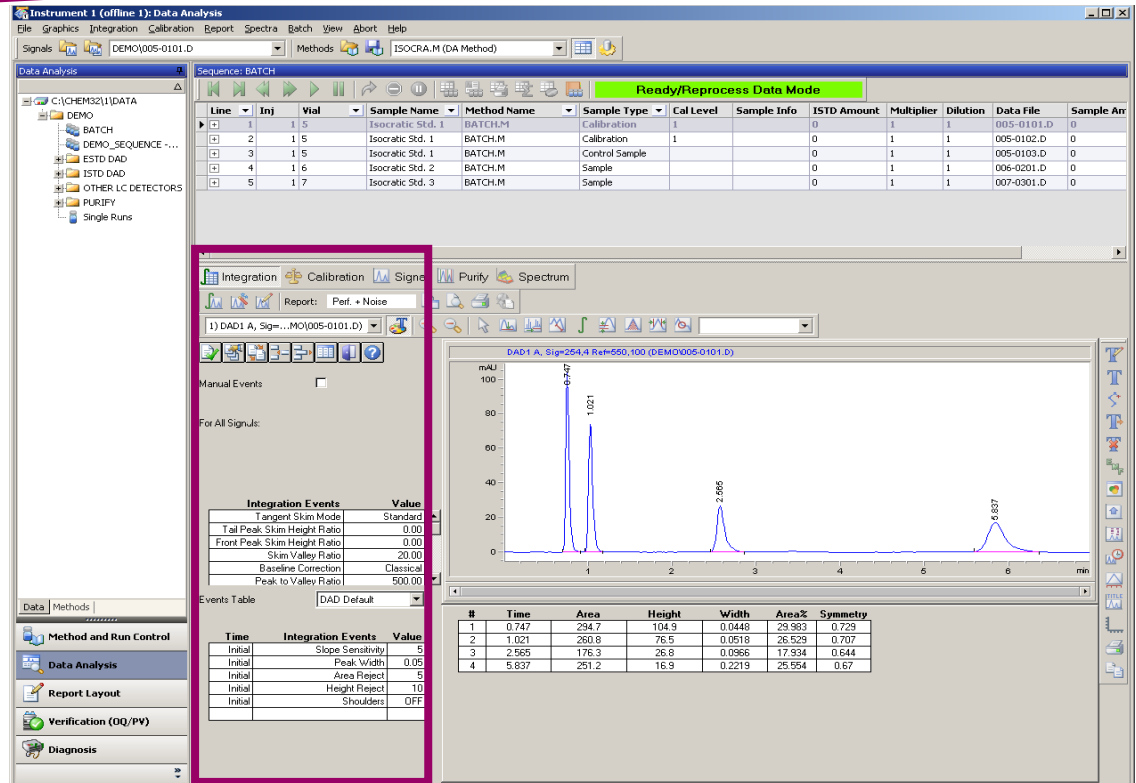
For All Signals:

Integration Events	Value
Tangent Skim Mode	Standard
Tail Peak Skim Height Ratio	0.00
Front Peak Skim Height Ratio	0.00
Skim Valley Ratio	20.00
Baseline Correction	Classical
Peak to Valley Ratio	500.00

Events Table DAD Default

Time	Integration Events	Value
Initial	Slope Sensitivity	5
Initial	Peak Width	0.05
Initial	Area Reject	5
Initial	Height Reject	10
Initial	Shoulders	OFF

Loads Integration Events



Instrument 1 (Offline 1): Data Analysis

File Graphics Integration Calibration Report Spectra Batch View Abort Help

Signals DEMO\005-0101.D Methods ISOCRAM (DA Method)

Data Analysis

Sequence: BATCH

Ready/Reprocess Data Mode

Line	Inj	Vial	Sample Name	Method Name	Sample Type	Cal Level	Sample Info	ISTD Amount	Multiplier	Dilution	Data File	Sample Arr
1	1	5	Isocratic Std. 1	BATCH.M	Calibration	1		0	1	1	005-0101.D	0
2	1	5	Isocratic Std. 1	BATCH.M	Calibration	1		0	1	1	005-0102.D	0
3	1	5	Isocratic Std. 1	BATCH.M	Control Sample			0	1	1	005-0103.D	0
4	1	6	Isocratic Std. 2	BATCH.M	Sample			0	1	1	006-0201.D	0
5	1	7	Isocratic Std. 3	BATCH.M	Sample			0	1	1	007-0301.D	0

Integration Calibration Signal Purify Spectrum

Report: Perf. + Noise

1) DAD1 A, Sig=...MO\005-0101.D

Manual Events

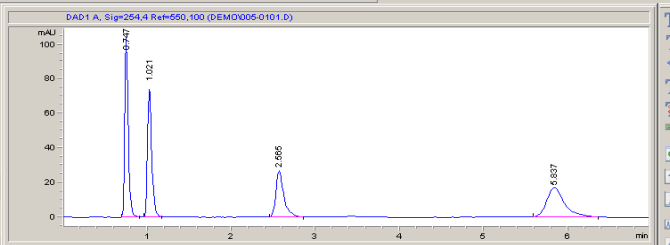
For All Signals:

Integration Events	Value
Tangent Skim Mode	Standard
Tail Peak Skim Height Ratio	0.00
Front Peak Skim Height Ratio	0.00
Skim Valley Ratio	20.00
Baseline Correction	Classical
Peak to Valley Ratio	500.00

Events Table DAD Default

Time	Integration Events	Value
Initial	Slope Sensitivity	5
Initial	Peak Width	0.05
Initial	Area Reject	5
Initial	Height Reject	10
Initial	Shoulders	OFF

DAD1 A, Sig=254.4 Ret=550,100 (DEMO\005-0101.D)



#	Time	Area	Height	Width	Area%	Symmetry
1	0.747	234.7	104.9	0.0448	25.983	0.725
2	1.021	260.8	76.5	0.0518	26.529	0.707
3	2.565	176.3	26.8	0.0566	17.934	0.644
4	5.837	251.2	16.9	0.2219	25.954	0.67

Set up integration for your method in the Data Analysis mode using a representative chromatogram.

# Initial Integration Events

# Initial integration events

Time	Integration Events	Value
Initial	Slope Sensitivity	5
Initial	Peak Width	0.05
Initial	Area Reject	5
Initial	Height Reject	1
Initial	Shoulders	OFF

- Use:**
- Slope Sensitivity* ⇒ to define peak sensitivity
  - Peakwidth* ⇒ to set an initial sampling interval for the integrator to distinguish peaks from baseline noise
  - Area Reject* ⇒ to filter small peaks
  - Height Reject* ⇒ to set noise rejection
  - Shoulder* ⇒ to specify the algorithm for shoulder detection

# Initial Settings - Events

For All Signals:

Integration Events	Value
Tangent Skim Mode	Standard
Tail Peak Skim Height Ratio	0.00
Front Peak Skim Height Ratio	0.00
Skim Valley Ratio	20.00
Baseline Correction	Classical
Peak to Valley Ratio	500.00

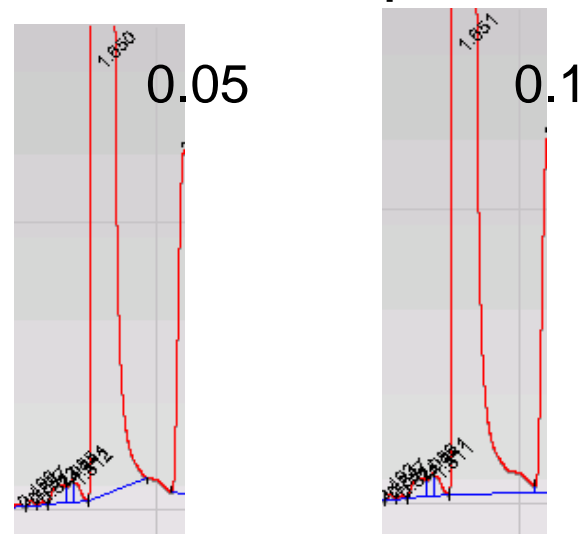
Events Table

Time	Integration Events	Value
Initial	Slope Sensitivity	5
Initial	Peak Width	0.05
Initial	Area Reject	5
Initial	Height Reject	1
Initial	Shoulders	OFF

**Slope sensitivity** – decreasing slope sensitivity will result in detecting smaller and broader peaks.

**Shoulder Detection Mode** – shoulders detected using the second derivative of peak

**Peak Width** – controls the ability of the integrator to distinguish peaks from baseline noise. In general, increasing the peak width will result in broader peaks.

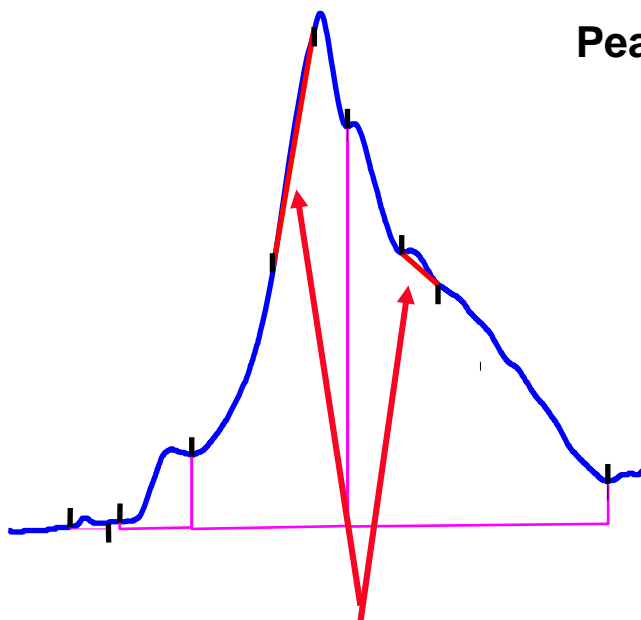


**Area reject**- All peaks whose areas are below this value will not be reported.

**Height reject**- All peaks whose heights are below this value will not be reported.

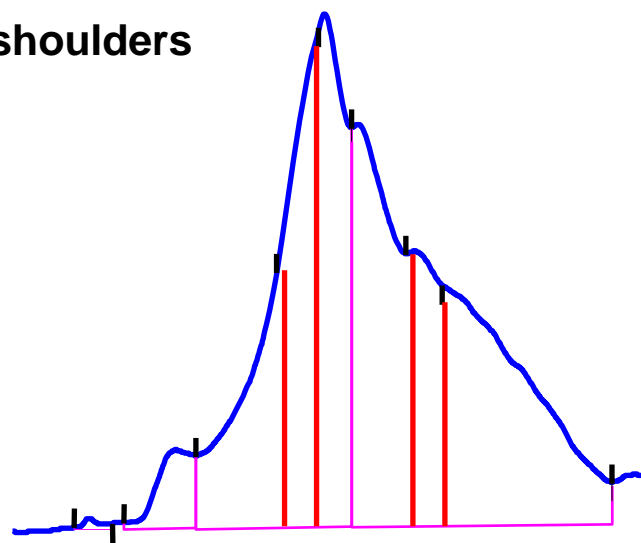
# Shoulder Detection

Shoulders occur when two peaks are so close together that no valley exists between them



**Tangent Shoulders**

**Peak with shoulders**



**Drop Line Shoulders**



# Initial Settings – For All Signals

For All Signals:

Integration Events	Value
Tangent Skim Mode	Standard
Tail Peak Skim Height Ratio	0.00
Front Peak Skim Height Ratio	0.00
Skim Valley Ratio	20.00
Baseline Correction	Classical
Peak to Valley Ratio	500.00

Events Table: DAD Default

Time	Integration Events	Value
Initial	Slope Sensitivity	5
Initial	Peak Width	0.05
Initial	Area Reject	5
Initial	Height Reject	1
Initial	Shoulders	OFF

Some Events in this table are dependent on other events in this table.

**Tangent Skim Mode** – only applies when conditions for the following settings are met:

Tail Peak Skim Height Ratio, or Front Peak Skim Height Ratio, and Skim Valley Ratio

**Peak To Valley Ratio**

Baseline Correction must be **Advanced**

# Tangent Skim Mode

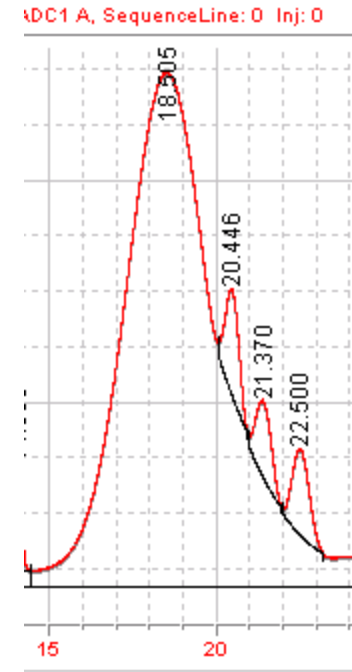
For All Signals:

Integration Events	Value
Tangent Skim Mode	Standard
Tail Peak Skim Height Ratio	0.00
Front Peak Skim Height Ratio	0.00
Skim Valley Ratio	20.00
Baseline Correction	Classical
Peak to Valley Ratio	500.00

Events Table

Time	Integration Events	Value
Initial	Slope Sensitivity	5
Initial	Peak Width	0.05
Initial	Area Reject	5
Initial	Height Reject	1
Initial	Shoulders	OFF

- Tangent Skim Modes
  - New Exponential
  - Exponential
  - Straight
  - Standard



The **Tail Peak Skim Height Ratio** and **Skim Valley Ratio** will be used to determine whether a tangent skim will be applied to calculate the area of a child peak on the trailing edge of a parent peak.

# Tail Peak Skim Height Ratio

For All Signals:

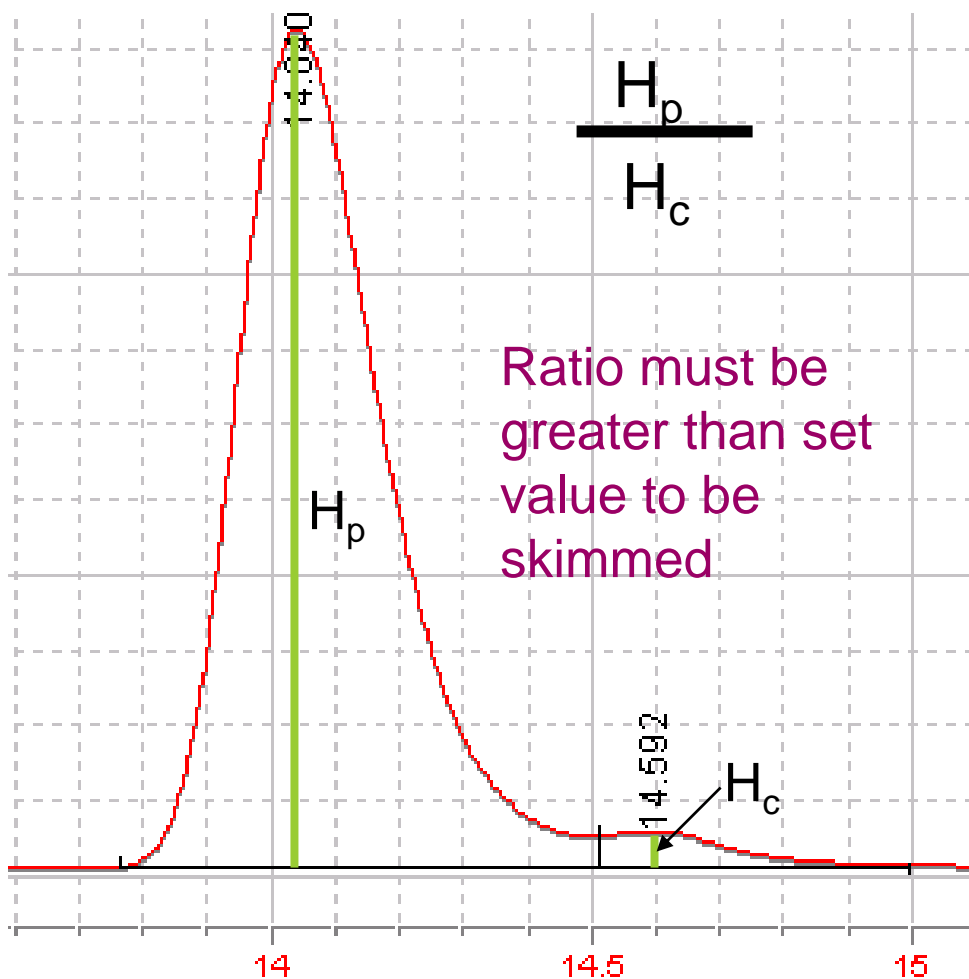
Integration Events	Value
Tangent Skim Mode	Standard
Tail Peak Skim Height Ratio	0.00
Front Peak Skim Height Ratio	0.00
Skim Valley Ratio	20.00
Baseline Correction	Classical
Peak to Valley Ratio	500.00

Events Table

Time	Integration Events	Value
Initial	Slope Sensitivity	5
Initial	Peak Width	0.05
Initial	Area Reject	5
Initial	Height Reject	1
Initial	Shoulders	OFF

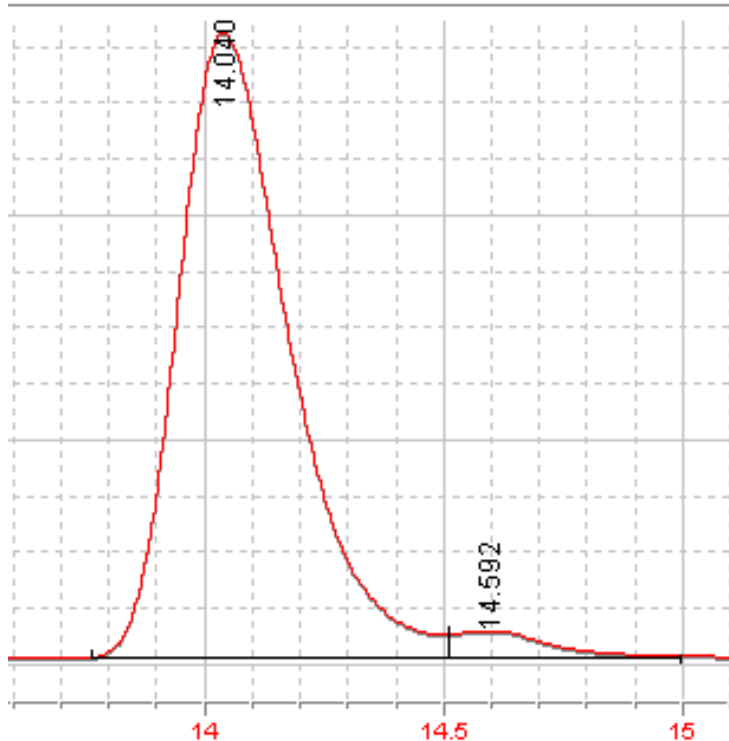
Setting the value to zero **disables** tangent skimming

AD1 A, SequenceLine: 0 Inj: 0



# Tail Peak Skim Height Ratio

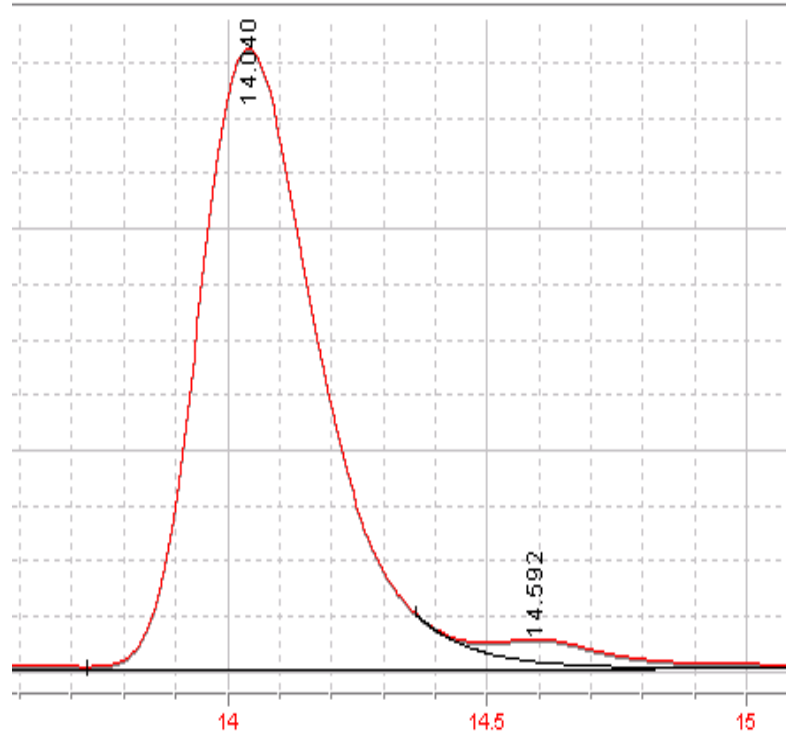
AD1 A, SequenceLine: 0 Inj: 0



No Tangent Skimming

Tail Peak Skim Height Ratio = 0  
Skim Valley Ratio = 20

DAD1 A, SequenceLine: 0 Inj: 0



Tangent Skimmed

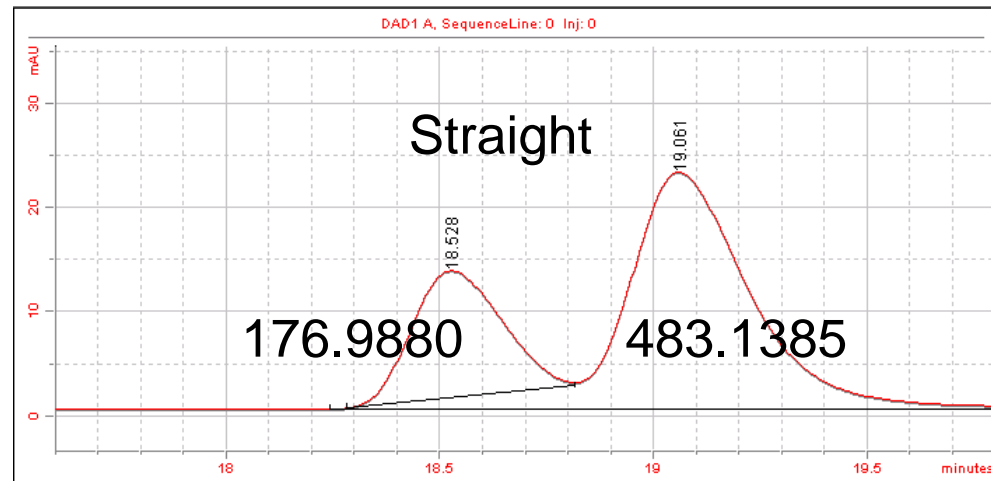
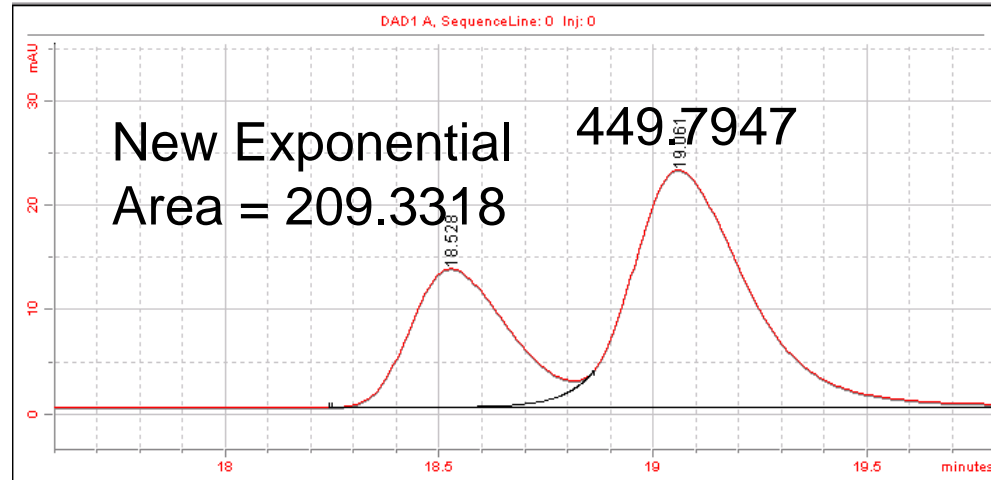
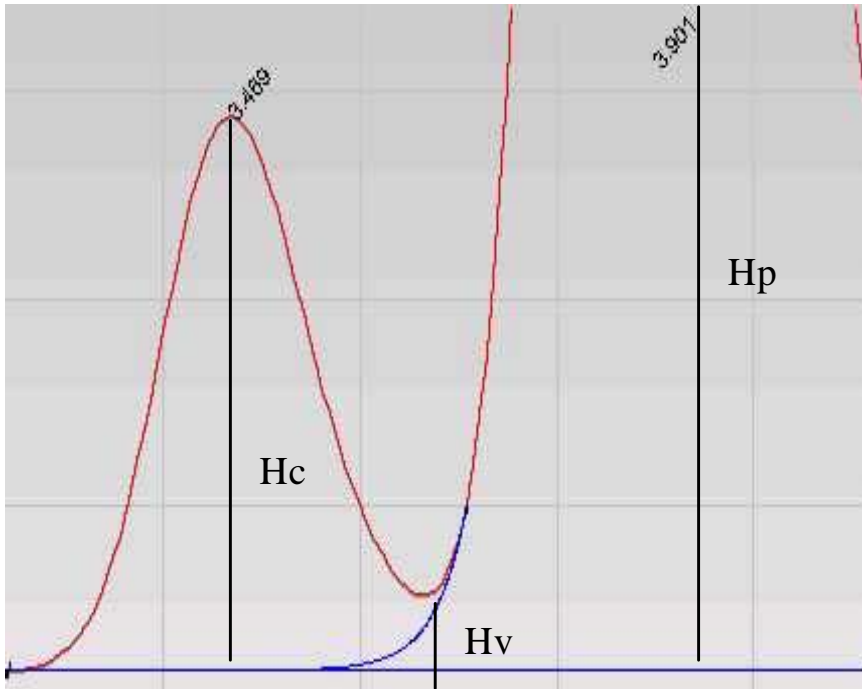
Tail Peak Skim Height Ratio = 3  
Skim Valley Ratio = 20

# Front Peak Skim Height Ratio

$\frac{H_p}{H_c} \rightarrow$  Set Front Skim Height Ratio

$\frac{H_c}{H_v} <$  Set Valley Height Ratio

Very similar to Tail Peak Skimming



# Skim Valley Ratio

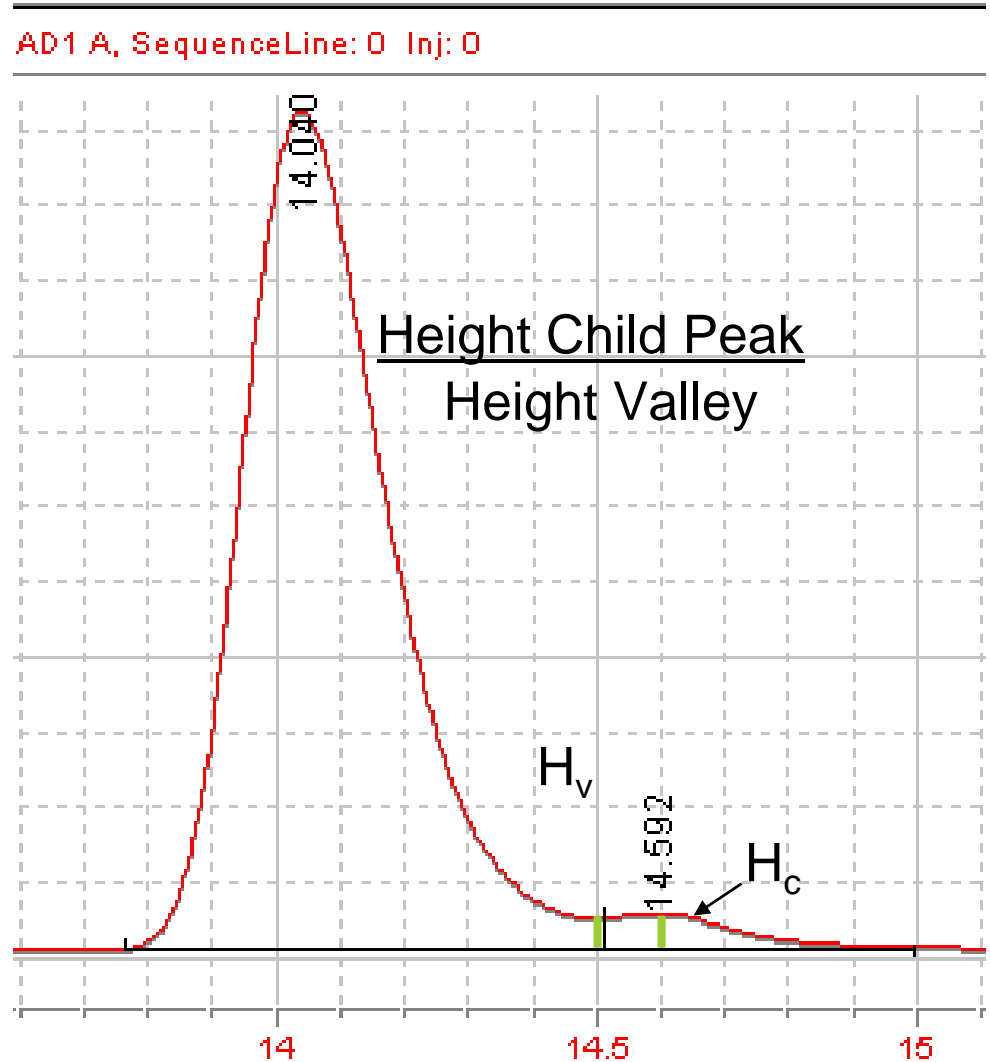
For All Signals:

Integration Events	Value
Tangent Skim Mode	Standard
Tail Peak Skim Height Ratio	0.00
Front Peak Skim Height Ratio	0.00
Skim Valley Ratio	20.00
Baseline Correction	Classical
Peak to Valley Ratio	500.00

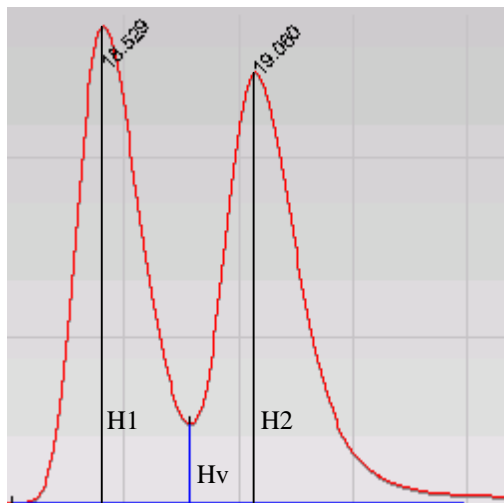
Events Table

Time	Integration Events	Value
Initial	Slope Sensitivity	5
Initial	Peak Width	0.05
Initial	Area Reject	5
Initial	Height Reject	1
Initial	Shoulders	OFF

When the ratio is less than the set value the child will be skimmed.



# Advanced Baseline Peak to Valley Ratio

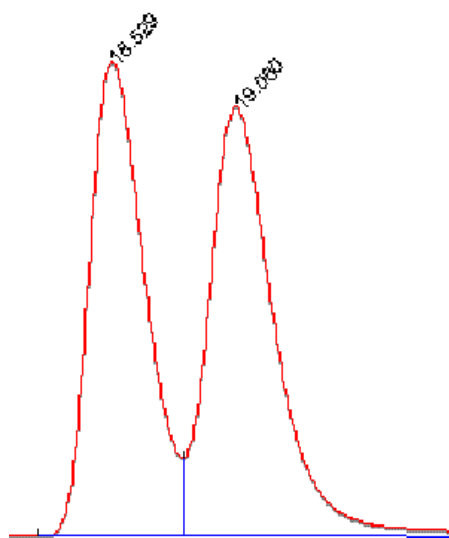


$H1 \geq H2$

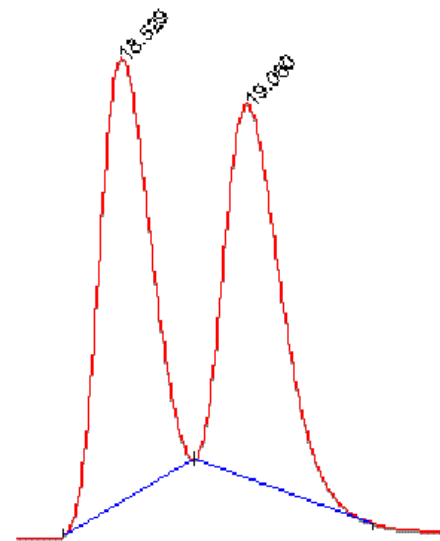
$PtoVRatio = H2 / H_v$

$H2 > H1$

$PtoVRatio = H1 / H_v$



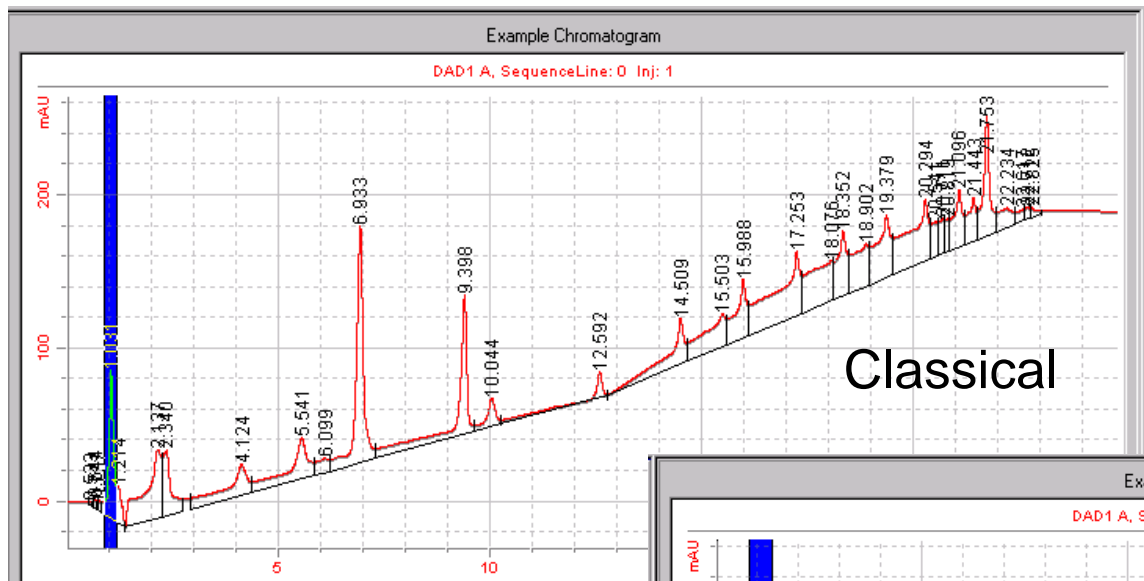
Peak to Valley Ratio  
**LOWER** than  
User Setting



Peak to Valley Ratio  
**HIGHER** than  
User Setting

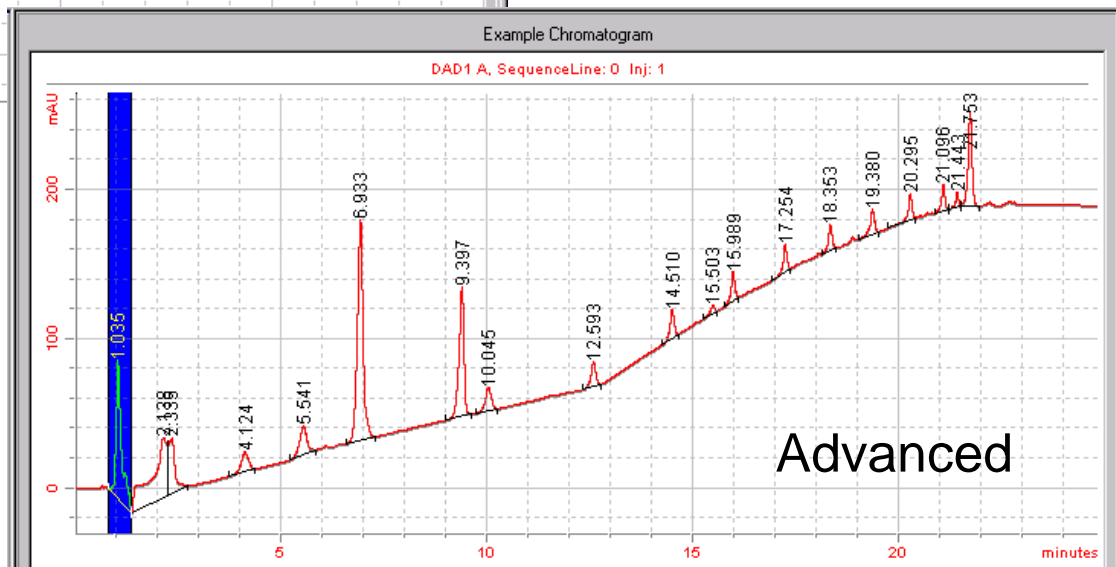
# Advanced Baseline

Integrator tries to improve the start and end points of a peak, re-establish the baseline for a cluster of peaks and remove baseline penetrations. Uses **Peak to Valley Ratio**



Classical

Advanced Baseline tracks the curvature of baselines better

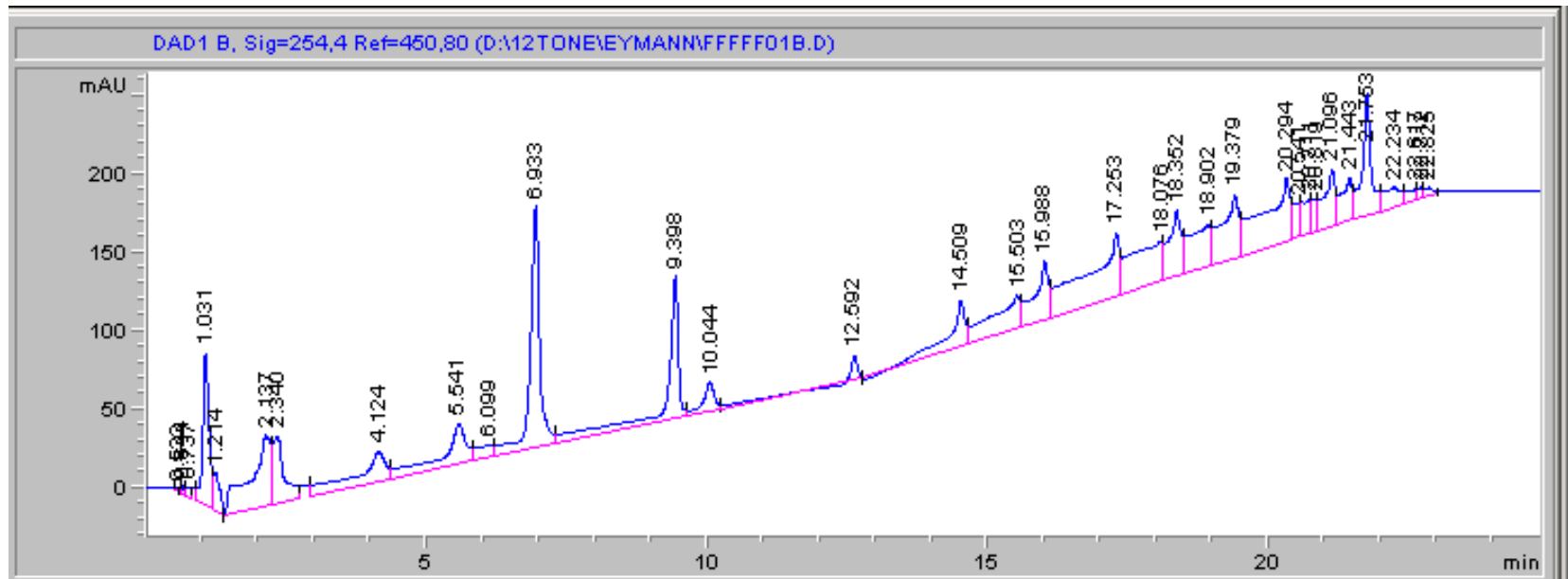


Advanced



# Integration Example

# Integration Example - Default Parameters



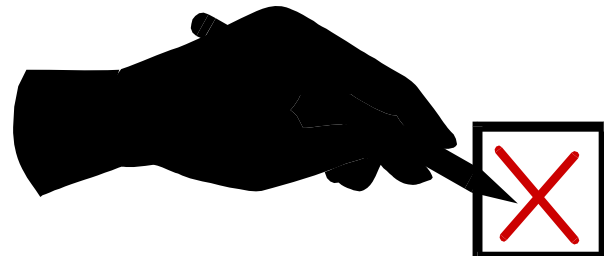
Default integration parameters may not always be the best for your analysis

## Possible Problems

- Noise selected as peaks
- Baseline tracking difficulties
- Drop lines inappropriate

# Practical Integration Advice – Starting Point

1. Set the **slope sensitivity** to 50.
2. Estimate the **peak width** from the initial integration. Use the smallest peak width from a real chromatographic peak, not noise. Set initial **height** and **area reject** to zero.
3. Set the **Tail Peak Skim Height Ratio** to 3, the **Front Peak Skim Height Ratio** to 6, and the **Skim Valley Ratio** to 20.
4. **Baseline correction** is Advanced with **Tangent Skim Mode New Exponential**.
5. Integrate and view the results.
6. If all peaks of interest were not integrated, lower the **slope sensitivity** until all real peaks are integrated.
7. If there are still peaks that cannot be integrated, lower the **peak width** setting.
8. Use **timed events** if necessary.
9. Remove undesired peaks with the **height** or **area reject**.



# Example – Initial

Instrument 1 (offline 1): Data Analysis

File Graphics Integration Calibration Report Spectra Batch View Abort Help

Signals C:\FFFFF01B.D Methods DEF\_LC.M

Data Analysis Sequence: BATCH

Ready/Reprocess Data Mode

Line	Inj	Vial	Sample Name	Method Name	Sample Type	Cal Level	Sample Info	ISTD Amount	Multiplier	Dilution	Data File	Sample
1	1	5	Isocratic Std. 1	BATCH.M	Calibration	1		0	1	1	005-0101.D	0
2	1	5	Isocratic Std. 1	BATCH.M	Calibration	1		0	1	1	005-0102.D	0
3	1	5	Isocratic Std. 1	BATCH.M	Control Sample			0	1	1	005-0103.D	0
4	1	6	Isocratic Std. 2	BATCH.M	Sample			0	1	1	006-0201.D	0
5	1	7	Isocratic Std. 3	BATCH.M	Sample			0	1	1	007-0301.D	0

Integration Calibration Signal Purify Spectrum

Report: Short

DAD1 B, Sig=254...C:\FFFFF01B.D

Manual Events

For All Signals:

Integration Events	Value
Tangent Skim Mode	New Exponential
Tail Peak Skim Height Ratio	3.00
Front Peak Skim Height Ratio	6.00
Skim Valley Ratio	20.00
Baseline Correction	Advanced
Peak to Valley Ratio	500.00

Events Table DAD Default

Time	Integration Events	Value
Initial	Slope Sensitivity	50
Initial	Peak Width	0.06
Initial	Area Reject	0
Initial	Height Reject	0
Initial	Shoulders	OFF

DAD1 B, Sig=254,4 Ret=450,80 (C:\FFFFF01B.D)

Current Integration from DEF\_LC.M default integration values

#	Time	Area	Height	Width	Area%	Symmetry
1	0.533	13.4	2.7	0.064	0.000	0.572
2	0.644	26.2	6.3	0.0537		
3	0.737	41.1	7	0.075		
4	1.031	777.5	97.1	0.1078		
5	1.214	165.8	24	0.0934		
6	2.137	1202.7	44.9	0.3447		
7	2.34	619.7	42.7	0.1999		
8	4.124	763.7	20.1	0.4962		
9	5.541	860	26.6	0.4215	4.237	2.406
10	6.099	182.5	9.1	0.2608	0.903	2.83
11	6.933	1887.7	154.5	0.173	9.343	1.345
12	9.398	1426.2	91.2	0.2081	7.059	3.326
13	10.044	279	18.6	0.21	1.381	1.609

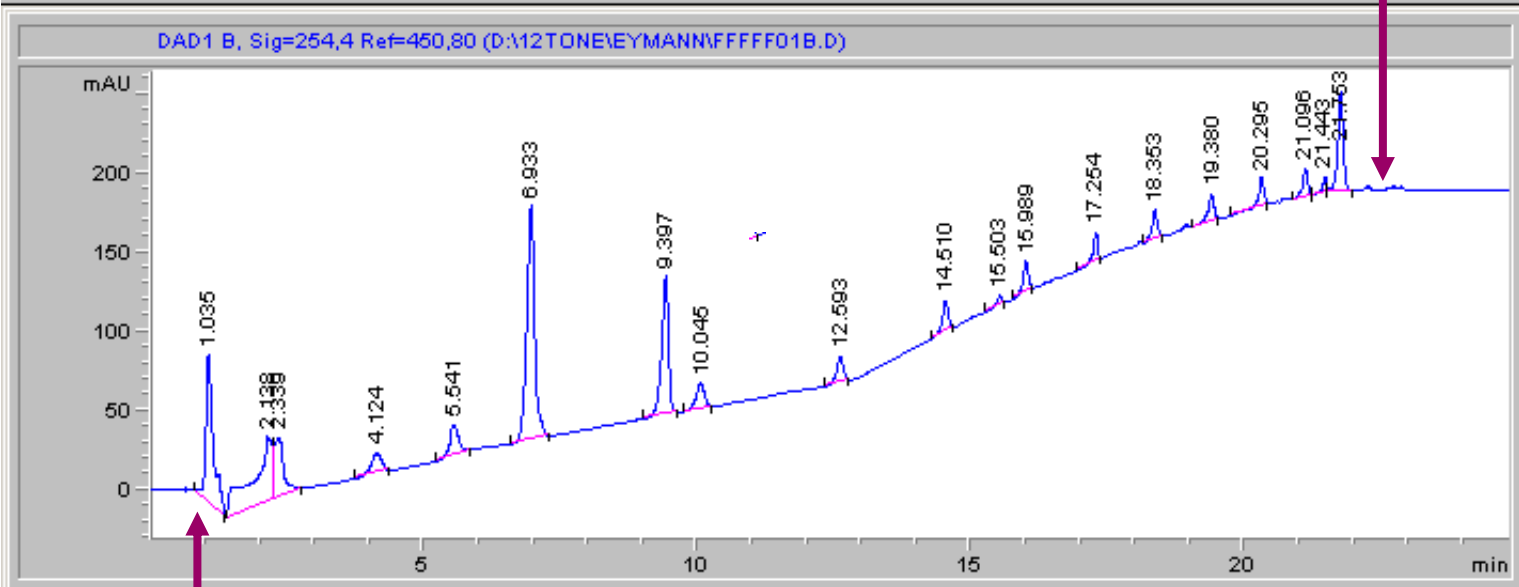
Pick narrowest real chromatographic peak for peak width.

Set initial values, then re-integrate

# First Integration Results

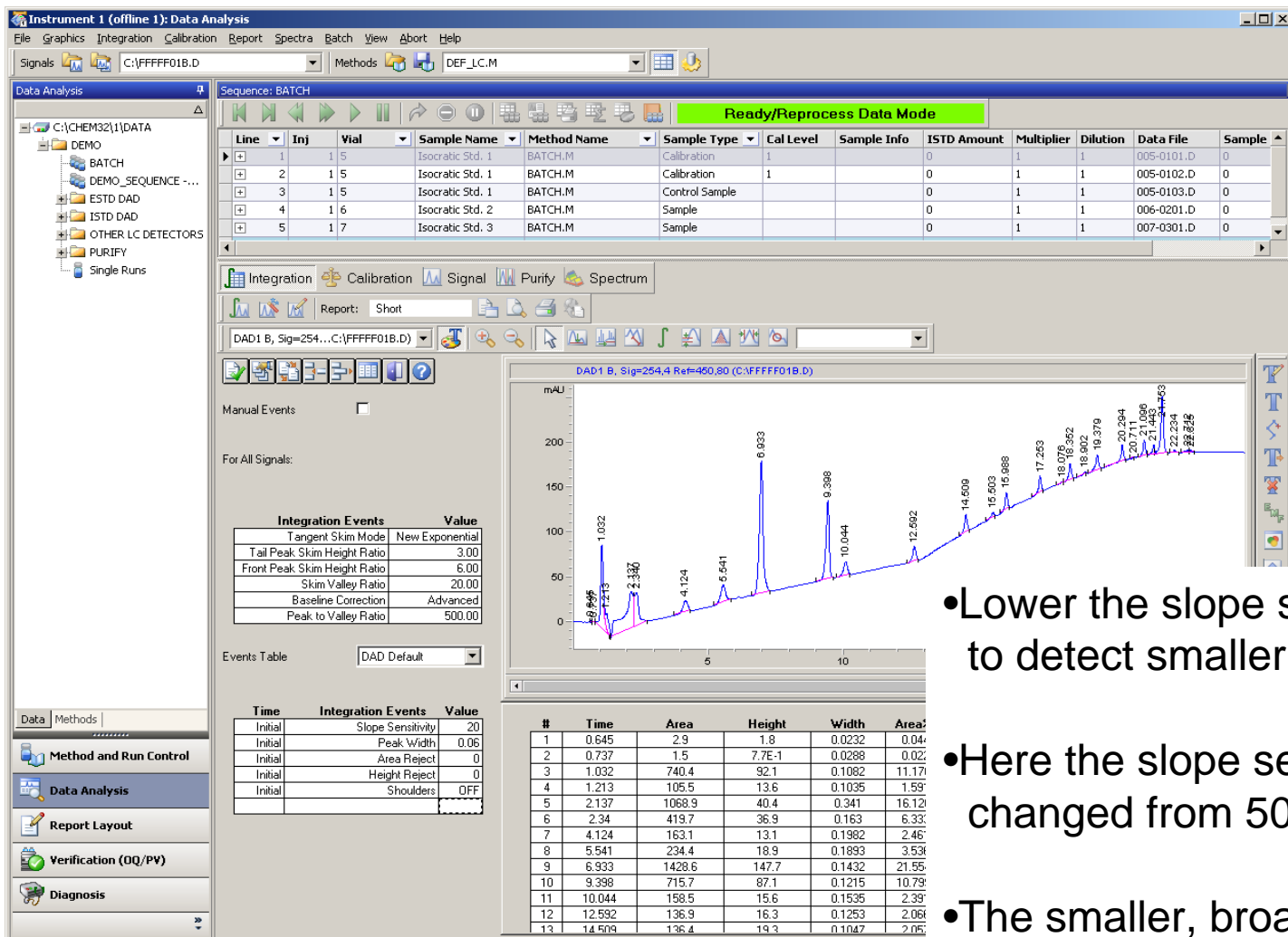
Better, but still needs work.

Desired peaks not Integrated



Baseline problems

# Adjust Initial Parameters



- Lower the slope sensitivity to detect smaller, broader peaks.
- Here the slope sensitivity was changed from 50 to 20.
- The smaller, broader peaks were detected.

# Save Integration Events as Part of a Method

When finished creating the integration events, save them to the method.

The screenshot shows the 'Instrument 1 (offline 1): Data Analysis' window. The 'File' menu is open, and the 'Save As' option is highlighted. A sub-menu is visible, showing 'Method...' and 'Library...' options. The 'Method...' option is highlighted. The 'Methods' dropdown menu is also open, showing 'DEF\_LC.M'. The main window displays a table of integration results and a chromatogram plot.

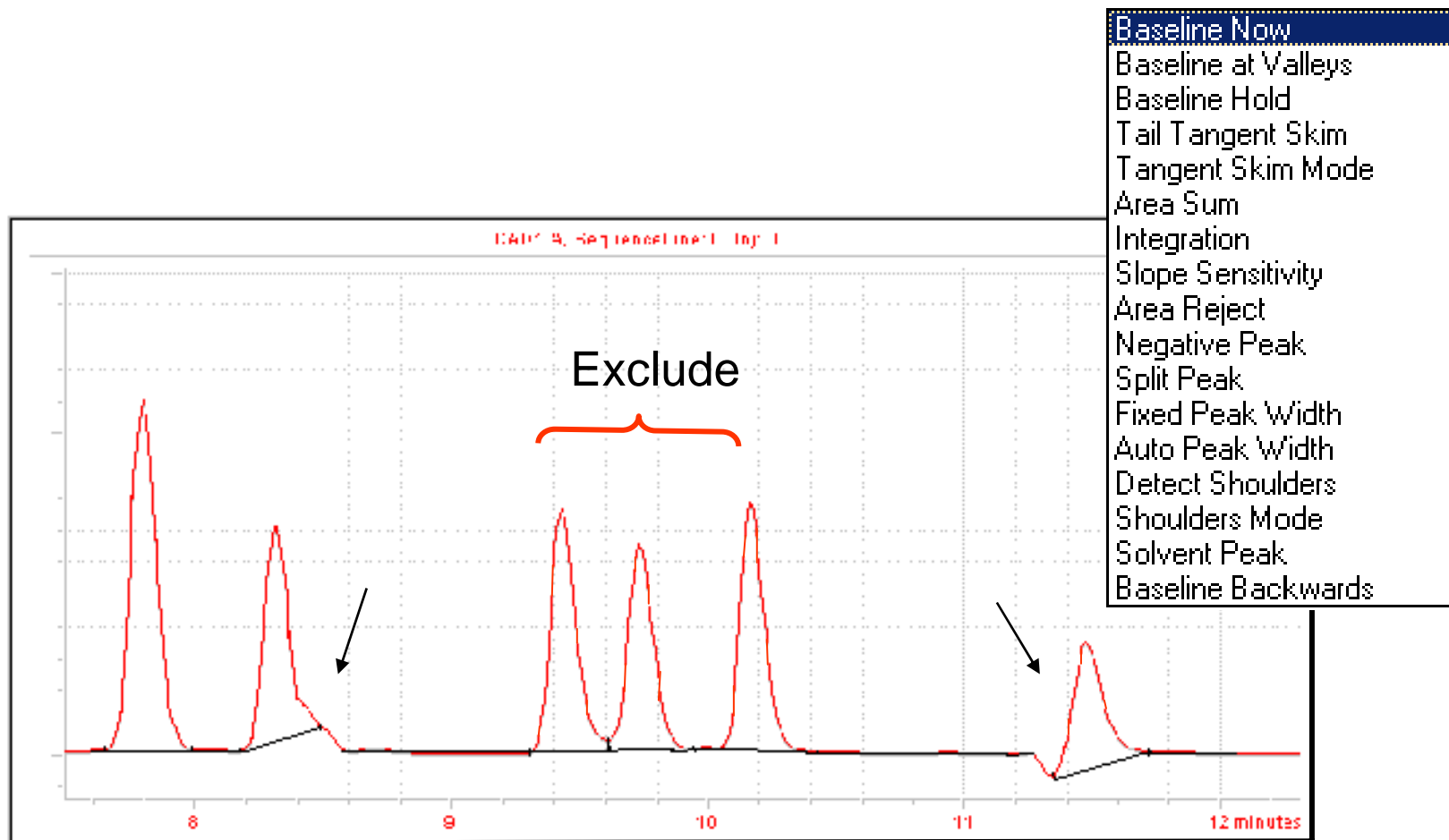
Vial	Sample Name	Method Name	Sample
1 5	Isocratic Std. 1	BATCH.M	Calibration
1 5	Isocratic Std. 1	BATCH.M	Calibration
1 5	Isocratic Std. 1	BATCH.M	Control S
1 6	Isocratic Std. 2	BATCH.M	Sample
1 7	Isocratic Std. 3	BATCH.M	Sample

Chromatogram Plot: DAD1 B, Sig=254,4 Ref=450  
mAU vs. Time (min)  
Peaks at 0.361 and 3.130

# Timed Integration Events



# What Timed Events are Useful for the Problems Below?



# Enhanced Integrator - Timed Events

Add Timed Events

Delete a Timed Event

Instrument 1 [offline 1]: Data Analysis

File Sequence Graphics Integration Calibration Report Spectra Batch View ECM Abort Help

Sequence: DEMO\_SEQUENCE -2006-02-24-1

Use current method

Ready/Reprocess Data Mode

Line	Inj	Vial	Sample Name	Method Name	Sample Type	Cal Level	Sample Info	Sample Am...	ISTD Amount	Multiplier	Dilution
1	1	Vial 1	isocratic standa...	ISOCRA.M	Calibration	1	DAD : slit 2n	0	0	1	1
2	1	Vial 1	isocratic standard ...	ISOCRA.M	Calibration	1	DAD : slit 2n - d	0	0	1	1

Integration Calibration Signal Purify Spectrum

Report: Short

DAD1 A, Sig=25...4-1\001-0101.D

Manual Events:

For All Signals:

Integration Events		Value
Tangent Skim Mode		Standard
Tail Peak Skim Height Ratio		0.00
Front Peak Skim Height Ratio		0.00
Skim Waller Ratio		20.00

Events Table: DAD Default

Time	Integration Events	Value
Initial	Slope Sensitivity	5
Initial	Peak Width	0.05
Initial	Area Reject	5
Initial	Height Reject	1
Initial	Shoulders	OFF

DAD1 A, Sig=254.4 Ref=off (DEMO\DEMO\_SEQUENCE -2006-02-24-1\001-0101.D)

#	Time	Area	Height	Width	Area%	Symmetry
1	1.315	311.1	147.5	0.0336	22.297	0.83
2	1.779	282.4	105.8	0.0411	20.238	0.84
3	3.595	335.4	70.3	0.0746	24.035	0.849
4	6.947	466.5	50	0.1449	33.430	0.871

Ready Integration done.

# Insert Timed Events

Sequence: BATCH

Line	Inj	Vial	Sample Name	Method Name	Sample Type	Cal Level	Sample Info	ISTD Amount	Multiplier	Dilution	Data File	Sample
1	1	5	Isocratic Std. 1	BATCH.M	Calibration	1		0	1	1	005-0101.D	0
2	1	5	Isocratic Std. 1	BATCH.M	Calibration	1		0	1	1	005-0102.D	0
3	1	5	Isocratic Std. 1	BATCH.M	Control Sample			0	1	1	005-0103.D	0
4	1	6	Isocratic Std. 2	BATCH.M	Sample			0	1	1	006-0201.D	0
5	1	7	Isocratic Std. 3	BATCH.M	Sample			0	1	1	007-0301.D	0

Integration Events

Integration Events	Value
Tangent Skim Mode	New Exponential
Tail Peak Skim Height Ratio	3.00
Front Peak Skim Height Ratio	6.00
Skim Valley Ratio	20.00
Baseline Correction	Advanced
Peak to Valley Ratio	500.00

Events Table

Time	Integration Events	Value
Initial	Slope Sensitivity	20
Initial	Peak Width	0.06
Initial	Area Reject	0
Initial	Height Reject	0
Initial	Shoulders	OFF

Chromatogram Data:

#	Time	Area	Height	Width	Area%	Symm
1	0.645	2.9	1.8	0.0232	0.044	0.61
2	0.737	1.5	7.7E-1	0.0288	0.022	0.7
3	1.032	740.4	92.1	0.1082	11.170	0.56
4	1.213	105.5	13.6	0.1035	1.591	0.32
5	2.137	1068.9	40.4	0.341	16.126	3.88
6	2.34	419.7	36.9	0.163	6.333	0.83
7	4.124	163.1	13.1	0.1982	2.461	1.09
8	5.541	234.4	18.9	0.1893	3.536	1.07
9	6.933	1428.6	147.7	0.1432	21.554	1.14
10	9.398	715.7	87.1	0.1215	10.799	1.44
11	10.044	158.5	15.6	0.1535	2.391	1.13
12	12.592	136.9	16.3	0.1253	2.066	1.07
13	14.509	136.4	19.3	0.1047	2.057	1.07

- Use drop box to select desired event.
- Click on chromatogram at the desired time.
- Or, use the insert timed event tool.
- Use the delete timed events tool to remove an event.

# Insert Timed Events

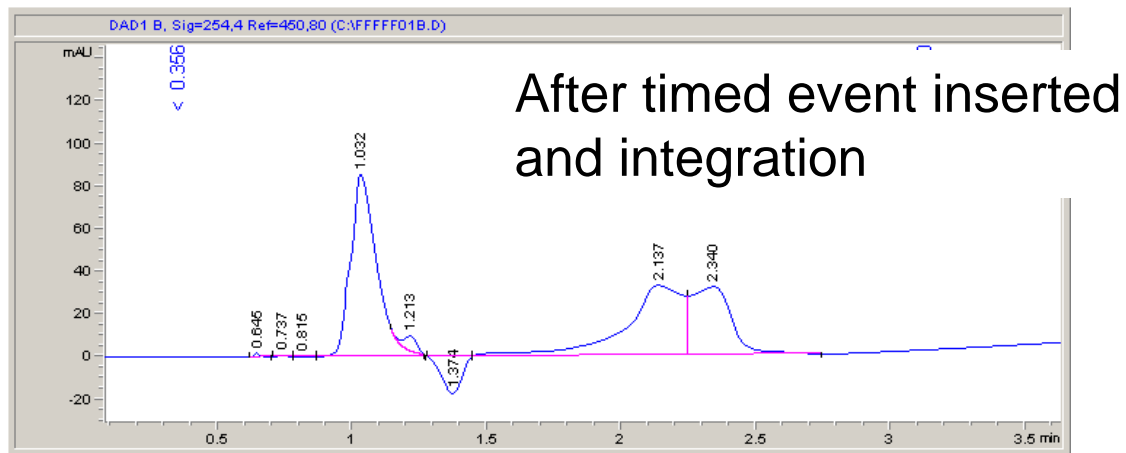
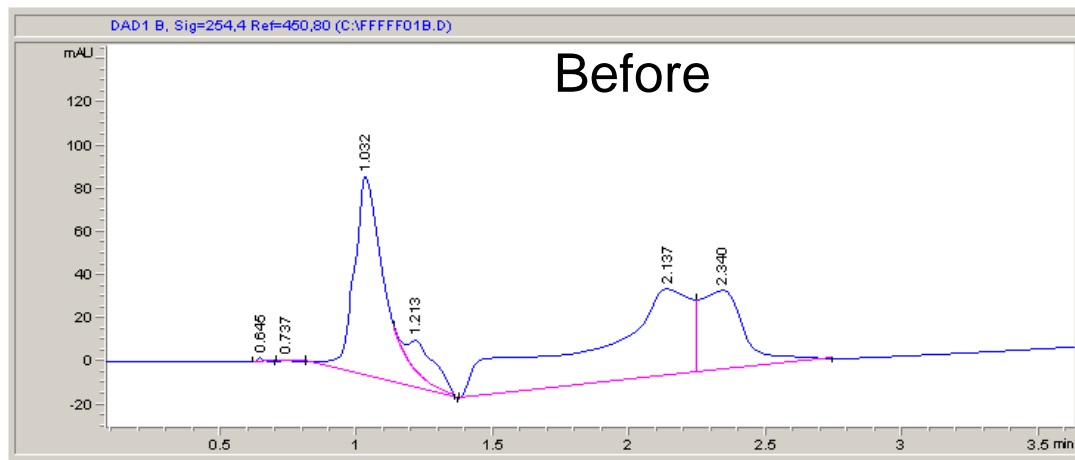
Manual Events

For All Signals:

Integration Events	Value
Tangent Skim Mode	New Exponential
Tail Peak Skim Height Ratio	3.00
Front Peak Skim Height Ratio	6.00
Skim Valley Ratio	20.00
Baseline Correction	Advanced
Peak to Valley Ratio	500.00

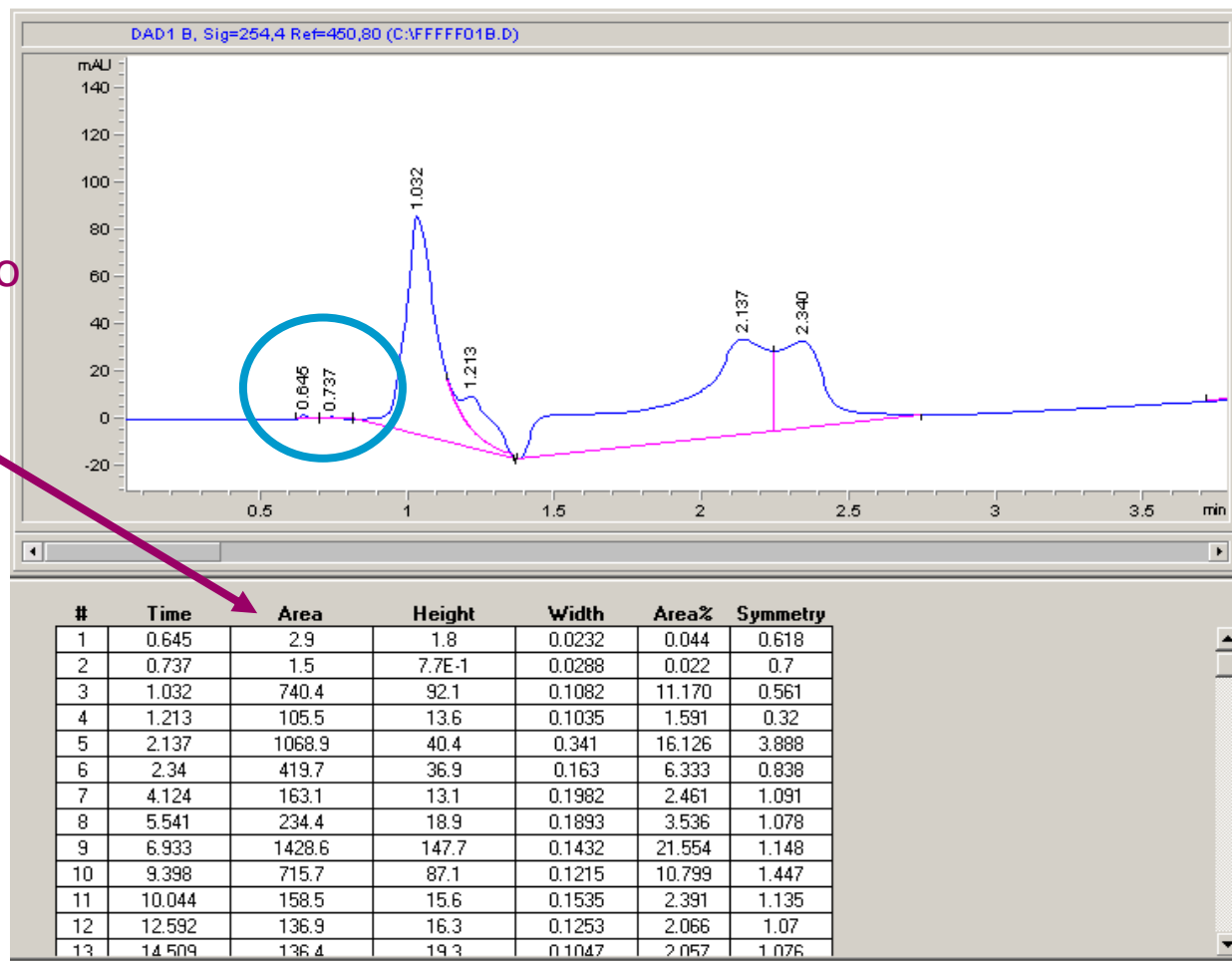
Events Table DAD Default

Time	Integration Events	Value
Initial	Slope Sensitivity	20
Initial	Peak Width	0.06
Initial	Area Reject	0
Initial	Height Reject	0
Initial	Shoulders	OFF
0.356	Negative Peak	ON
3.130	Negative Peak	OFF



# Finish Integration

Use area and height reject to ignore unwanted peaks



# Save and Close

The screenshot displays the Agilent ChemStation interface for 'Instrument 1 (offline 1): Data Analysis'. The main window shows a chromatogram titled 'DAD1 B, Sig=254.4 Ref=450.80 (C:\FFFFF01B.D)'. The x-axis represents time in minutes (0 to 25), and the y-axis represents intensity in mAU (0 to 200). Numerous peaks are labeled with their retention times. A red arrow points to a save icon (a document with a green checkmark) in the left sidebar.

**Sequence: BATCH**

Line	Inj	Vial	Sample Name	Method Name	Sample Type	Cal Level	Sample Info	ISTD Amount	Multiplier	Dilution	Data File	Sample
1	1	5	Isocratic Std. 1	BATCH.M	Calibration	1		0	1	1	005-0101.D	0
2	1	5	Isocratic Std. 1	BATCH.M	Calibration	1		0	1	1	005-0102.D	0
3	1	5	Isocratic Std. 1	BATCH.M	Control Sample			0	1	1	005-0103.D	0
4	1	6	Isocratic Std. 2	BATCH.M	Sample			0	1	1	006-0201.D	0
5	1	7	Isocratic Std. 3	BATCH.M	Sample			0	1	1	007-0301.D	0

**Integration Events**

Integration Events	Value
Tangent Skim Mode	New Exponential
Tail Peak Skim Height Ratio	3.00
Front Peak Skim Height Ratio	6.00
Skim Valley Ratio	20.00
Baseline Correction	Advanced
Peak to Valley Ratio	500.00

**Events Table** (DAD Default)

Time	Integration Events	Value
Initial	Slope Sensitivity	20
Initial	Peak Width	0.06
Initial	Area Reject	5
Initial	Height Reject	0
Initial	Shoulders	OFF
0.351	Negative Peak	ON
3.130	Negative Peak	OFF

**Chromatogram Data Table**

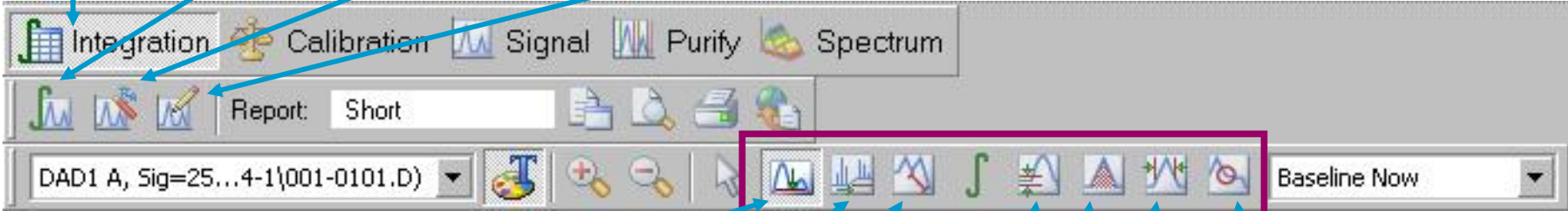
#	Time	Area	Height	Width	Area%	Symmetry
1	1.032	576.6	85.2	0.0933	9.948	0.629
2	1.213	24.3	7	0.0577	0.418	0.899
3	1.374	92.8	17.7	0.0853	1.601	1.299
4	2.137	491.1	32.6	0.2079	8.472	1.675
5	2.34	322.5	31.7	0.1696	5.564	1.069
6	4.124	163.1	13.1	0.1982	2.814	1.091
7	5.541	234.4	18.9	0.1893	4.043	1.078
8	6.933	1428.6	147.7	0.1432	24.646	1.148
9	9.398	715.7	87.1	0.1215	12.348	1.447
10	10.044	158.5	15.6	0.1535	2.734	1.135
11	12.592	136.9	16.3	0.1253	2.362	1.07
12	14.509	136.4	19.3	0.1047	2.352	1.076
13	15.503	38.9	5.6	0.1031	0.671	1.858

# Summary: Integration Task Tool Bar



Use **Auto Integrate** to find suitable integration events

Integration Tasks    Integrate Current Chromatogram    Auto Integrate    Integration Events



Set Baseline Now

Set Baseline Hold

Set Tail Tangent Skim

Set Integration

Set Area Reject  
Set Slope Sensitivity

Set Fixed Peak Width

Set Detect Shoulders

Only visible with  
integration events  
open

# Manual Integration Events



# Manual Integration Events vs. Timed Integration Events

The image displays two screenshots of the ChemStation software interface. The top screenshot, labeled '1.', shows a chromatogram with three peaks. The first two peaks are highlighted with a red box, and a red '1.' is placed next to them. The bottom screenshot, labeled '2.', shows a zoomed-in view of two peaks with a red box around them and a red '2.' next to them. Below the screenshots are two tables: 'Integration Events' and 'Events Table'.

**Integration Events**

Integration Events	Value
Tangent Skim Mode	Standard
Tail Peak Skim Height Ratio	0.00
Front Peak Skim Height Ratio	0.00
Skim Valley Ratio	20.00

**Events Table**

Time	Integration Events	Value
Initial	Slope Sensitivity	4
Initial	Peak Width	0.05
Initial	Area Reject	5
Initial	Height Reject	10
Initial	Shoulders	OFF
1.200	Integration	OFF
1.400	Integration	ON

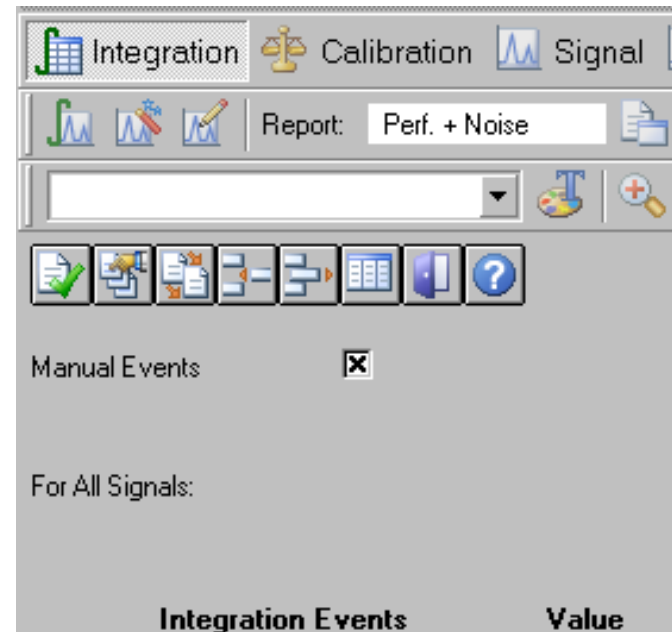
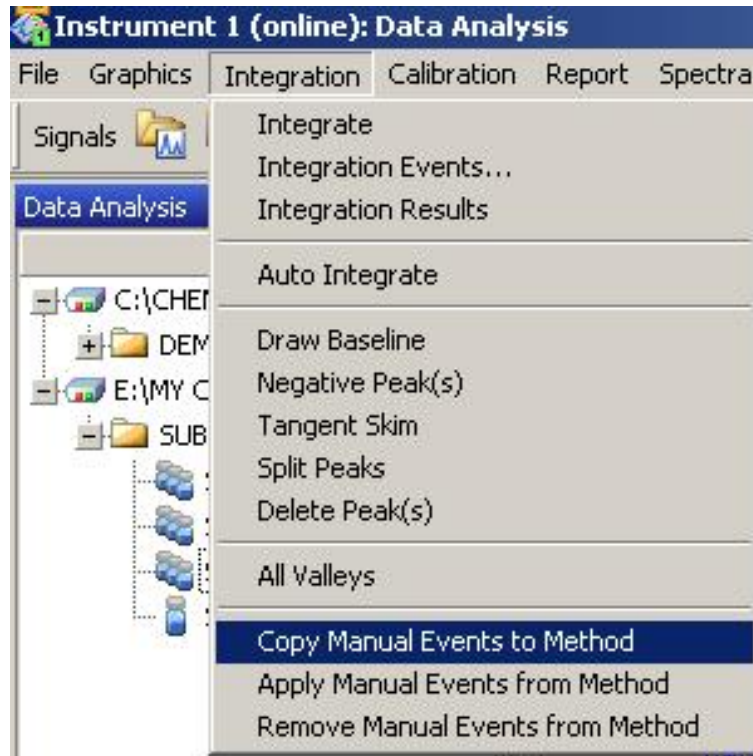
1. Manual Integration Events

2. Timed Integration Events

# Treatment of Manual Integration Events in ChemStation

- Manual integration events are stored in a method.  
(Always possible)
- Manual events are specific for a certain data file, so you would save these events in DA.M.  
(Possible since ChemStation B.02.01)
- However, this method cannot be used for reprocessing (especially interesting for calibration runs).
- During reprocessing, all manual changes in DA.M get overwritten.

# Saving Manual Integration Events with a Method



1. Copy manual events to the method.
2. The manual events have to be applied manually, or you have to select “Manual Events” in the integration events table to always apply the manual events.

# Solution in ChemStation B.04.01: Manual Integration Events Stored in the Data File

- Automatically applied during data review and reprocessing
- Indicator in Navigation Table when present
- Icons to handle manual events:
  - “Save” to data file
  - “Delete” all manual baselines from file
  - “Undo”: Stepwise removal of unsaved manual baselines
- It is still possible to store manual events in a method.

# Copying Manual Integration Events to Other Data Files

The screenshot displays the 'Instrument 1 (online): Data Analysis' interface. The top menu bar includes File, Method, Sequence, Graphics, Integration, Calibration, Report, Spectra, Batch, View, Abort, and Help. The 'Sequence: BATCH' window shows a table of integration events. A red box highlights the second row, which is selected. Below the table, the 'Integration' tab is active, showing a chromatogram with peaks labeled at retention times 0.47, 0.92, 1.82, and 2.569. A red box highlights the peak at 0.47 minutes. The chromatogram is titled 'DAD1 A, Sig=254,4 Ref=550,100 (DEMOV005-0102.D)'. The y-axis is labeled 'mAU' and the x-axis is labeled 'min'.

...	Line	Inj	Vial	Sample Name	Method Name	Sample Type	Man...	Cal Level
<input type="checkbox"/>	1	1	5	Isocratic Std. 1	BATCH.M	Calibration	<input checked="" type="checkbox"/>	1
<input checked="" type="checkbox"/>	2	1	5	Isocratic Std. 1	BATCH.M	Calibration	<input checked="" type="checkbox"/>	1
<input type="checkbox"/>	3	1	5	Isocratic Std. 1	BATCH.M	Control Sample	<input checked="" type="checkbox"/>	
<input checked="" type="checkbox"/>	4	1	6	Isocratic Std. 2	BATCH.M	Sample	<input type="checkbox"/>	
<input checked="" type="checkbox"/>	5	1	7	Isocratic Std. 3	BATCH.M	Sample	<input type="checkbox"/>	

Integration Calibration Signal Purify Spectrum

Report: Short All Loaded Signals

DAD1 A, Sig=254,4 Ref=550,100 (DEMOV005-0102.D)

mAU

50

0

0.47 0.92 1.82 2.569

min

DAD1 B, Sig=230,4 Ref=550,100 (DEMOV005-0102.D)

mAU

0.47 0.92

# Deleting Manual Integration Events

The screenshot displays the 'Instrument 1 (online): Data Analysis' window. The menu bar includes File, Method, Sequence, Graphics, Integration, Calibration, Report, Spectra, Batch, View, Abort, and Help. The 'Sequence: BATCH' panel shows a table of integration events. The table has columns for Line, Inj, Vial, Sample Name, Method Name, Sample Type, Man..., and Cal L. The first row is highlighted, showing Line 1, Inj 1, Vial 5, Sample Name 'Isocratic Std. 1', Method Name 'BATCH.M', Sample Type 'Calibration', Man... '-', and Cal L '1'. Below the table, the 'Integration' tab is active, showing a chromatogram plot with peaks at retention times 0.547, 1.021, and 2.565. The plot title is 'DAD1 A, Sig=254,4 Ref=550,100 (DEMO\005-0101.D)'. The y-axis is labeled 'mAU' and the x-axis is labeled with retention times 1, 2, 3, and 4. A peak at 1.021 is labeled with 'Area: 172.04'. The bottom of the window shows another plot titled 'DAD1 B, Sig=230,4 Ref=550,100 (DEMO\005-0101.D)'.

...	Line	Inj	Vial	Sample Name	Method Name	Sample Type	Man...	Cal L
▶	1	1	5	Isocratic Std. 1	BATCH.M	Calibration	-	1
	2	1	5	Isocratic Std. 1	BATCH.M	Calibration	-	1
	3	1	5	Isocratic Std. 1	BATCH.M	Control Sample	-	
	4	1	6	Isocratic Std. 2	BATCH.M	Sample	-	
	5	1	7	Isocratic Std. 3	BATCH.M	Sample	-	

# Information on ChemStation

Documentation can be found on the Agilent website

<http://www.chem.agilent.com/Scripts/PDS.asp?IPage=61233>

- Product Datasheet
- Specification
- Application Notes
- Manuals

Manuals can be found your ChemStation Installation DVD

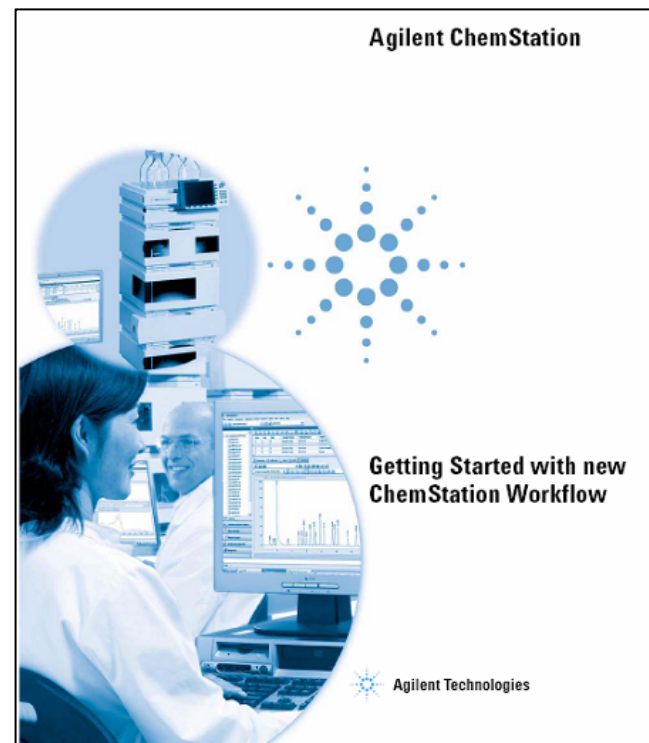
- Getting Started with New ChemStation Workflow  
PartNo.G2170-90042
- Understanding your ChemStation  
PartNo. G2070-91125
- OpenLAB Option  
PartNo.G2170-90233

Software Status Bulletin

<http://www.chem.agilent.com/en-US/Support/Downloads/Patches/Pages/default.aspx>

Customer Trainings (NorthAmerica)

<http://www.chem.agilent.com/cag/training/northam/index.asp>



# Learning Products – North America Course Catalog

Users needing to increase productivity in the lab by utilizing both standard and advanced features available in the Agilent GC/LC ChemStation may want to attend one of the following courses:

- H2606A - ChemStation for GC Data Analysis and Reporting (2 days)
- H5928A - Agilent HPLC (2D) Data Analysis and Reporting (2 days)
- H4039A - Agilent HPLC (3D) Data Analysis and Reporting (3 days)

## **Course Features**

- Data acquisition and method creation
- Data analysis including integration and calibration
- Sequencing
- Reporting

**For more information concerning course content, dates and locations, please visit:**

<http://www.chem.agilent.com/en-US/education/en-us/classroomtraining/Pages/Courselisting.aspx>



# Learning Products – European Course Catalog

Users needing to increase productivity in the lab by utilizing both standard and advanced features available in the Agilent GC/LC ChemStation may want to attend one of the following courses:

- H4033A – Agilent HPLC (3D) Method&Run Control, Data Analysis and Reporting (4 days)
- H8718A - Agilent HPLC (3D) Data Analysis and Reporting (2 days)
- H5928A – Advanced User Training, Quantification and Result Reporting (2 days)

## Course Features

- Data acquisition and method creation
- Data analysis including integration and calibration
- Sequencing
- Reporting

**For more information concerning course content, dates and locations, please visit:**

<http://www.chem.agilent.com/en-US/education/pages/homepage.aspx>

# QUESTIONS?

