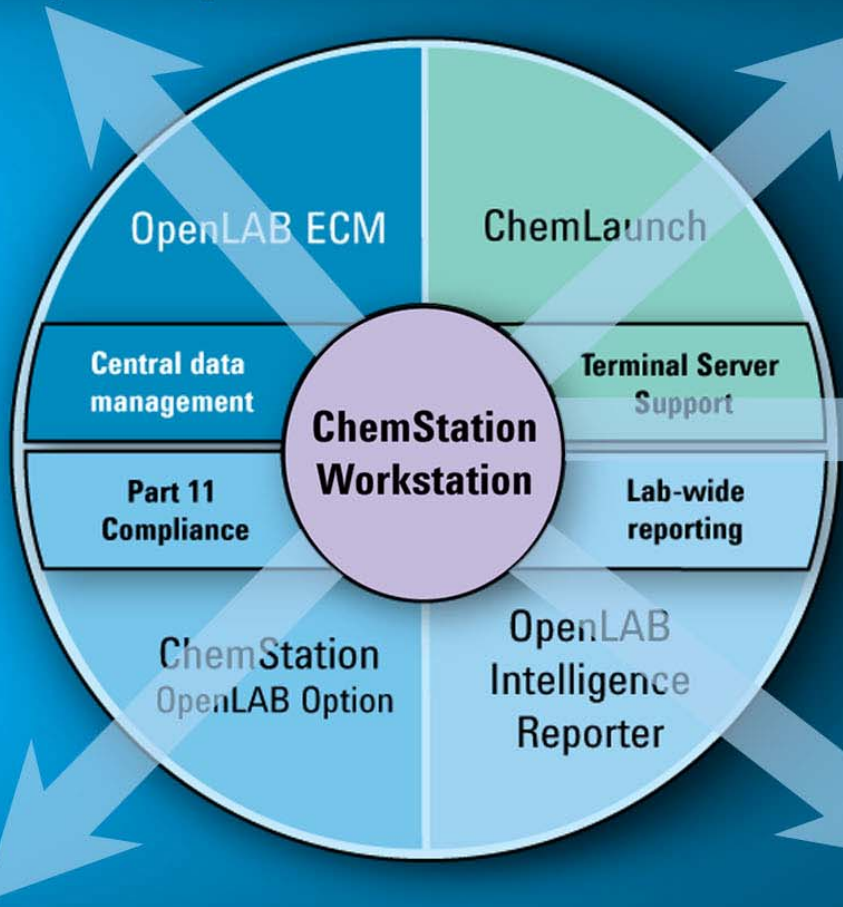
ChemStation E-Seminars

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

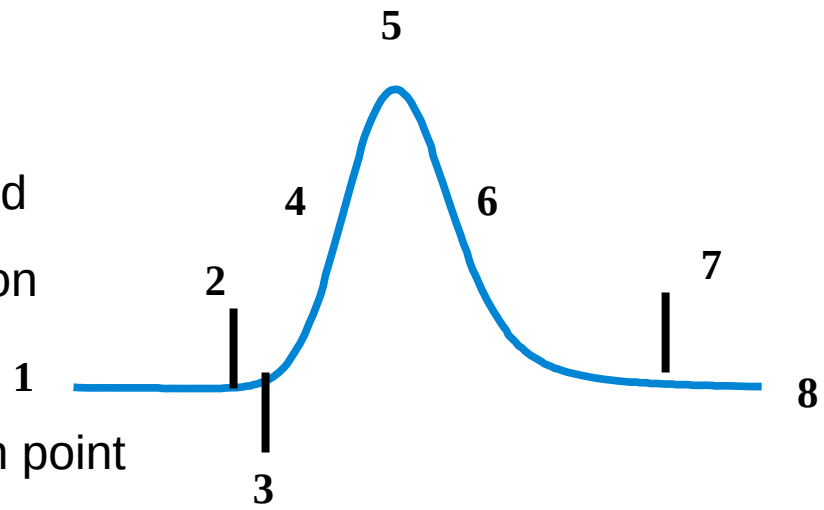
4 Curvature becomes negative: front inflection

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.

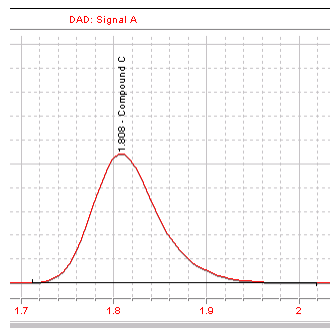
3) Identifies the start time for a peak and marks this point with a vertical tick mark.

4) Finds the apex of each peak, creates a parabolic fit for the peak top, and stores the retention time.

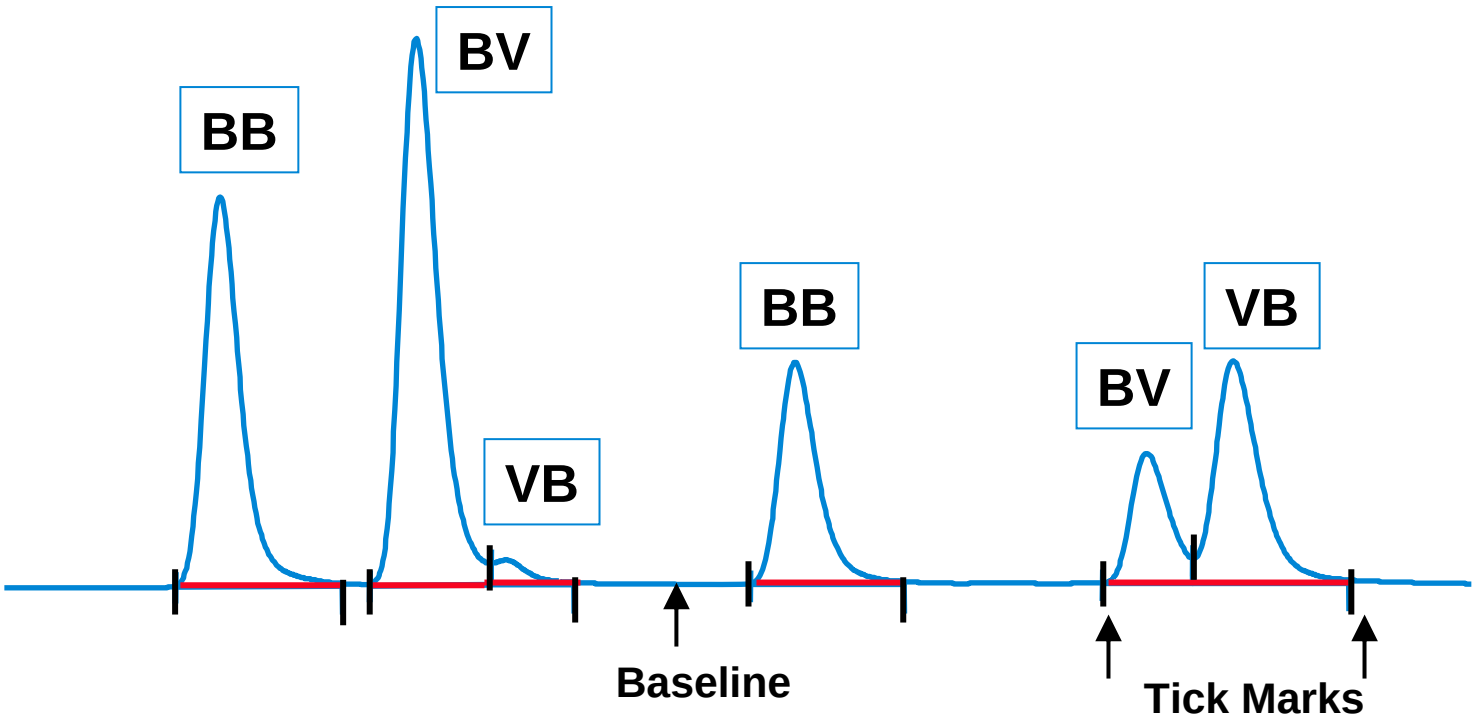
5) Identifies the end time for the peak, and marks this point with a vertical tick mark.

6) Constructs a baseline.

7) Calculates the area, height, and peak width for each peak.



Default Baseline Construction

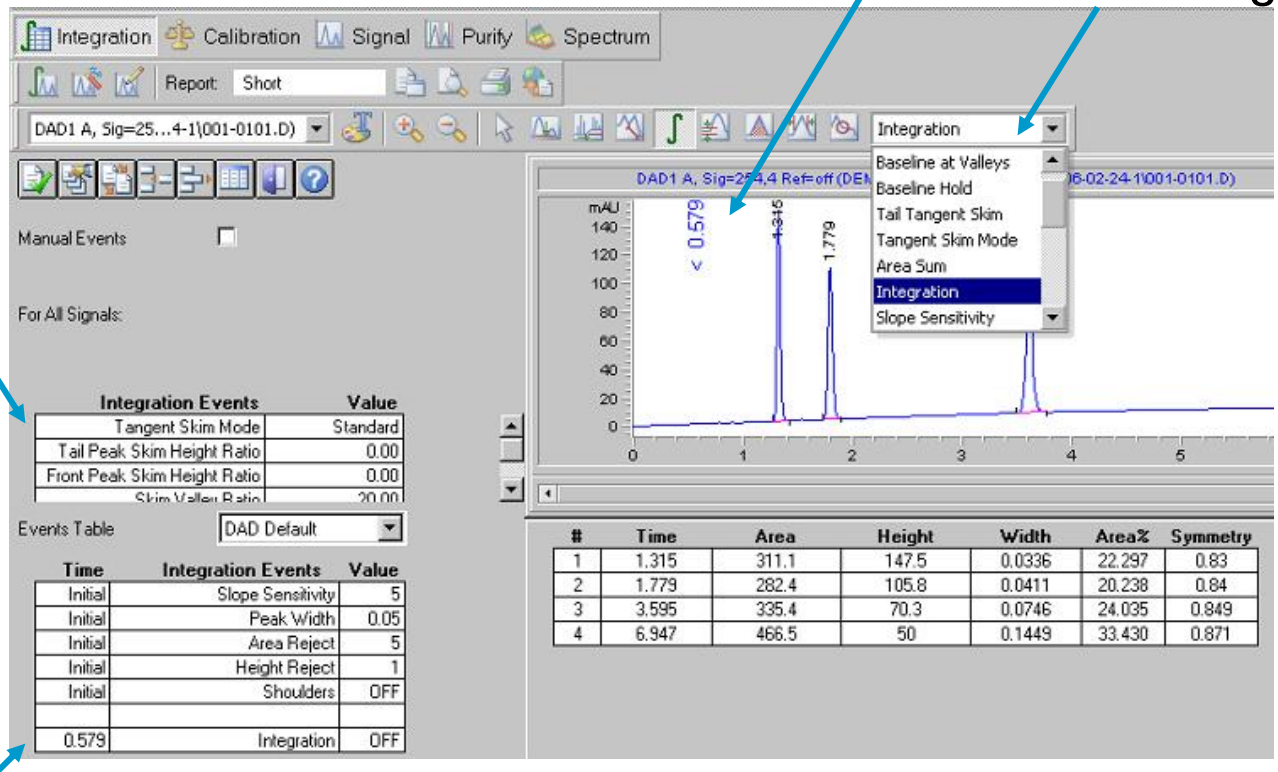


The default baseline is constructed as a series of straight line segments between: the start of the baseline, the tick marks, and the end of the peak.

Integrator UI

Define integration parameters just by selecting and clicking on the chromatogram

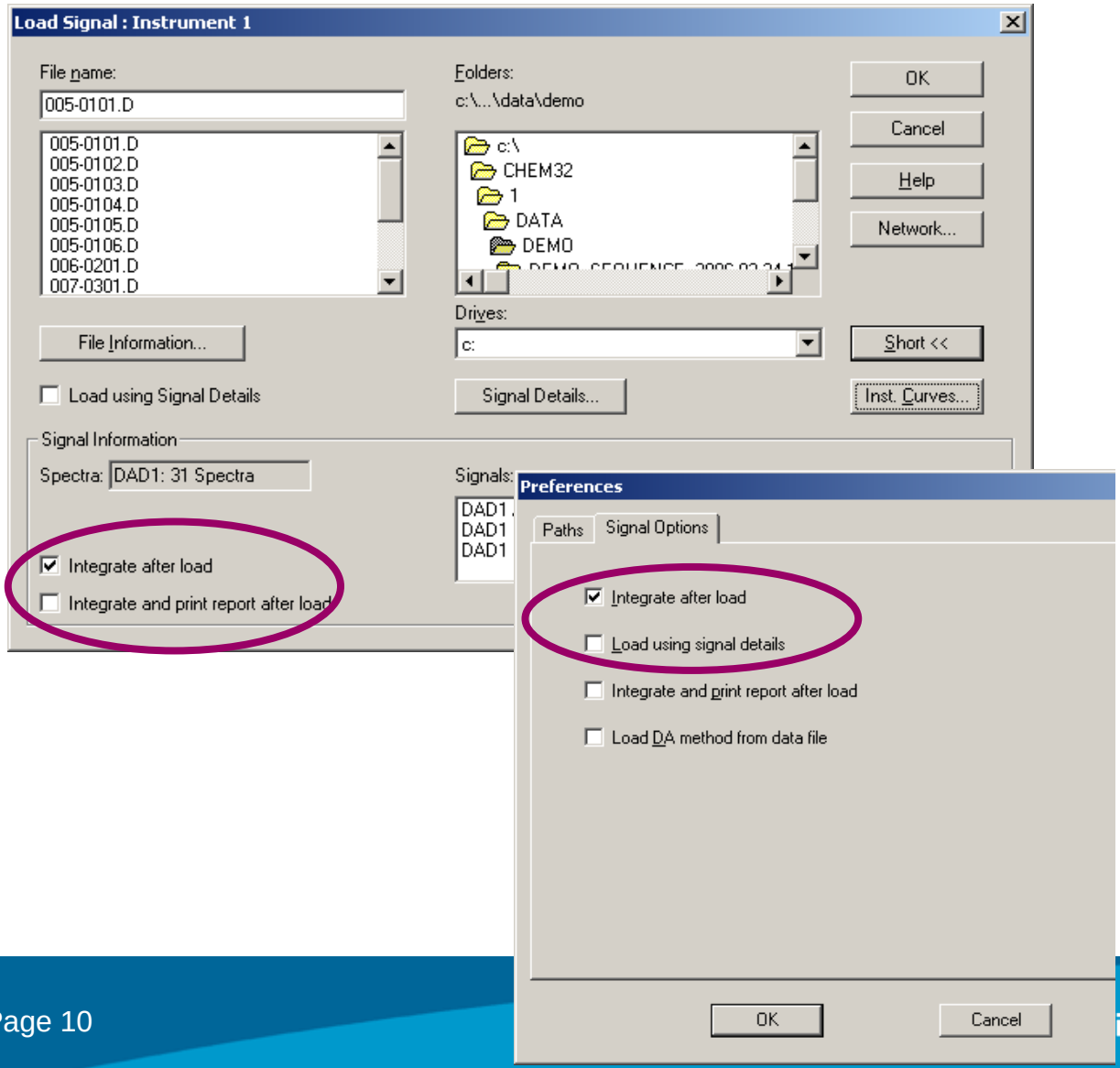
Integration events for all signals



There are two sets of detector specific integration events:

initial events and *time-based*

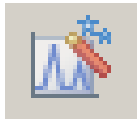
When a Signal is Loaded, Integration May Occur Automatically



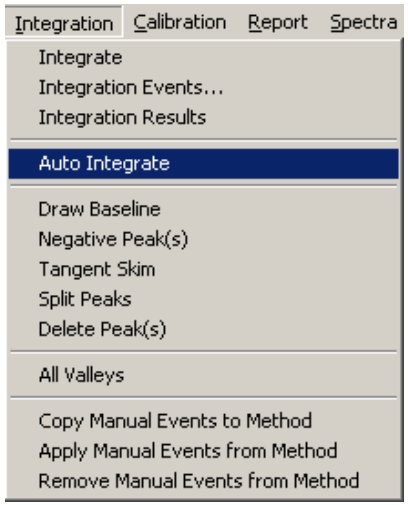
Integrate by:

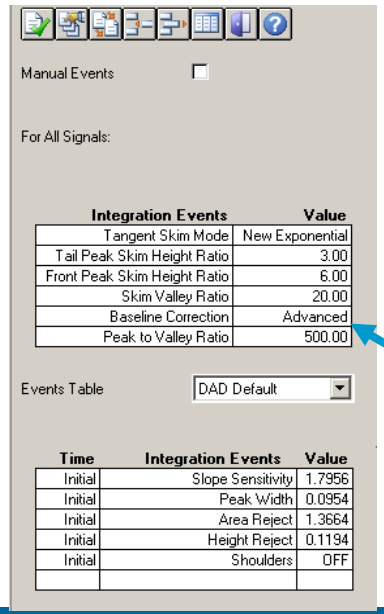
- Selecting Integrate after load in *Load Signal* dialog box or *Preferences* dialog box.
- Selecting *Integrate* or *Auto Integrate* from the menu.
- Selecting the Integration or Auto Integration Tool.
- Running a method where the Run Time Checklist includes Data Analysis.

Auto Integrate



Can be a good starting point for integration events.

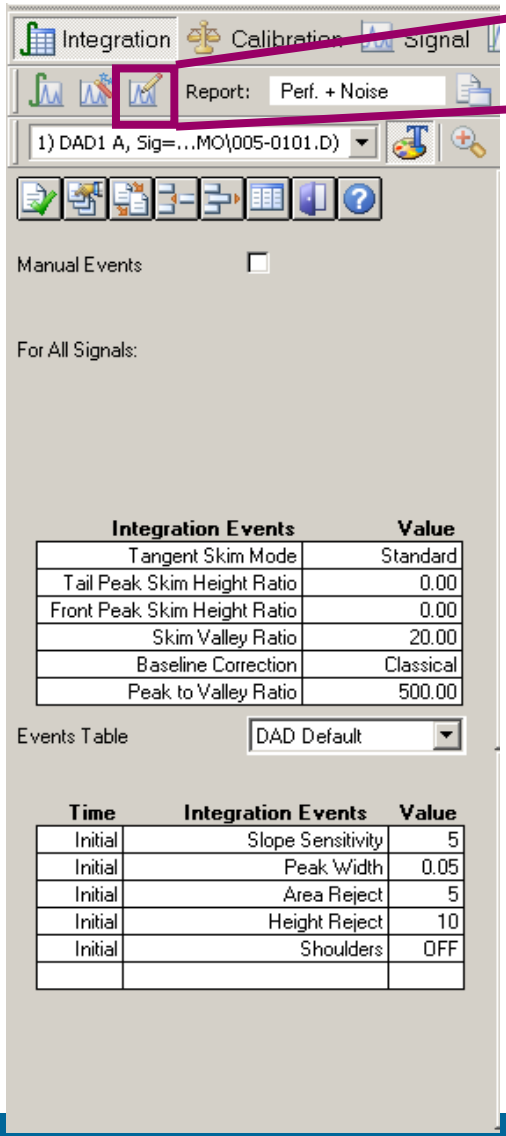


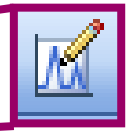


- Examines beginning and end regions to estimate noise.
- Assigns initial Slope Sensitivity and Height Reject.
- Assigns temporary Peak Width value for first pass integration.
- Sets Area Reject to zero.
- Performs trial integration, may be repeated several times.
- Calculates Peak Width based on early eluting peaks.
- Refines Slope Sensitivity and Height Reject.
- Computes Area Reject as 90% area of most symmetrical peak.

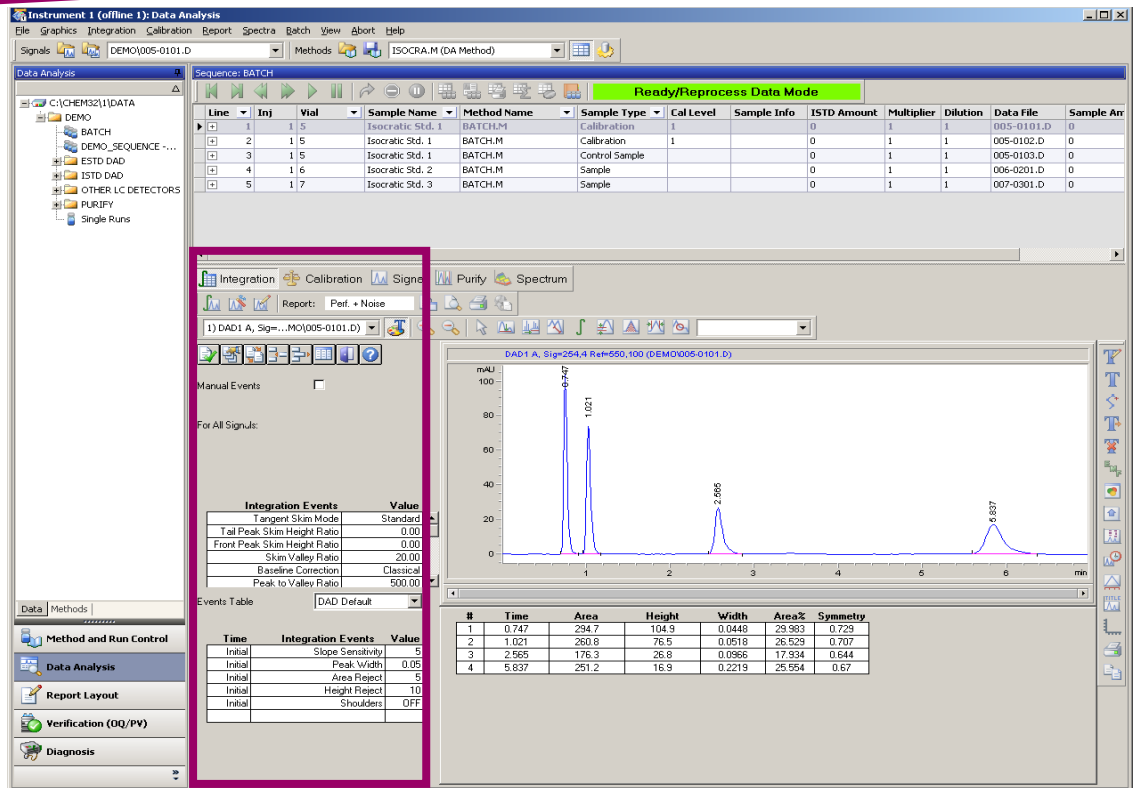
Autointegrates based on your settings in **For All Signals.**

Set Up Integration





Loads Integration Events



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

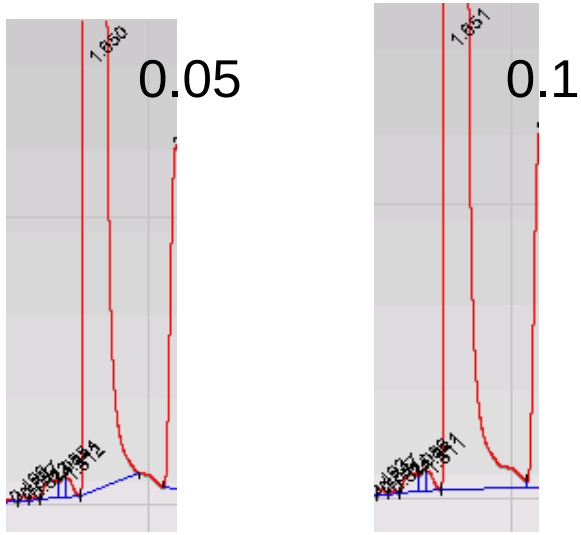
Events Table: DAD Default

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

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.

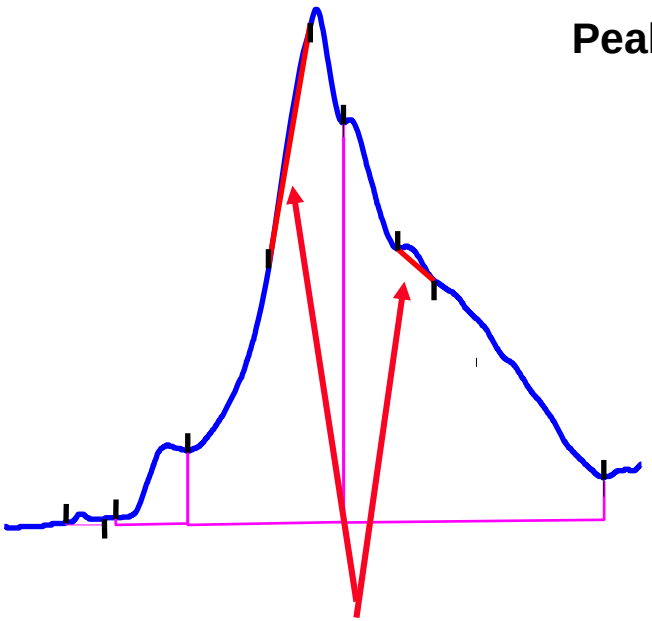


Area reject- All peaks whose areas are below this value will not be reported.

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

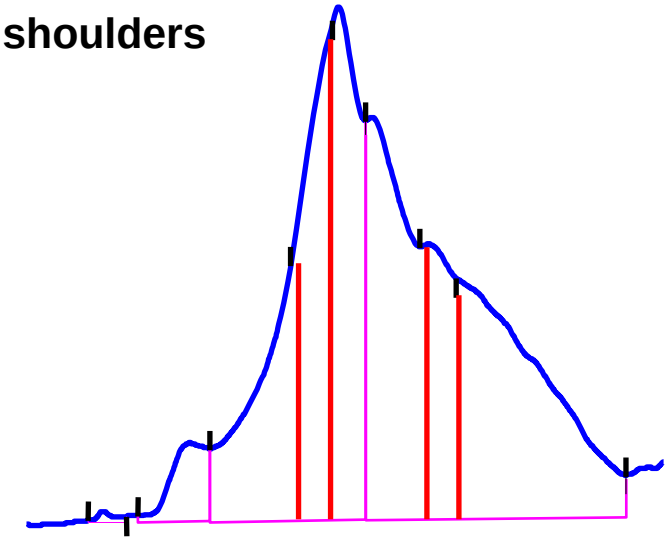
Shoulder Detection

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



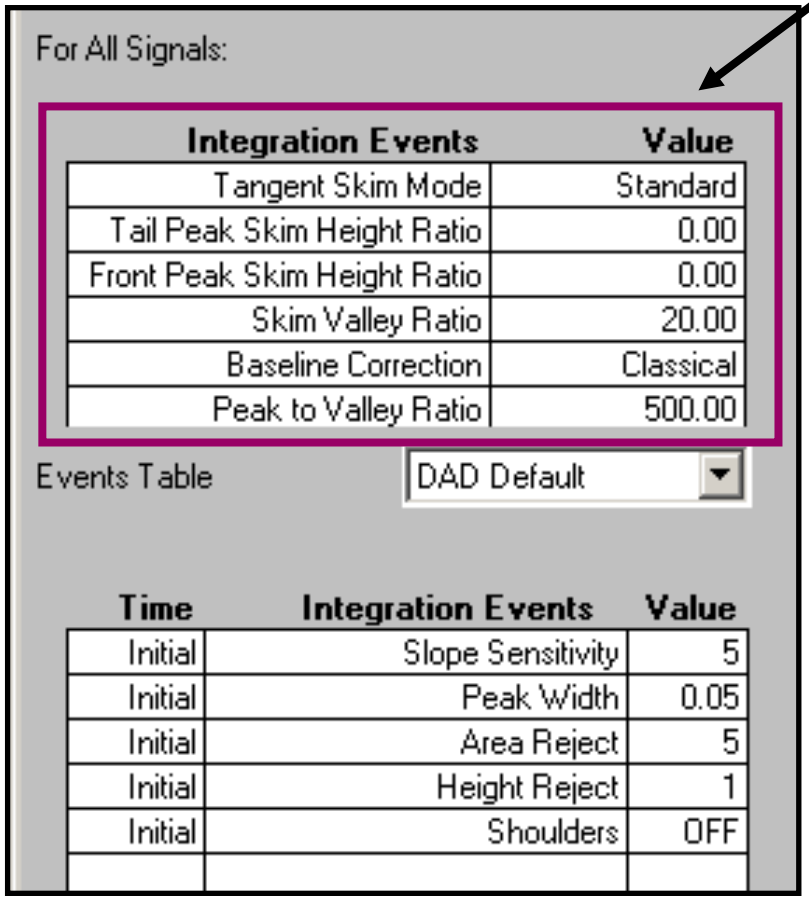
Tangent Shoulders

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

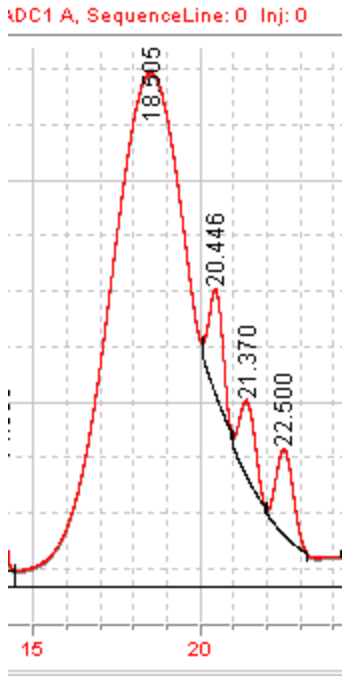
For All Signals:

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: 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

- 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

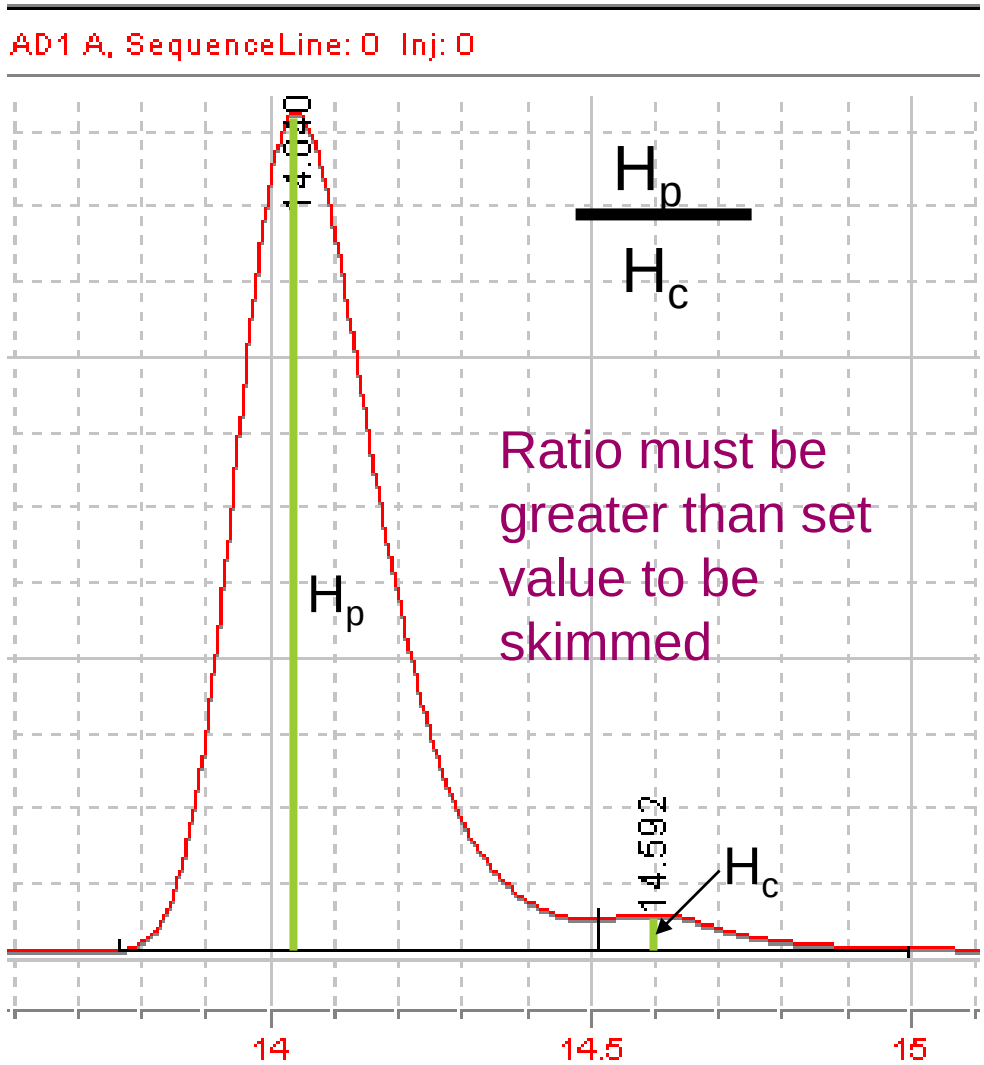
For All Signals:

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

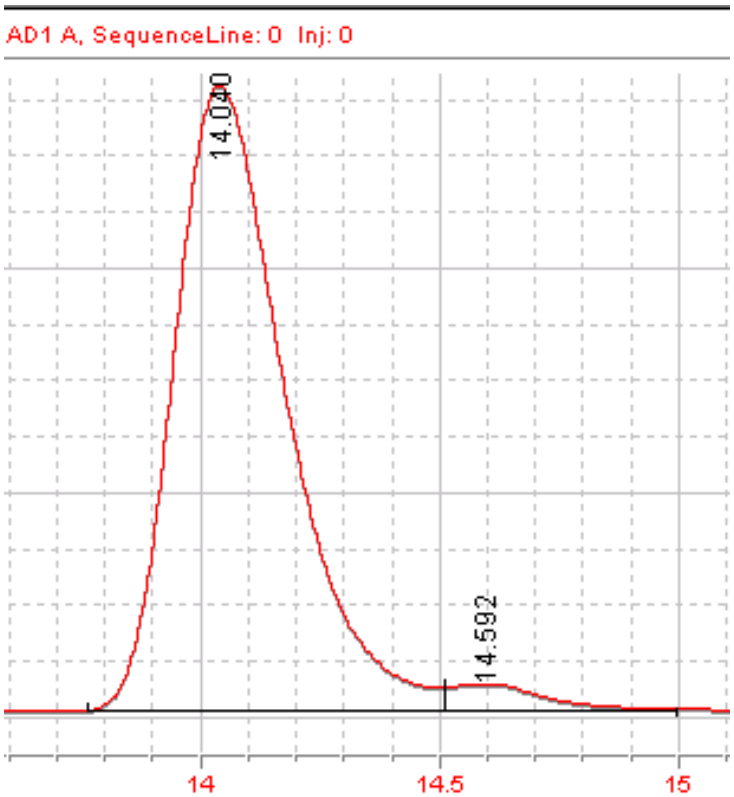
Events Table: DAD Default

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

Setting the value to zero disables tangent skimming

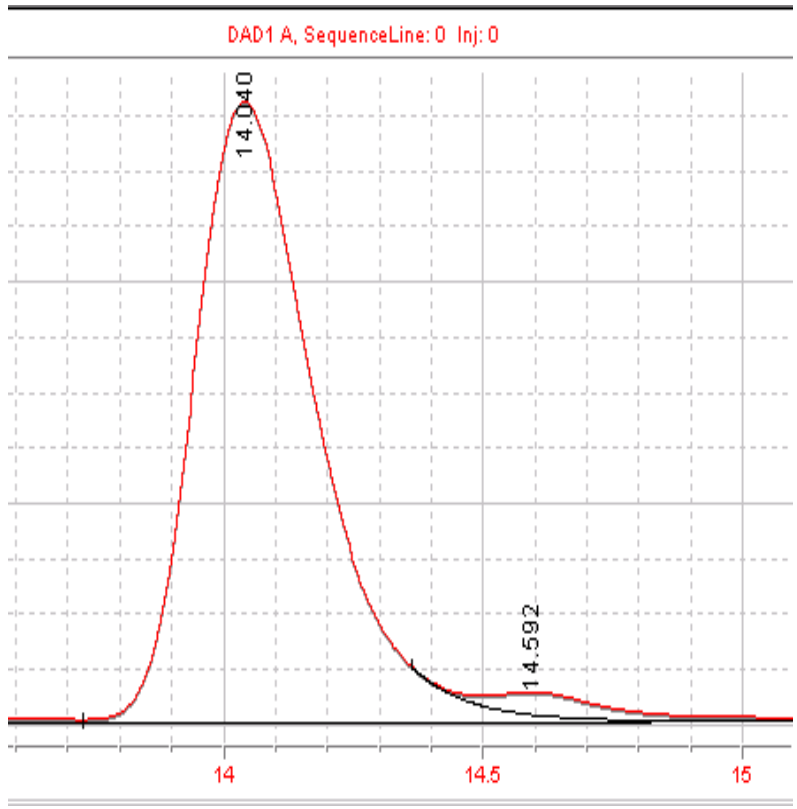


Tail Peak Skim Height Ratio



No Tangent Skimming

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



Tangent Skimmed

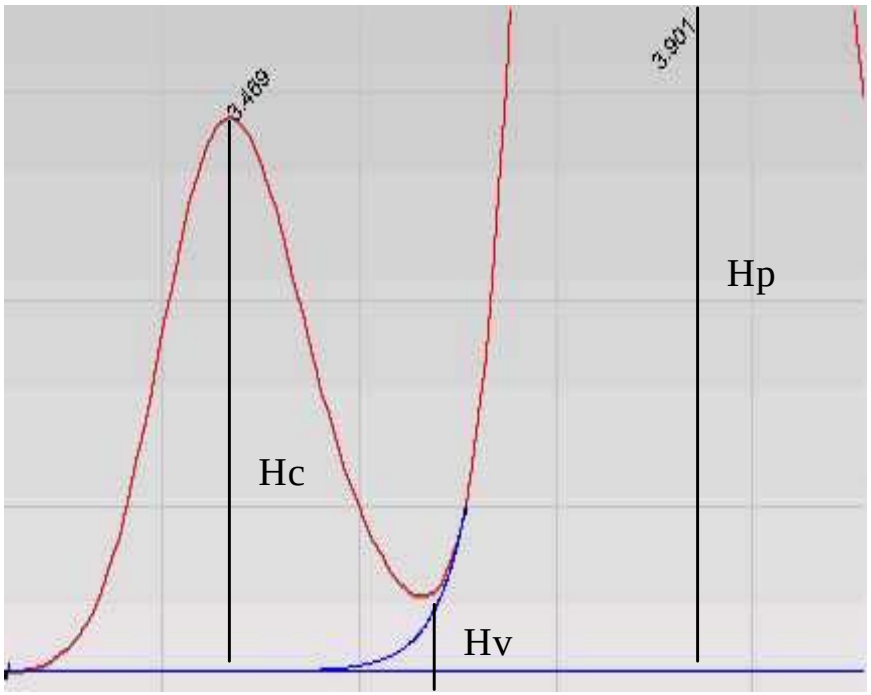
Tail Peak Skim Height Ratio = 3
Skim Valley Ratio = 20

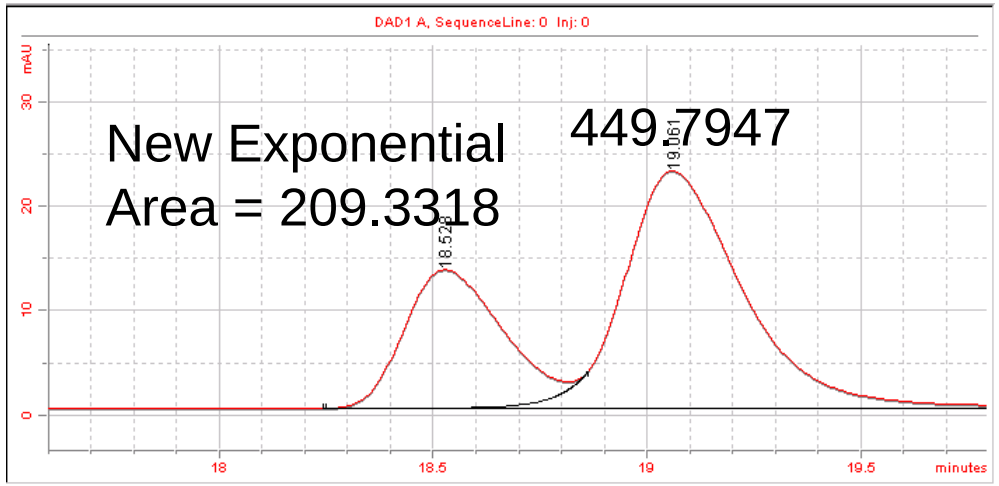
Front Peak Skim Height Ratio

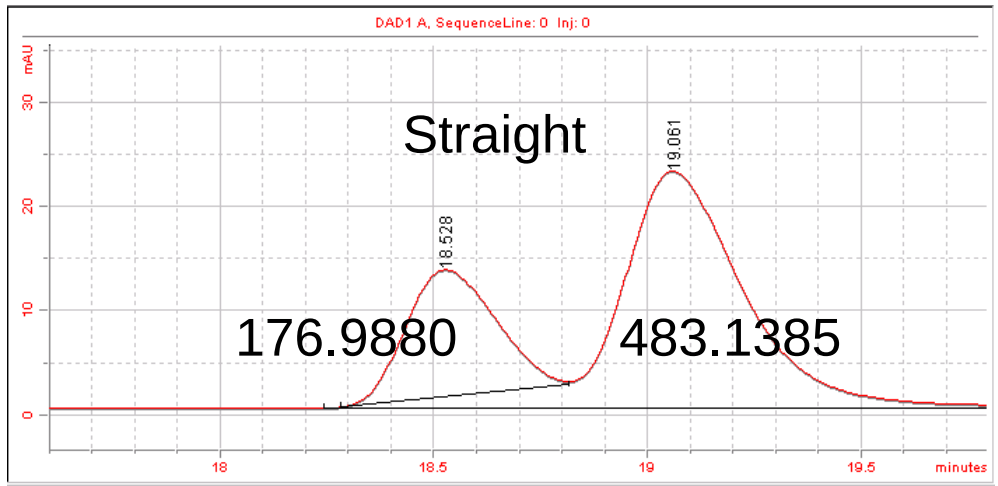
$\frac{H_p}{H_c} \rightarrow$ Set Front Skim Height Ratio

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

Very similar to Tail Peak Skimming







Skim Valley Ratio

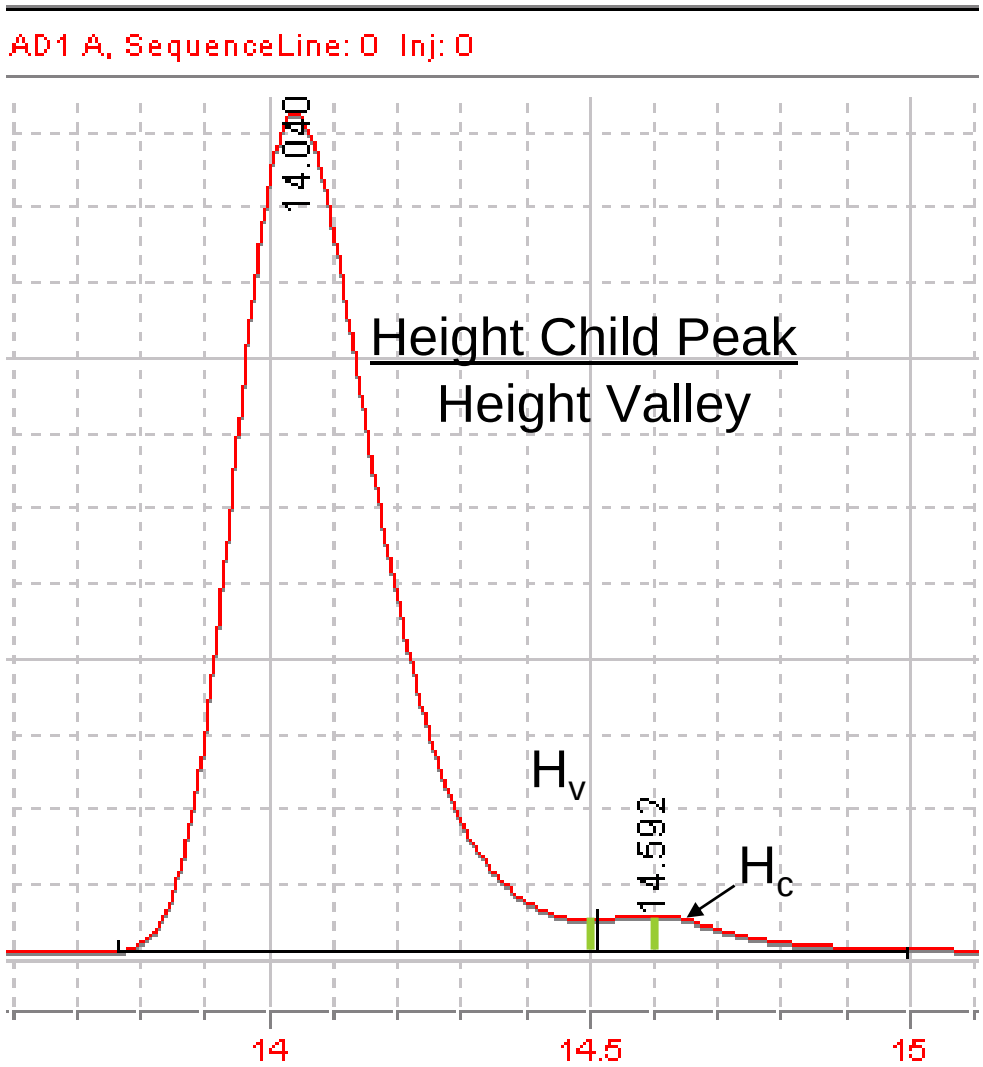
For All Signals:

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

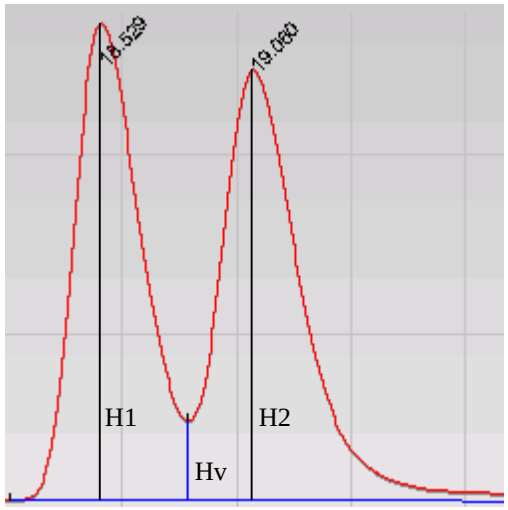
Events Table: DAD Default

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

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

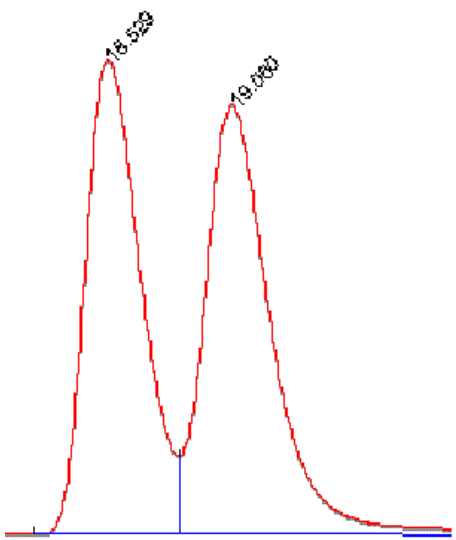


Advanced Baseline Peak to Valley Ratio

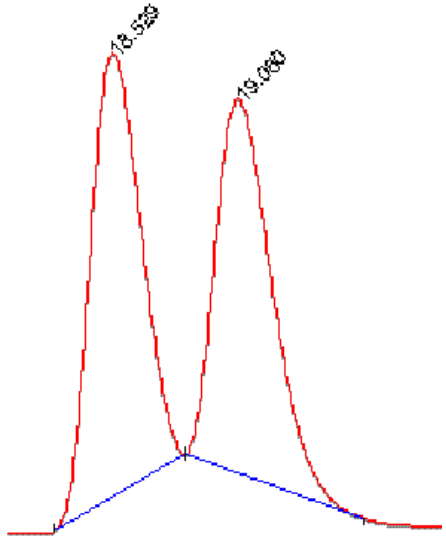


H1>=H2
PtoVRatio = H2 / Hv

H2>H1
PtoVRatio = H1 / Hv

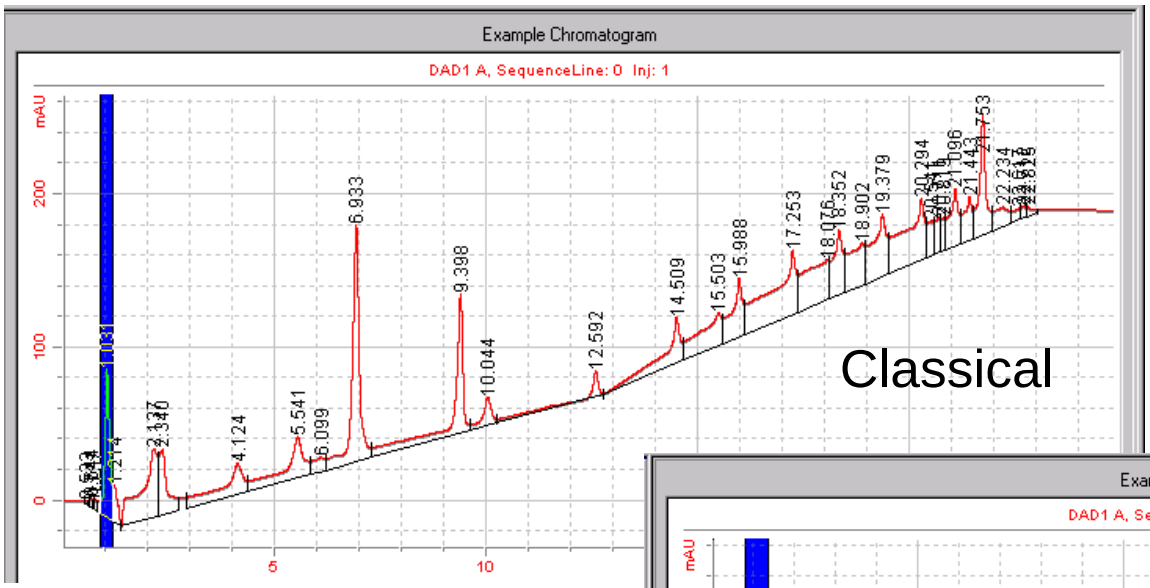


Peak to Valley Ratio
LOWER than
User Setting

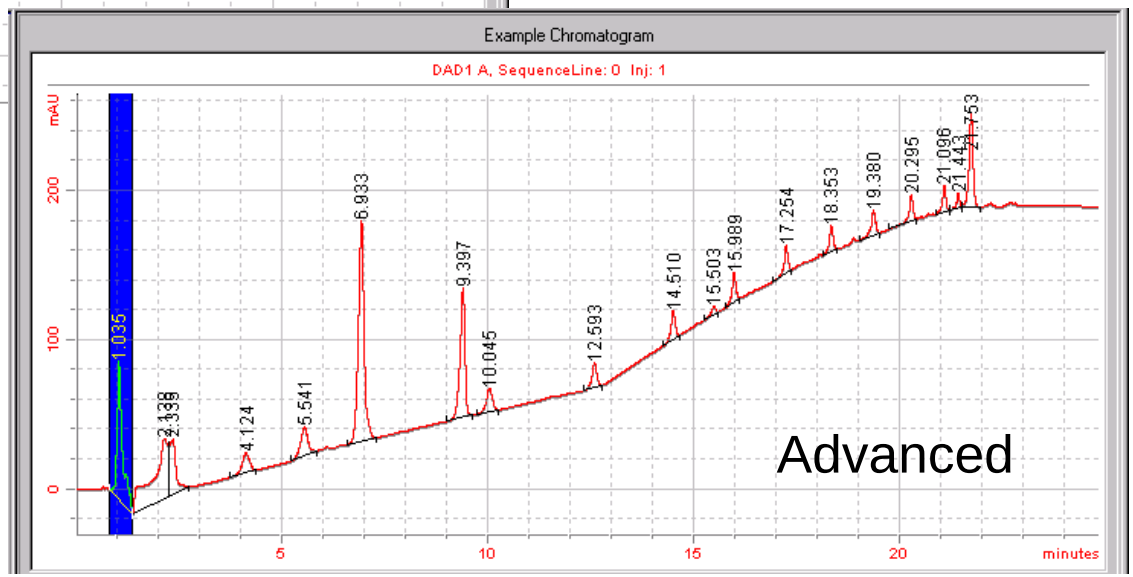


Peak to Valley Ratio
HIGHER than
User Setting

Advanced Baseline



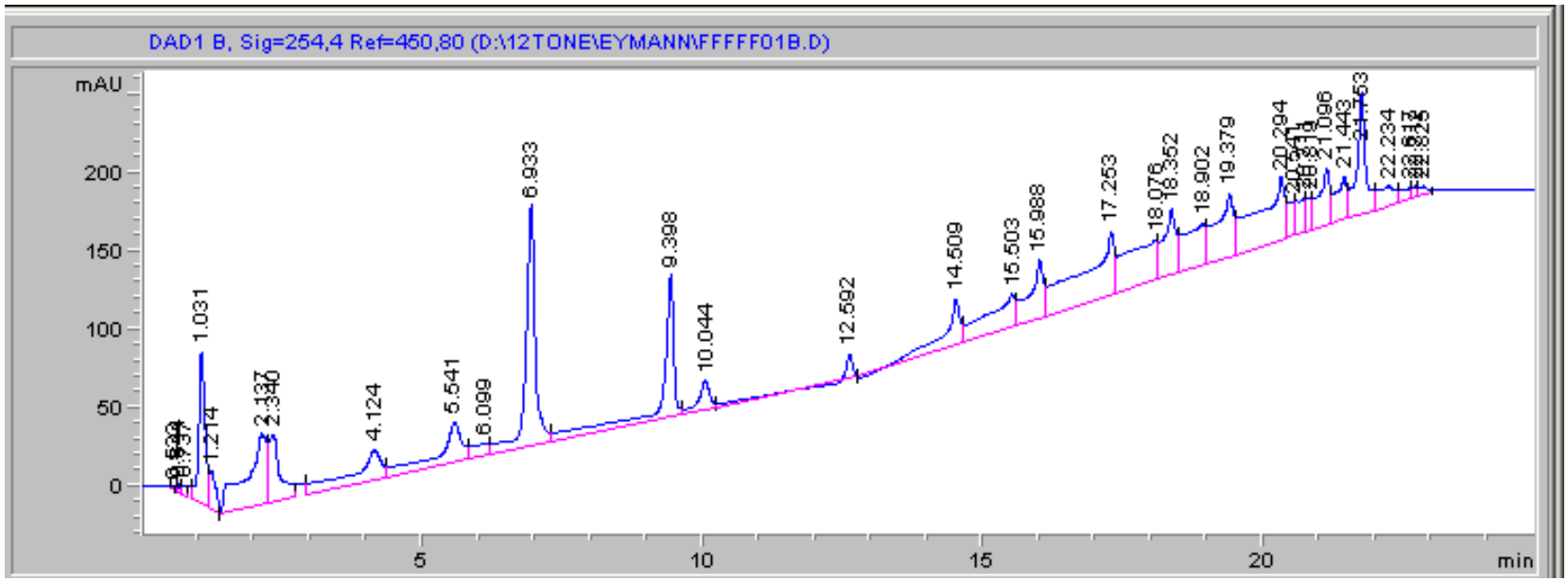
Integrator tries to improve the start and end points of a peak, re-establish the baseline for a cluster of peaks and remove baseline penetrations. Uses Peak to Valley Ratio



Advanced Baseline tracks the curvature of baselines better

Integration Example

Integration Example - Default Parameters



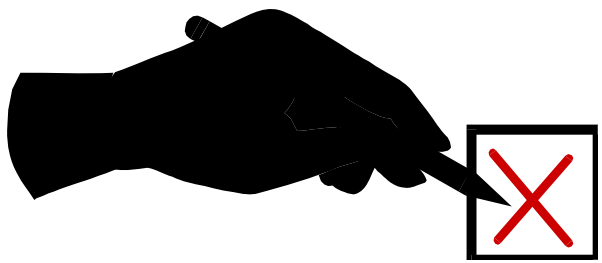
Default integration parameters may not always be the best for your analysis

Possible Problems

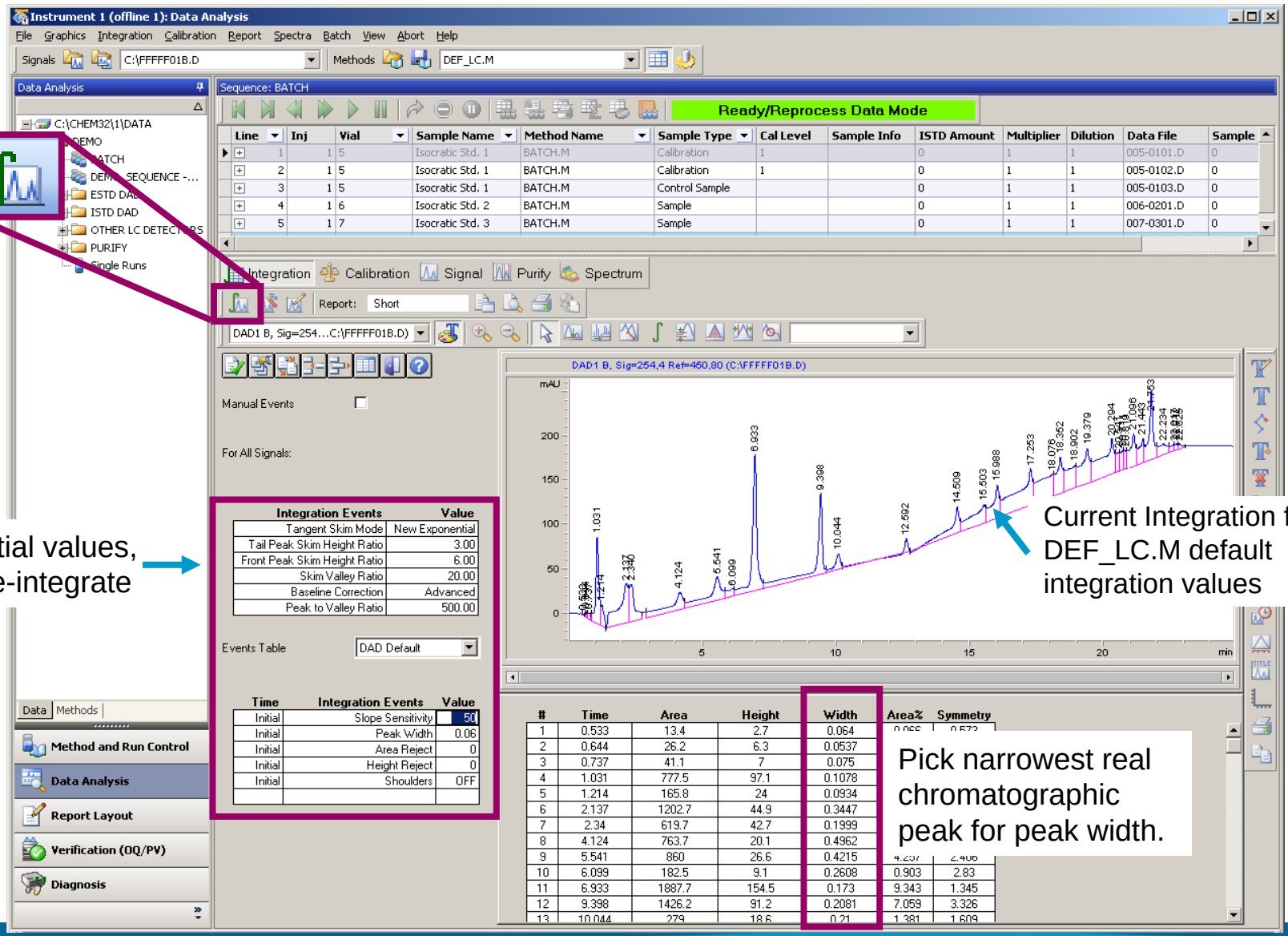
- Noise selected as peaks
- Baseline tracking difficulties
- Drop lines inappropriate

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



Set initial values, then re-integrate

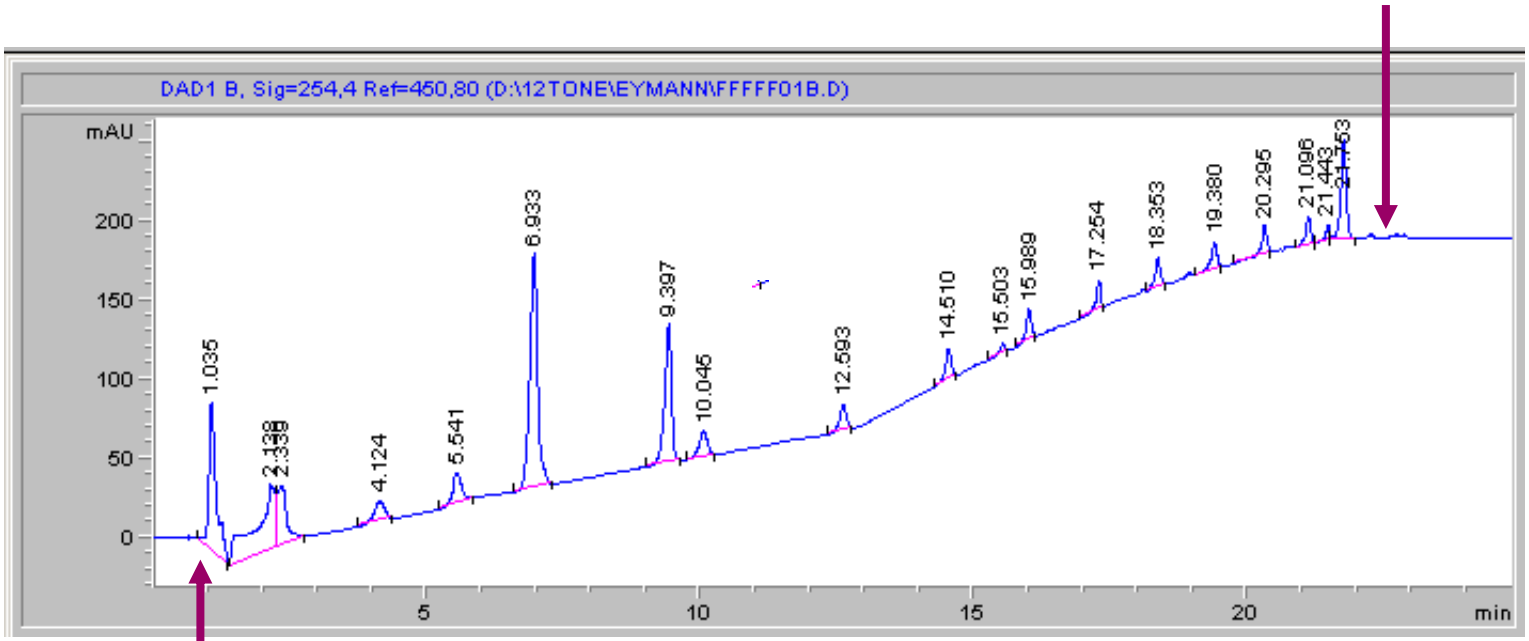
Current Integration from DEF_LC.M default integration values

Pick narrowest real chromatographic peak for peak width.

First Integration Results

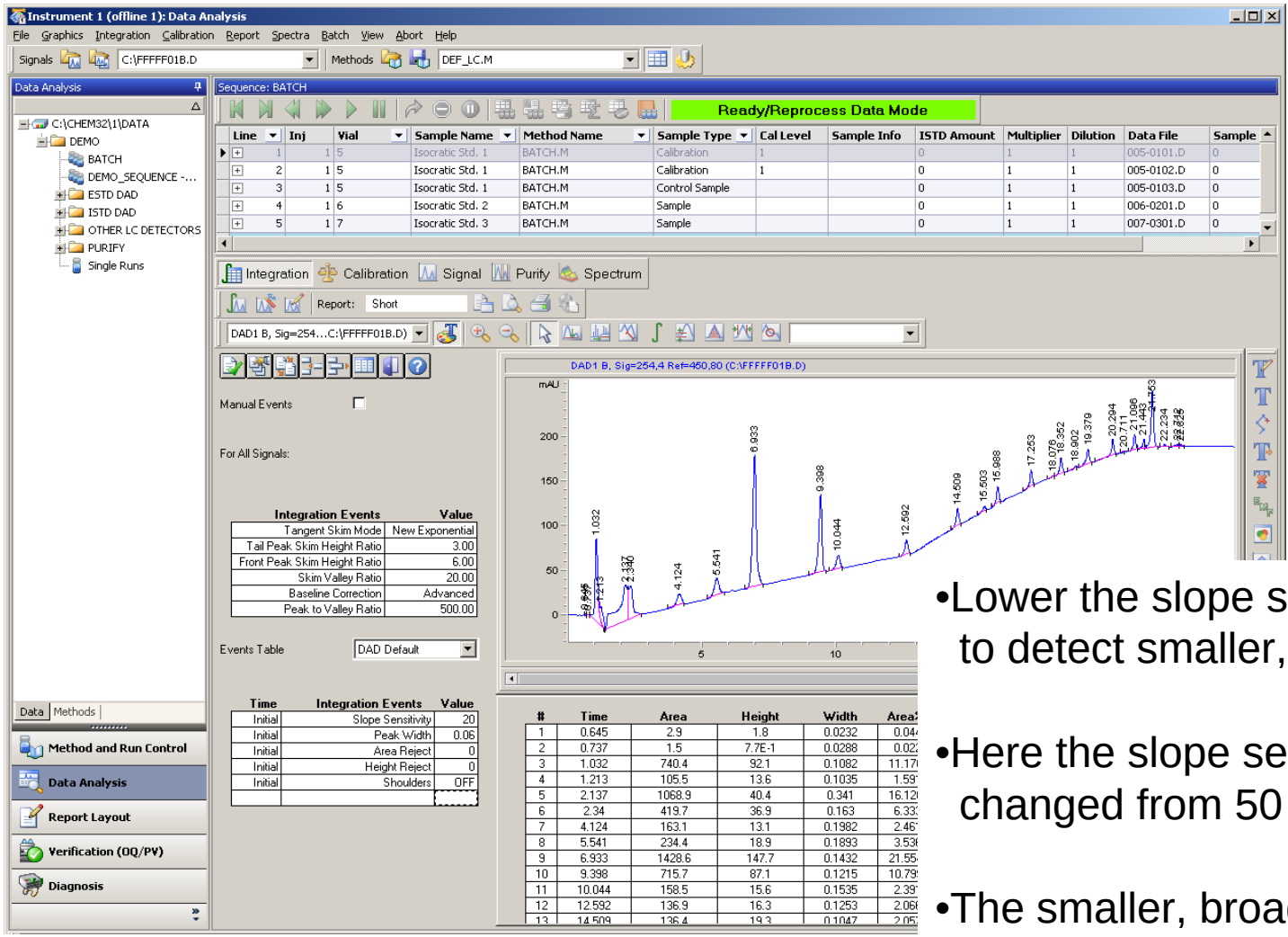
Desired peaks not Integrated

Better, but still needs work.



Baseline problems

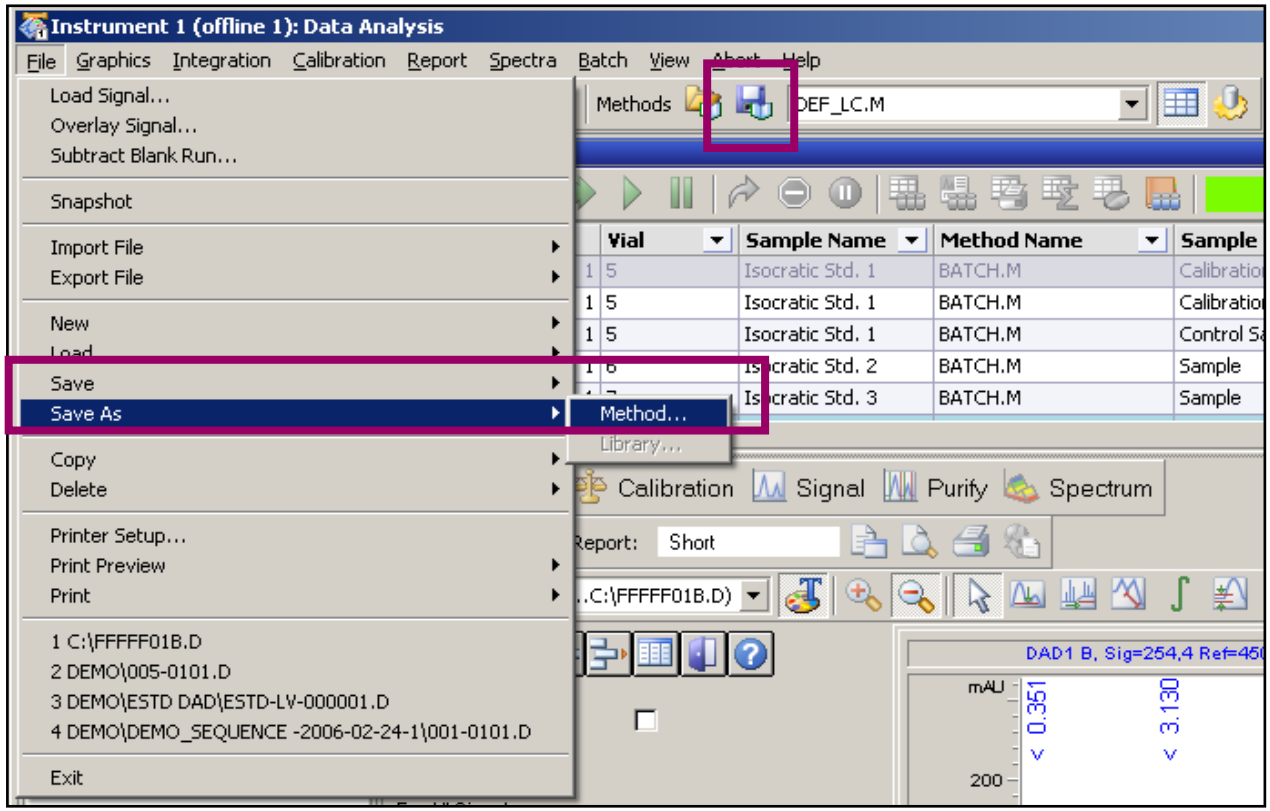
Adjust Initial Parameters



- 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.

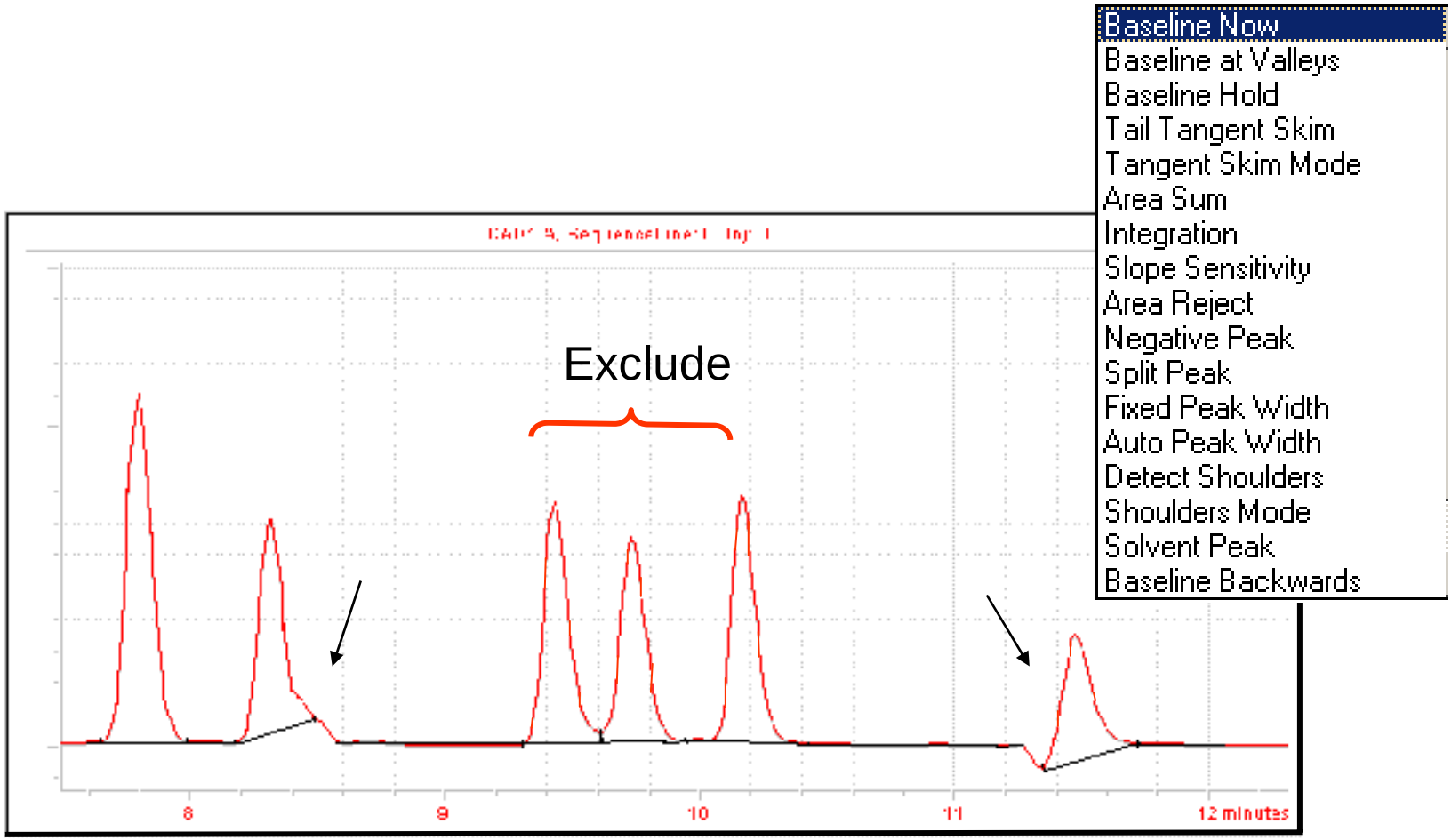
Save Integration Events as Part of a Method

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



Timed Integration Events

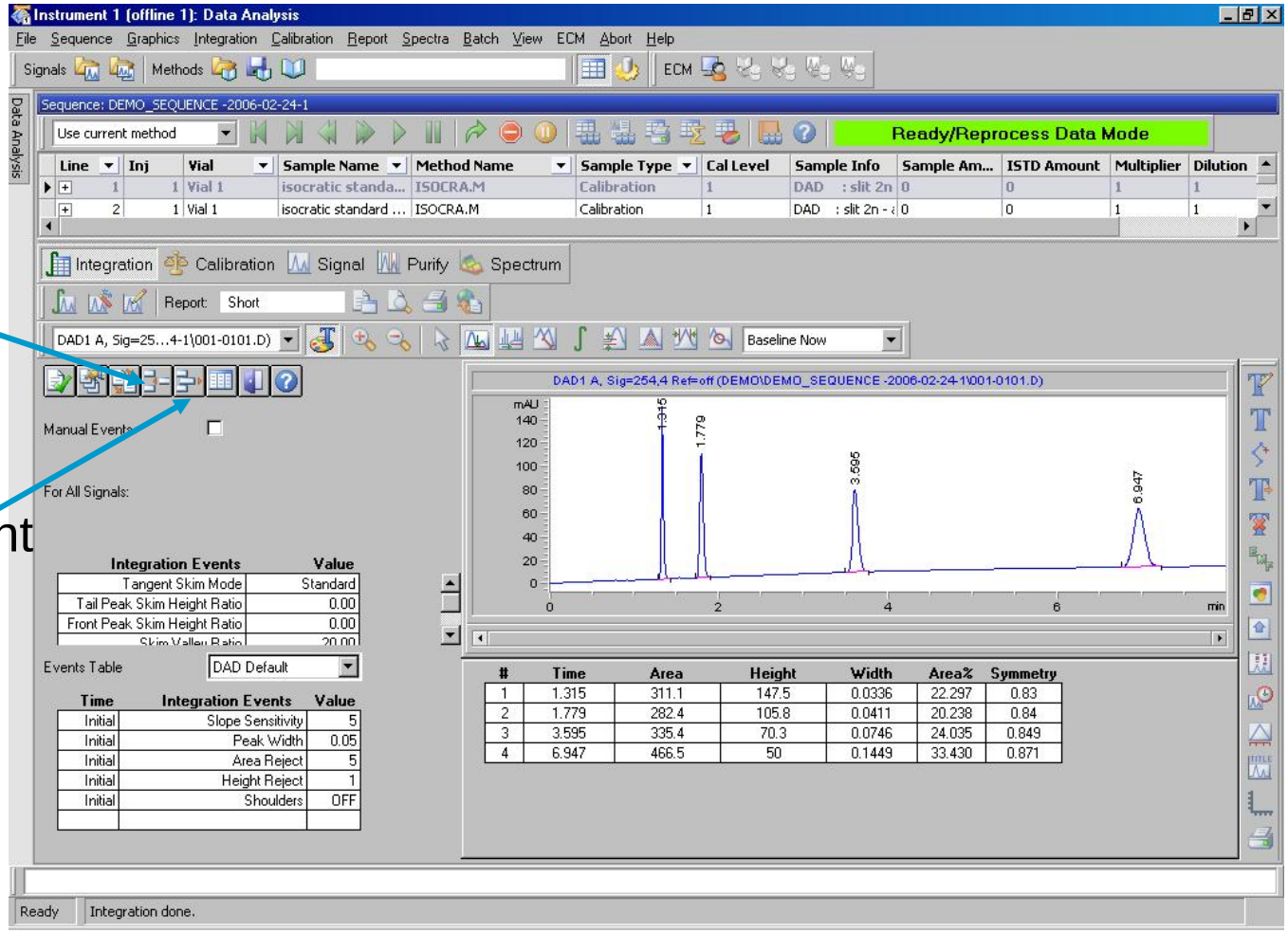
What Timed Events are Useful for the Problems Below?



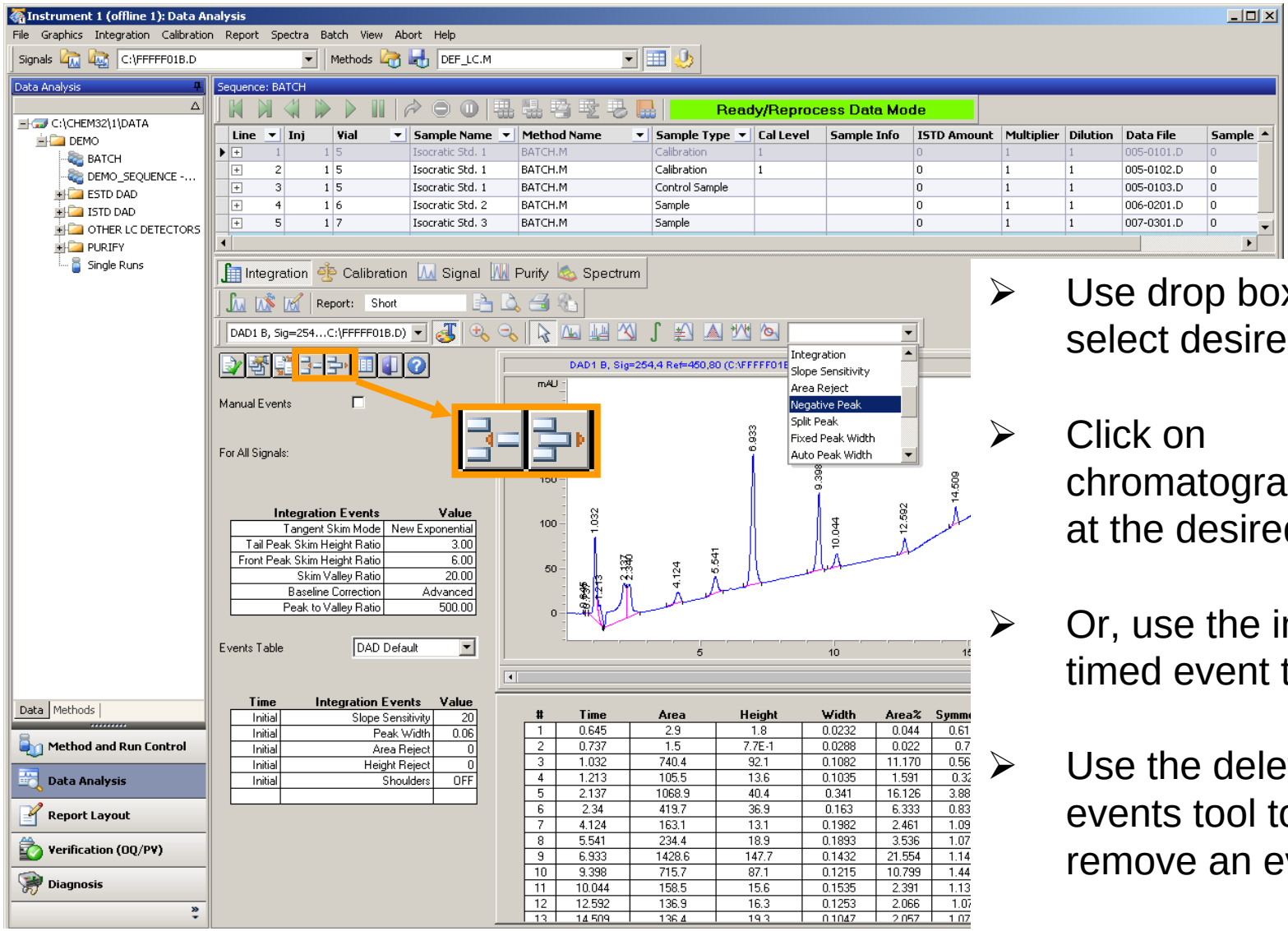
Enhanced Integrator - Timed Events

Add Timed Events

Delete a Timed Event



Insert Timed Events



- 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

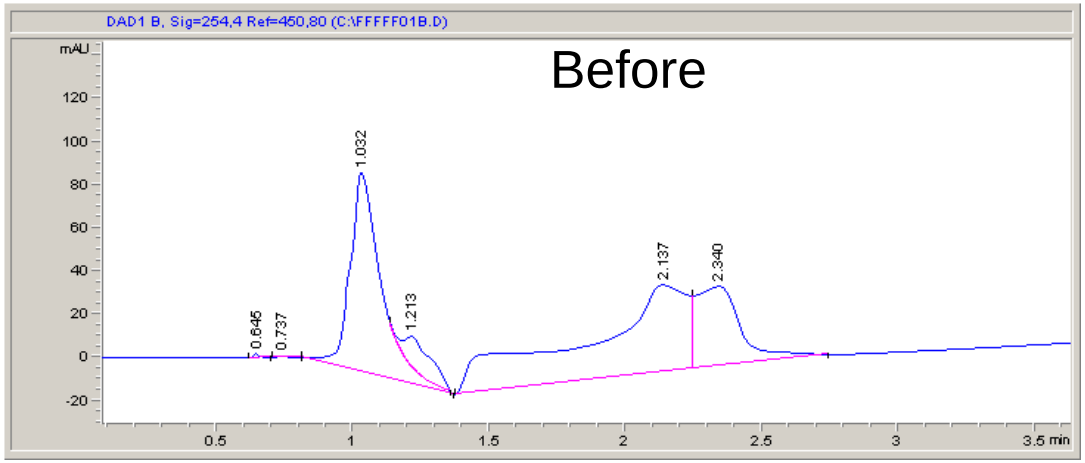
Manual Events ☐

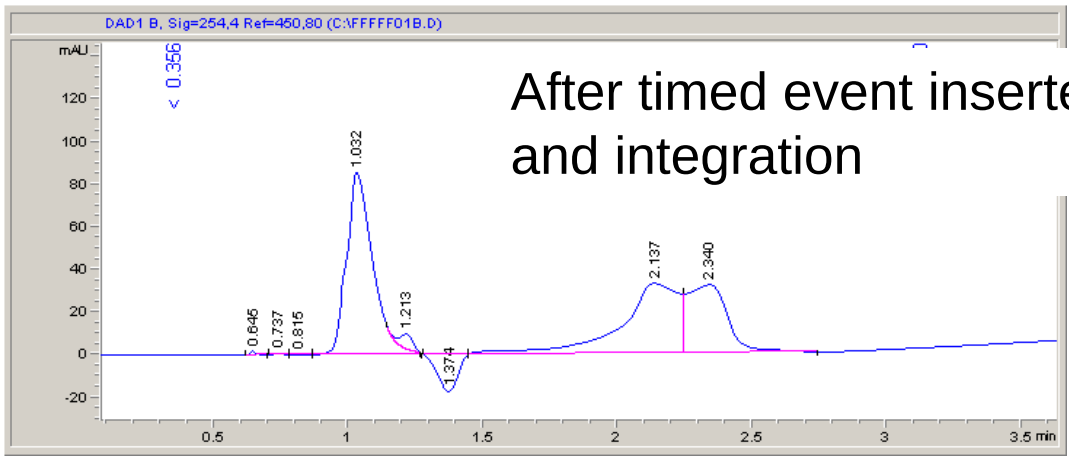
For All Signals:

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

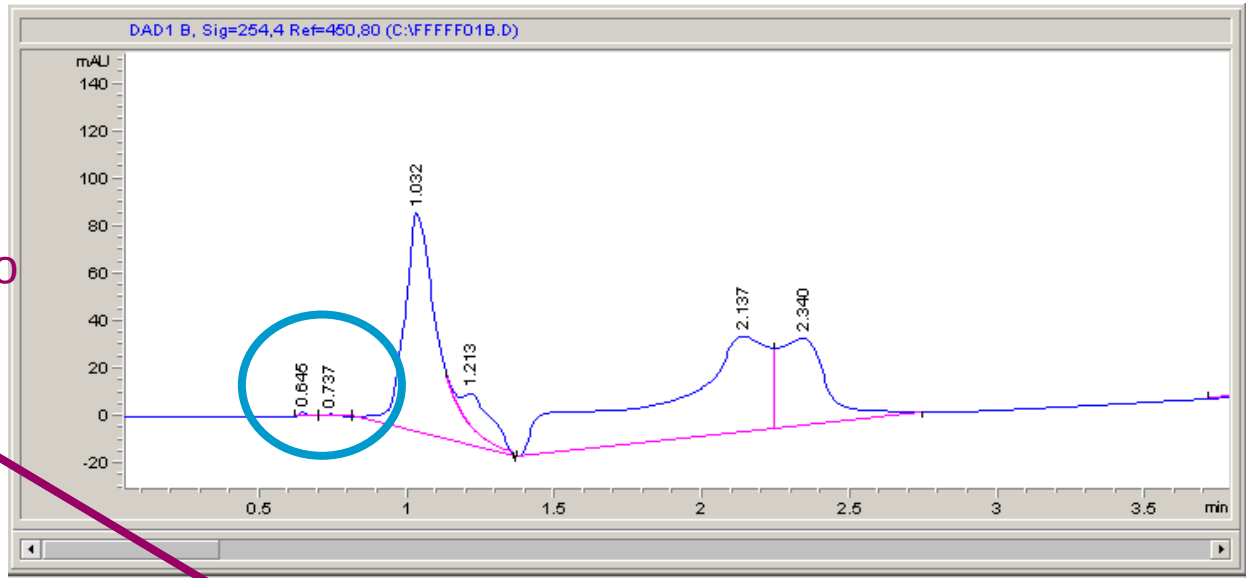




After timed event inserted and integration

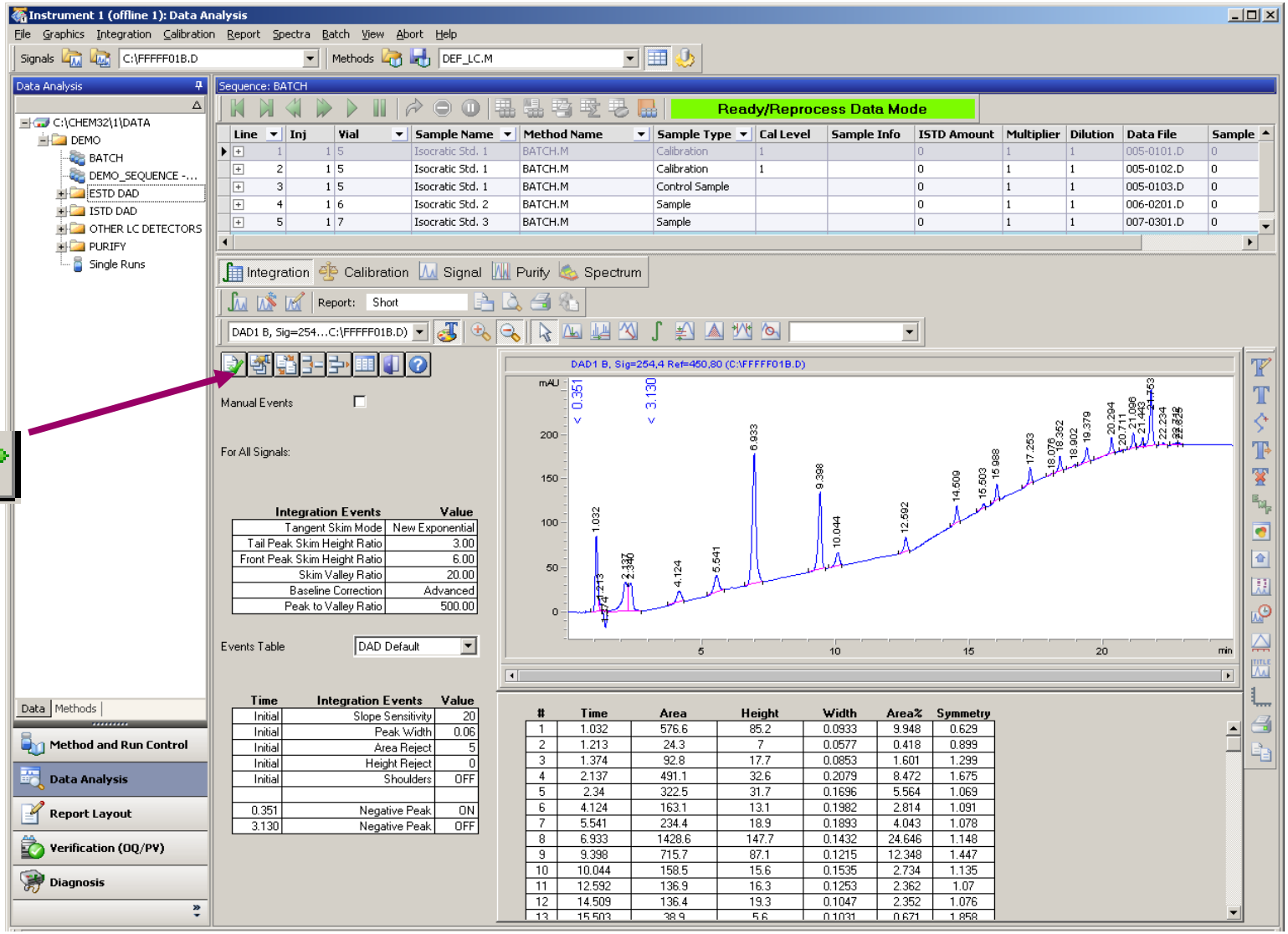
Finish Integration

Use area and height reject to ignore unwanted peaks



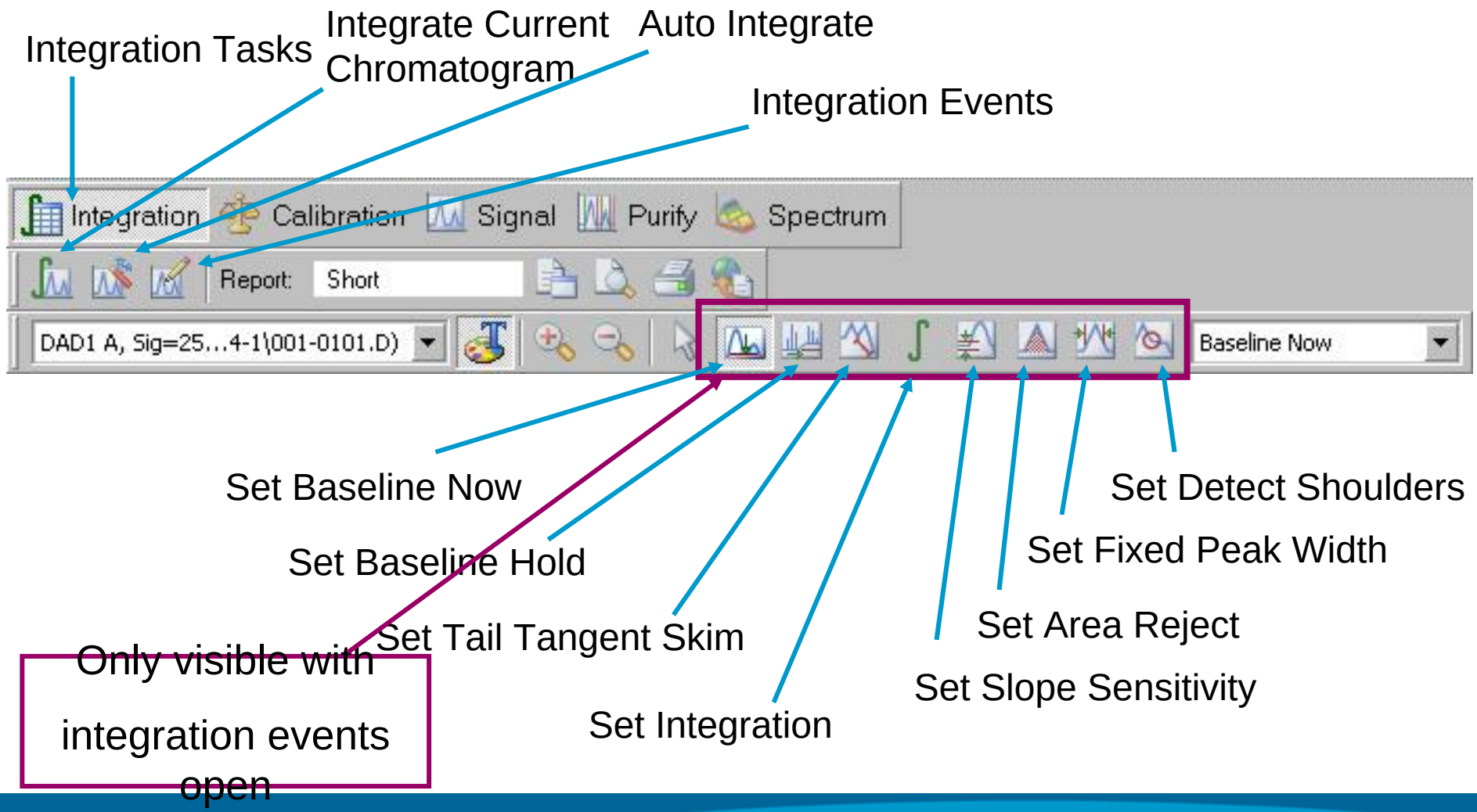
#	Time	Area	Height	Width	Area%	Symmetry
1	0.645	2.9	1.8	0.0232	0.044	0.618
2	0.737	1.5	7.7E-1	0.0288	0.022	0.7
3	1.032	740.4	92.1	0.1082	11.170	0.561
4	1.213	105.5	13.6	0.1035	1.591	0.32
5	2.137	1068.9	40.4	0.341	16.126	3.888
6	2.34	419.7	36.9	0.163	6.333	0.838
7	4.124	163.1	13.1	0.1982	2.461	1.091
8	5.541	234.4	18.9	0.1893	3.536	1.078
9	6.933	1428.6	147.7	0.1432	21.554	1.148
10	9.398	715.7	87.1	0.1215	10.799	1.447
11	10.044	158.5	15.6	0.1535	2.391	1.135
12	12.592	136.9	16.3	0.1253	2.066	1.07
13	14.509	136.4	19.3	0.1047	2.057	1.076

Save and Close



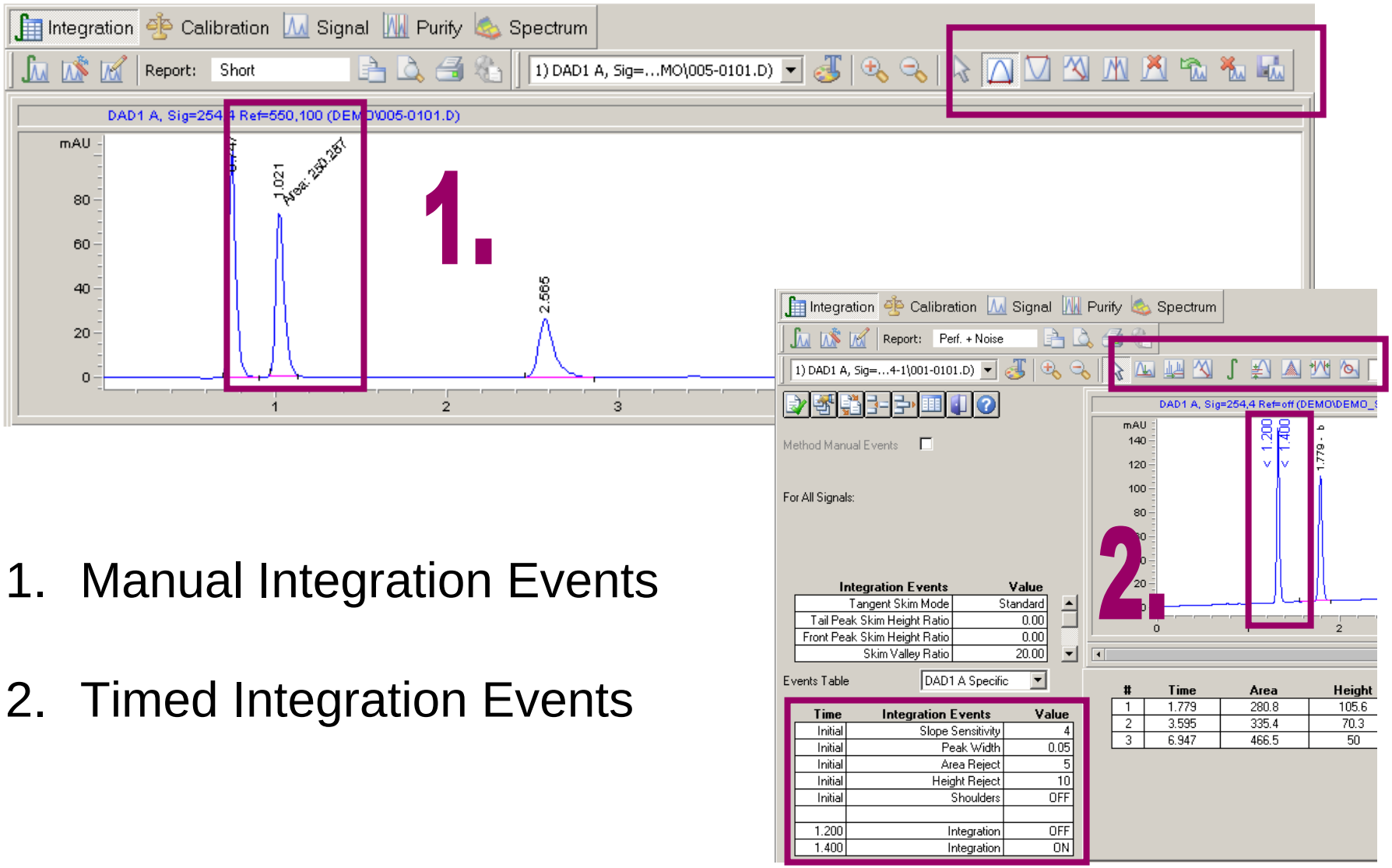
Summary: Integration Task Tool Bar

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



Manual Integration Events

Manual Integration Events vs. Timed Integration Events

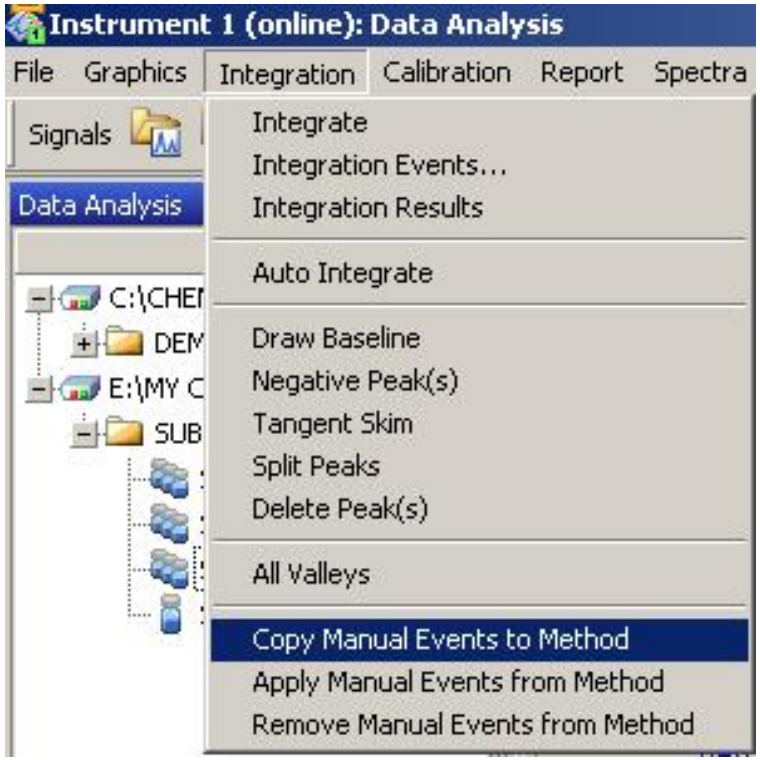


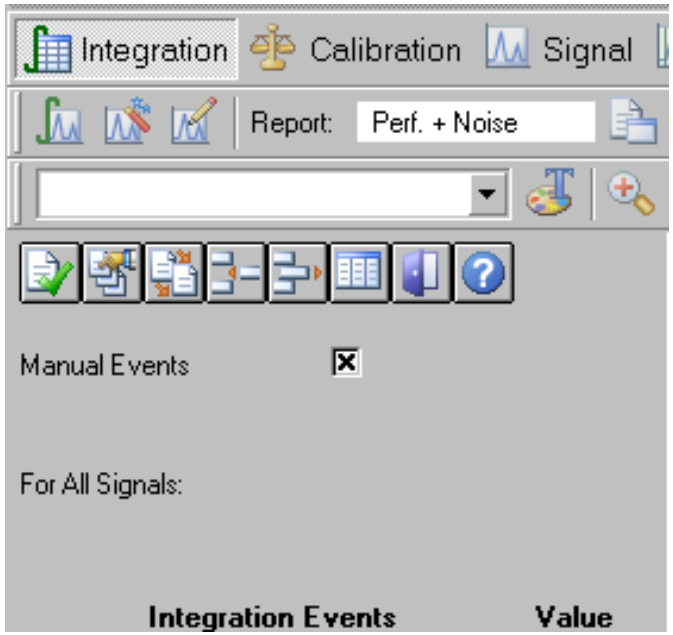
1. Manual Integration Events
2. Timed Integration Events

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





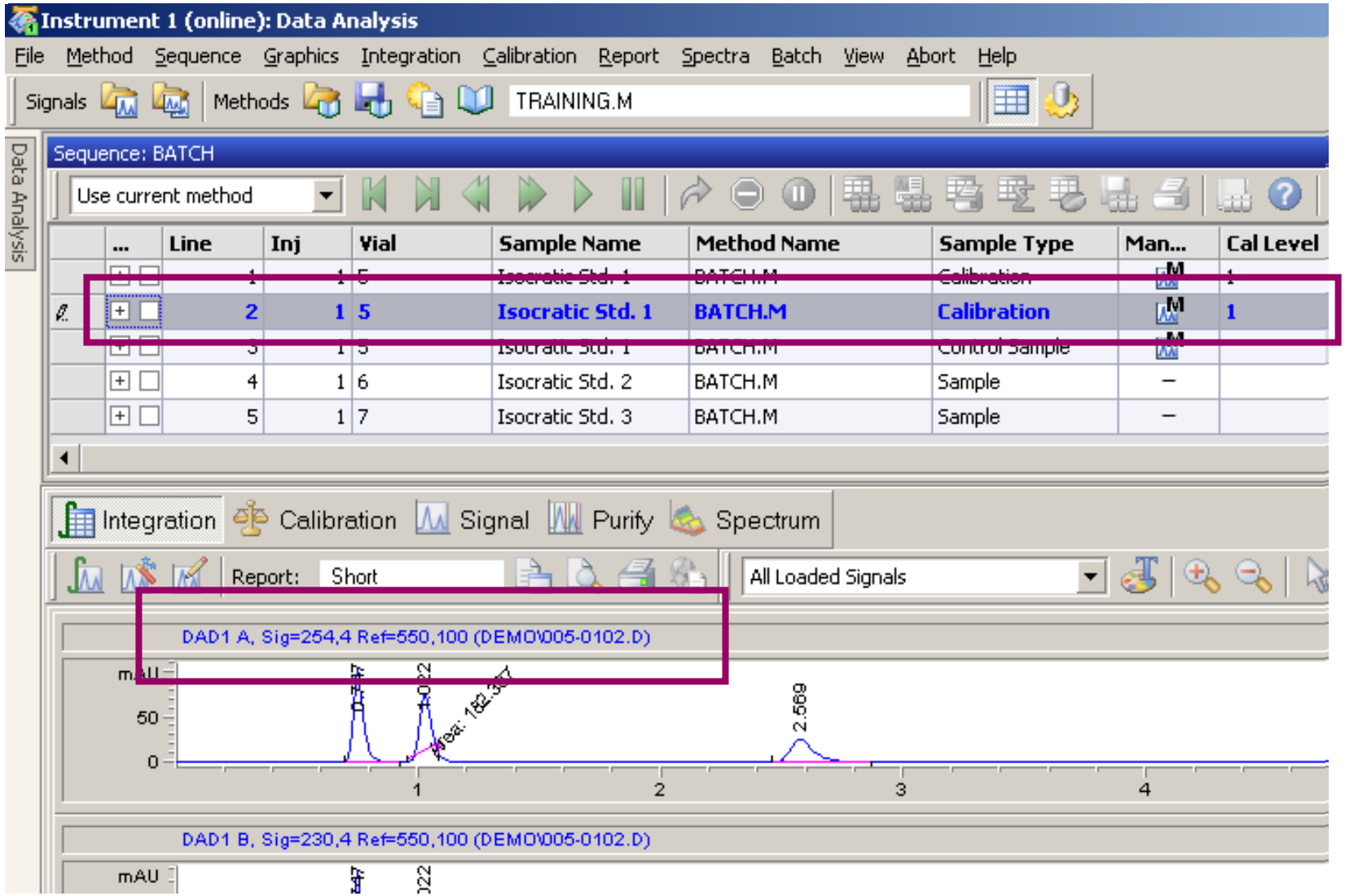
1. Copy manual events to the method.
2. The manual events have to be applied manually, or you have to select “Manual Events” in the integration events table to always apply the manual events.

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

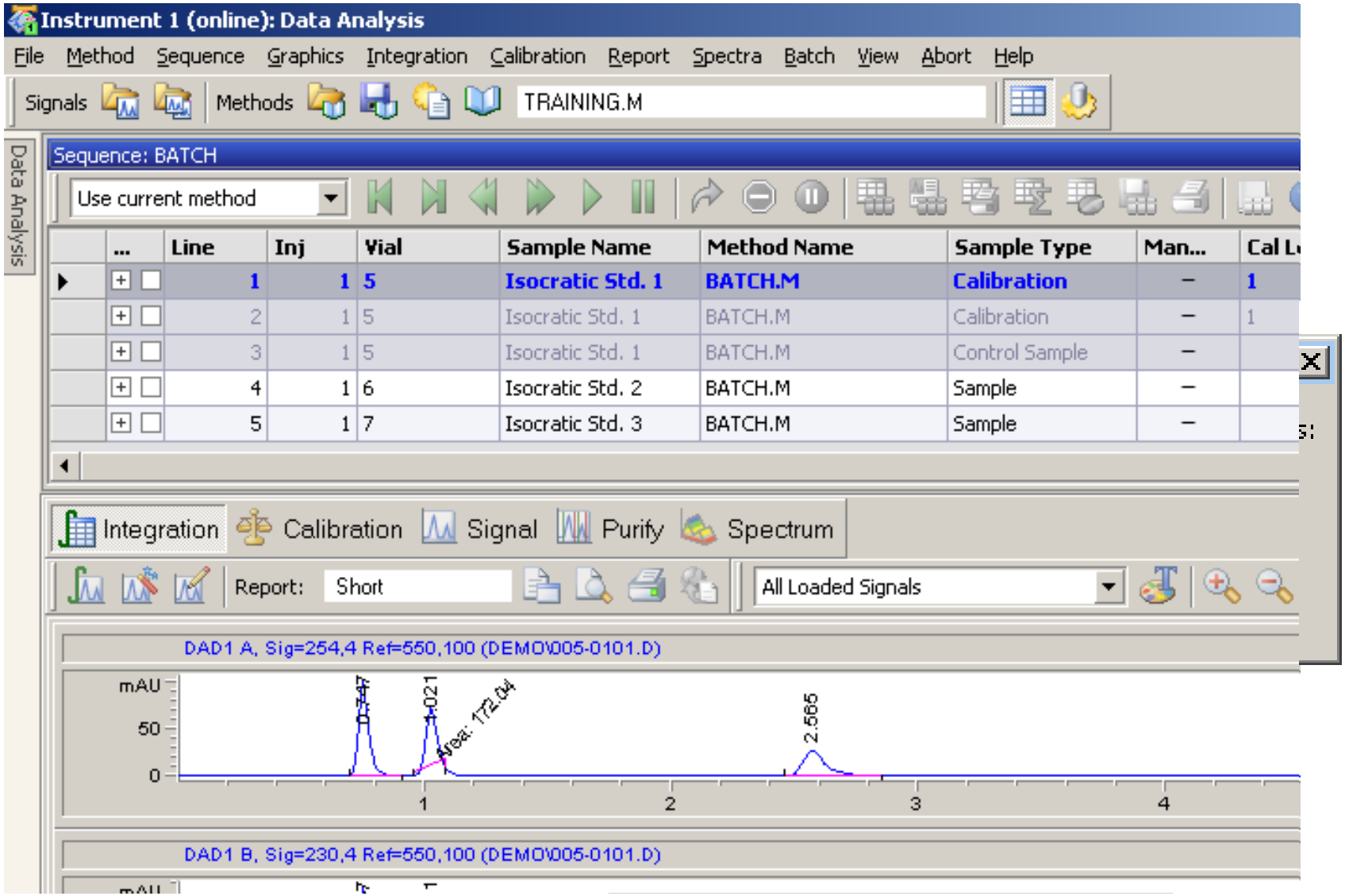
- Automatically applied during data review and reprocessing
- Indicator in Navigation Table when present
- Icons to handle manual events:
  - “Save” to data file
  - “Delete” all manual baselines from file
  - “Undo”: Stepwise removal of unsaved manual baselines

- It is still possible to store manual events in a method.

Copying Manual Integration Events to Other Data Files



Deleting Manual Integration Events



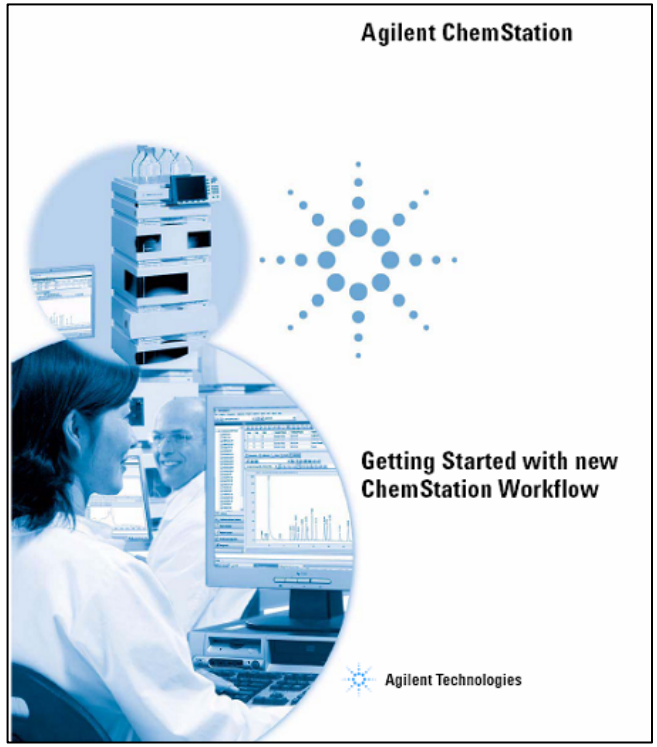
Information on ChemStation

Documentation can be found on the Agilent website
http://www.chem.agilent.com/Scripts/PDS.asp?lPage=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?