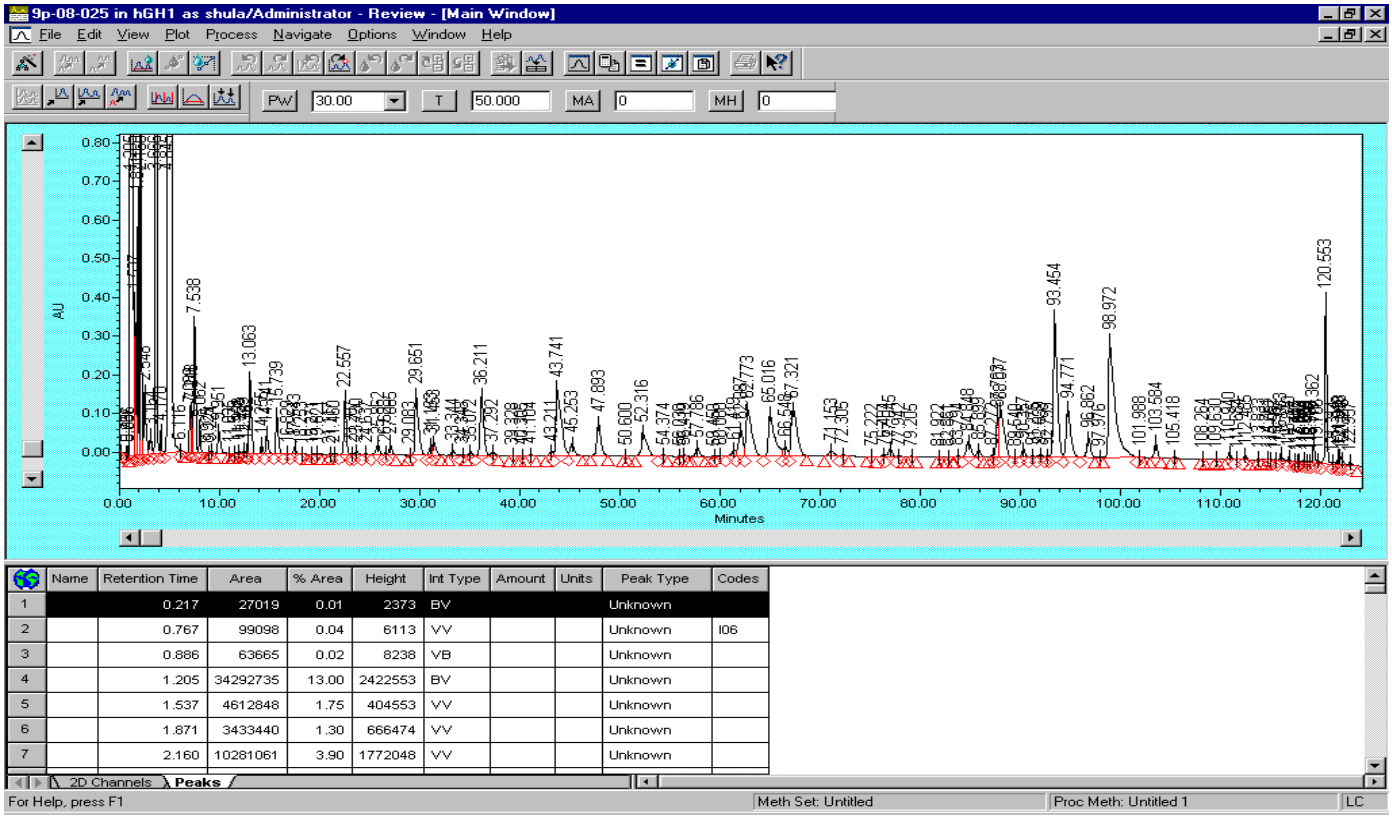
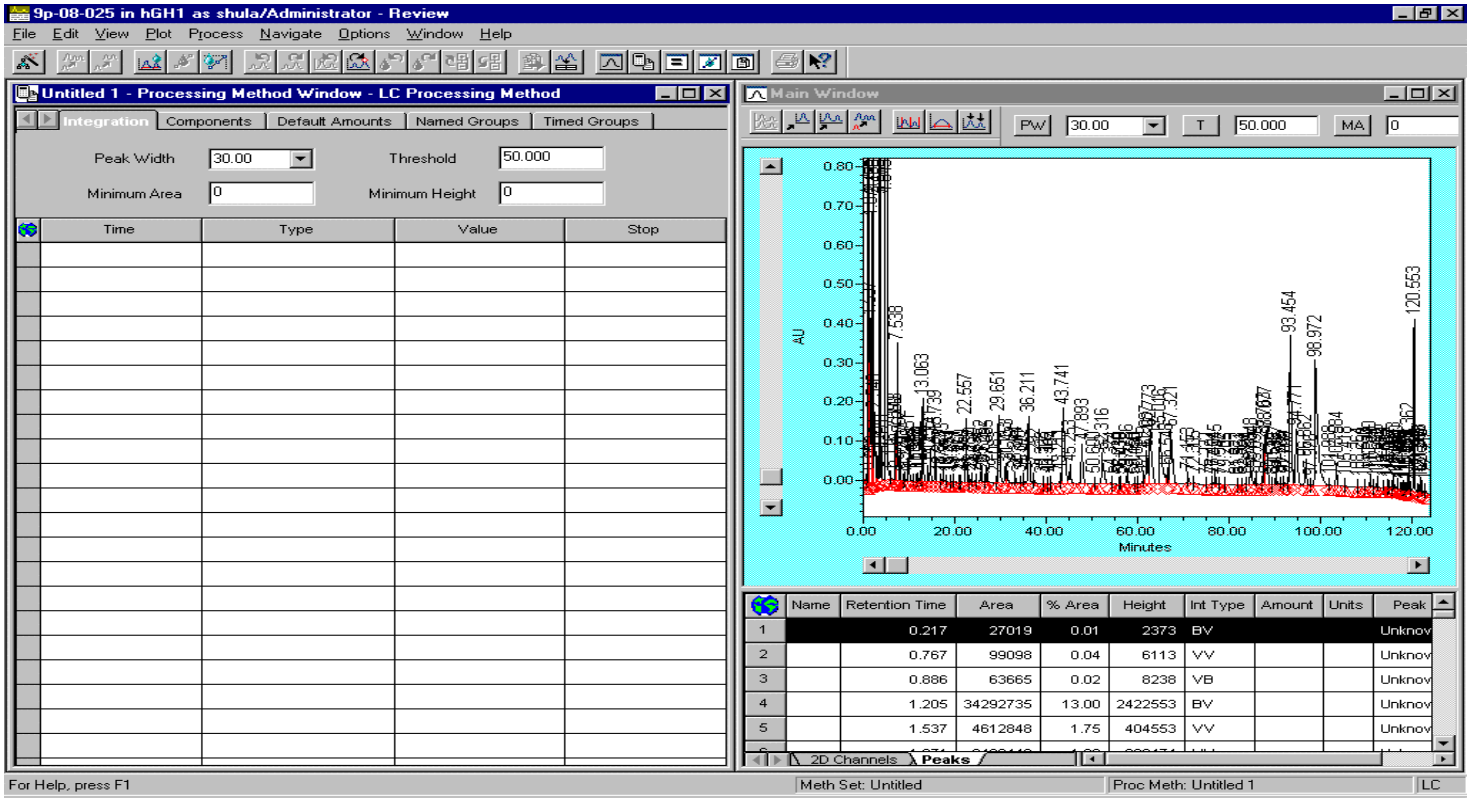
INTEGRATION EVENTS

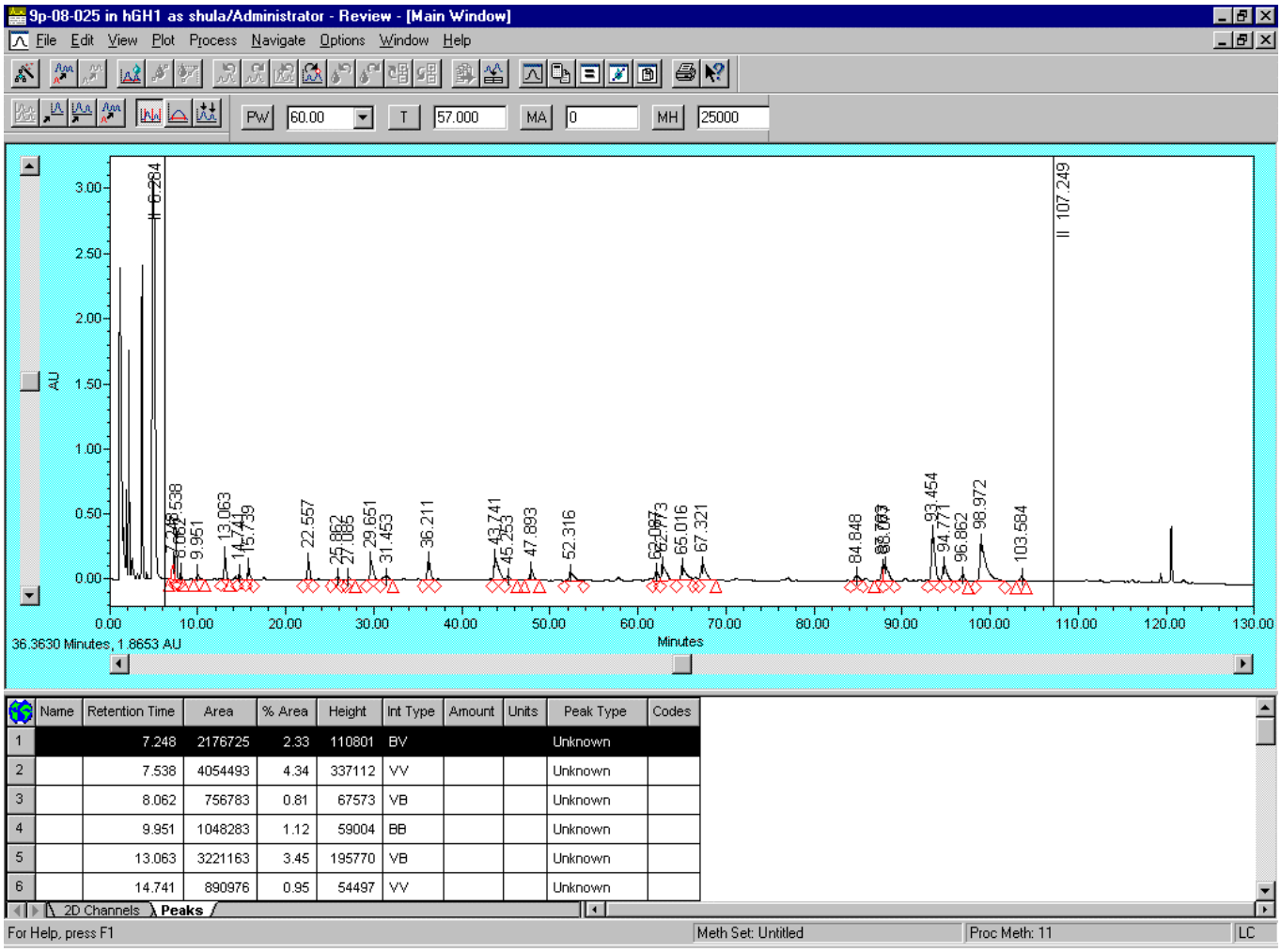
Select a *channel* and look at it in the *Review* window. Open the appropriate *Processing Method*. The *Default* Processing method that is opened automatically, *Untitled*, in the *Review* window yields too many peaks and wrong integration in the example here:



Therefore there is a need for many changes in the Integration Event Table in the Processing method. Tile the two windows, the *Main* and the *Processing Metho*d's to be able to see the results of the change immidiately.



For an existing method, first change the *Inhibit Integration* ranges and then the global integration events: *Threshold* and *Peak Width* (*T* and *PW*). Use the icon (or menu Option) "*Show Events*" so you can change them graphically. Do not use the *Threshold* value to omit unwanted peaks, if you have too many peaks, make sure to use the appropriate T value to define all of them properly (Start and end are OK), then choose the "*Minimum Height*" (*MH*) option, by choosing the highest peak you want to omit, then clicking the *MH* button and adding 20% to the value, or use the "*Minimum Area*" option in the same manner:



Now that you have just the peaks of interest, you can start using the more advanced features of the integration events table in the Processing method window. The Table has four columns:

Time

Specifies the time at which the event type is to start. Integration event table rows are sorted automatically by the entered start times. To activate an event for an entire chromatogram, enter a value of 0.000 in the Time field and leave the Stop field blank. Valid entries: 0.000 to 655.000 minutes. Default: 0.000.

Type

Specifies the type of integration event to occur at the specified time. You can choose from 18 event types. The selected event begins at the time specified in the Time field and stops at the time specified in the Stop field.

Value

Specifies the value of an additional parameter required by some event types (for example, the Set Minimum Area event type requires the value of the minimum peak area in µV/sec).

Stop

Specifies the time at which the event is to stop. Events that use a Stop time also allow a blank value, indicating activation of the event until the end of the run. Certain events (such as Set Lift off, Set Touchdown) do not use a Stop time. Valid entries: 0.000 to 655.000 minutes. Default: Blank.

The Types column includes the following:
Integration events are classified as detection events and integration events.
Detection events affect single peak or fused-peak start and/or end points.
Detection events include:

- Inhibit Integration
- Allow Negative Peaks
- Set Lift-off
- Set Touchdown
- Set Peak Width

Integration events adjust the baseline, affect height or area values within a single peak or fused peak, or define the criteria for an integrated peak to be considered valid.

Integration events include:

- Exponential Skim
- Force Baseline by Time
- Force Baseline by Peak
- Force Drop Line
- Force Peak
- Forward Horizontal by Peak
- Forward Horizontal by Time
- Reverse Horizontal by Peak
- Reverse Horizontal by Time

- Set Minimum Area
- Set Minimum Height
- Tangential Skim
- Valley-to-Valley

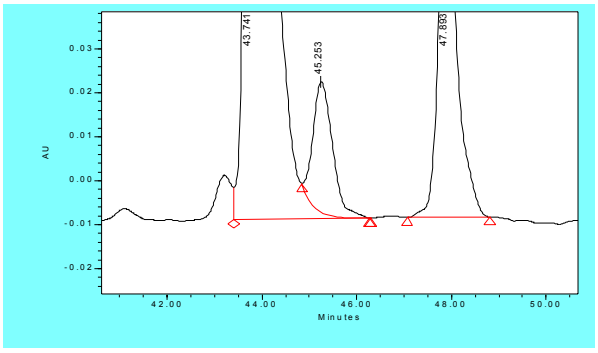
Use the Millennium help to illustrate each of them.

We will look one by one at the effect of the events as they appear in the list:
When a riding peak needs to be skimmed from the slope, there are two options, exponential or tangential skim:

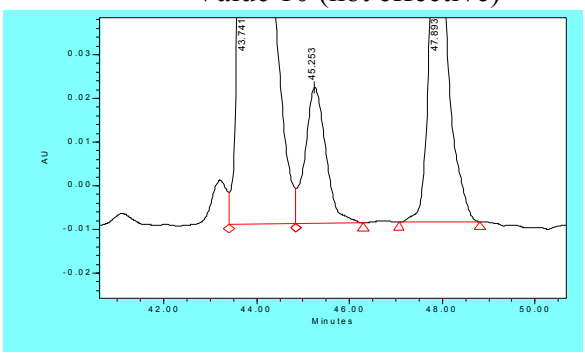
Exponential Skim

For example, skim at 42 min. The value is a detection type of value - minimum slope to detect the riding peak.

Value 4.

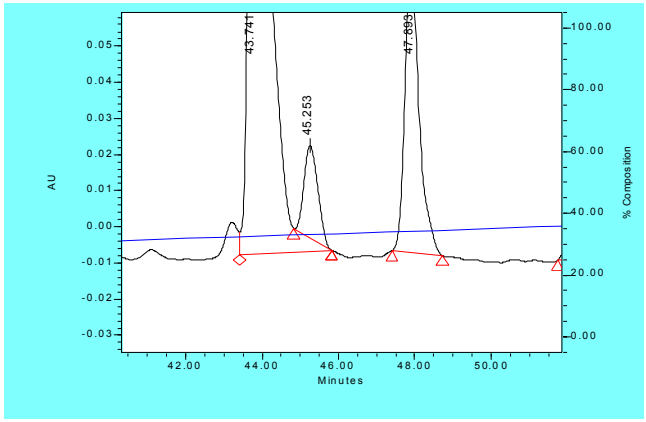


Value 10 (not effective)



Tangential Skim:

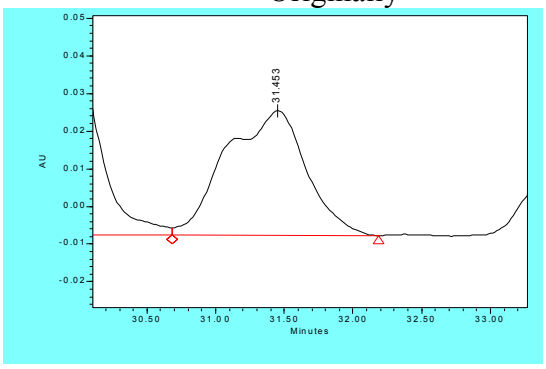
This event just connects the two starting and ending points of the riding peak



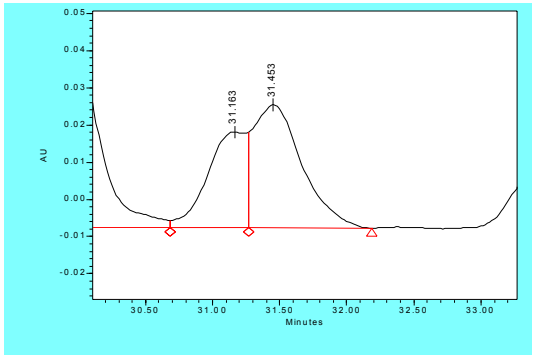
Force Drop line

For example: Force drop-line at 31.26 min to force fused peaks to split:

Originally



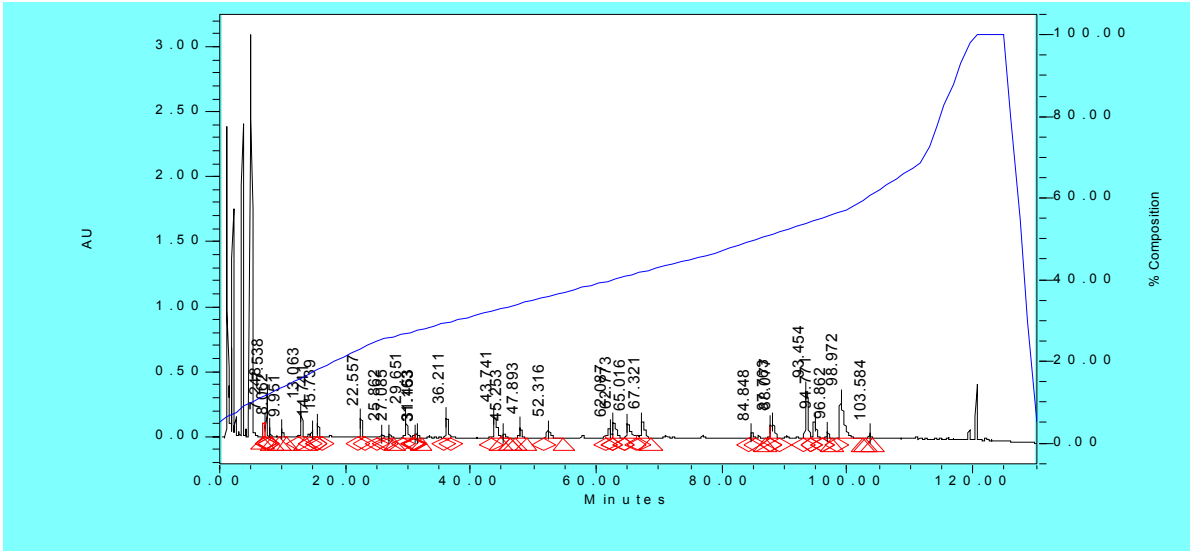
Force Drop line



Remember that the place of the dropped line fits just the currently used chromatogram, it is very specific for a chromatogram. It is used frequently in GPC to define broad fused peaks, not so much in regular LC.

Force Baseline by Time & Force Baseline by Peak

Since we are dealing with a gradient chromatogram, the baseline might be drifting:



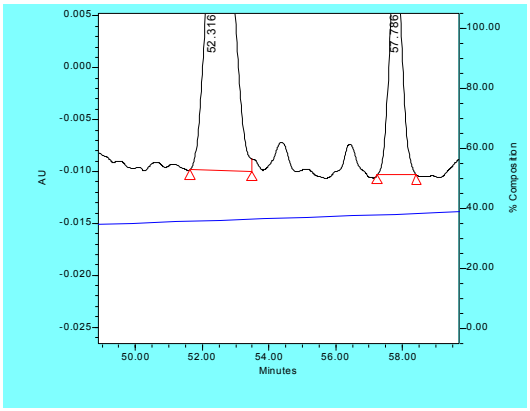
Projects a baseline based on either of the following:

- Event start and end times (Force Baseline by Time)
- Peak start and end points within this range (Force Baseline by Peak)

(Note: The forced baseline does not have to be horizontal.)

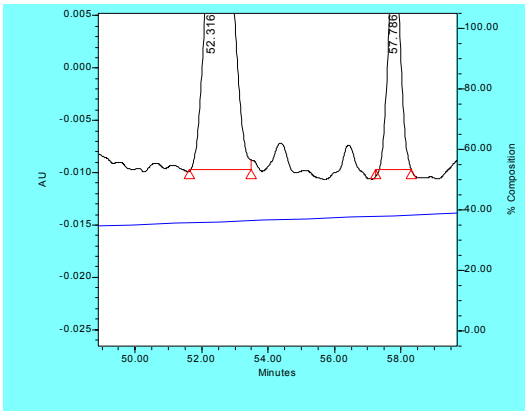
Force Base line by Peak at 50-59.4 min

Base line starts at the starting point of the first peak.



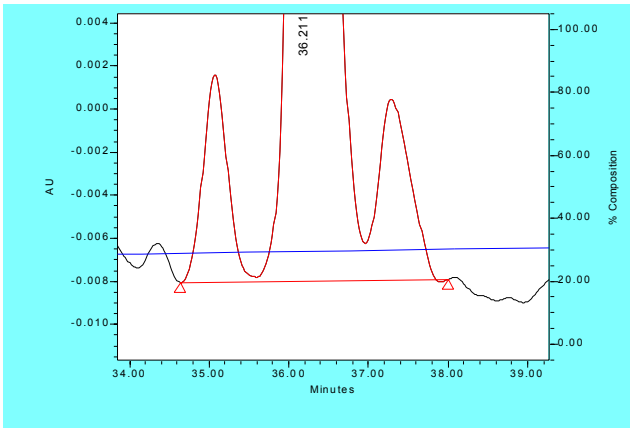
Force Base line by Time at 50-59.4 min

Base line starts at the start of the event, 50 min, and ends at 50.4



Force Peak

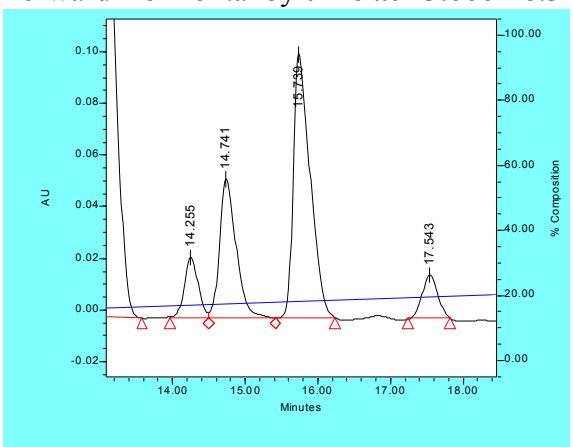
When adjacent peaks have to be integrated together and reported as a sum of areas:



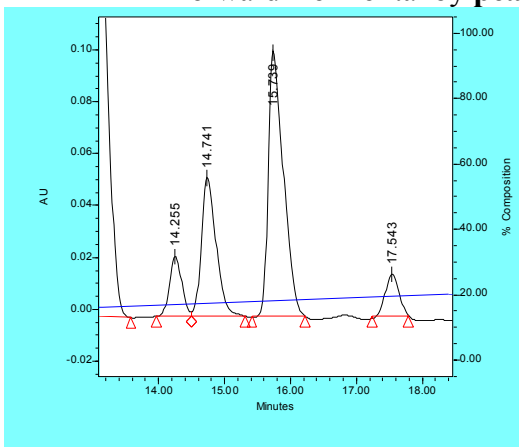
Forward Horizontal by Peak (FHP) & Forward Horizontal by Time (FHP)

Baseline is forced to be horizontal from the first point in time forward (FHP) or from the first peak (FHP)

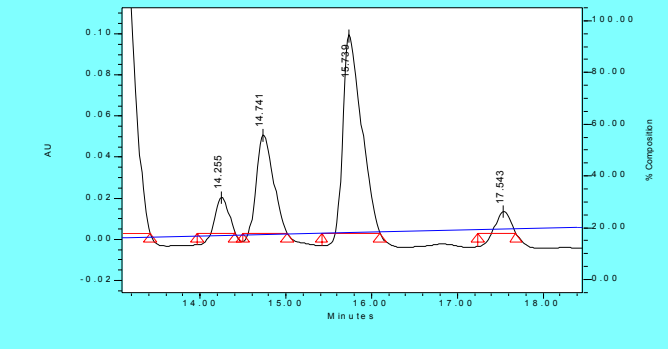
Forward Horizontal by **time at** 13.666-18.3



Forward Horizontal by **peak at** 13.666-18.3



Note that the "Force horizontal by Time" is very sensitive to the base line at the starting point of the event: When the event started at 13.4 the picture was distorted due to the preceding peak:



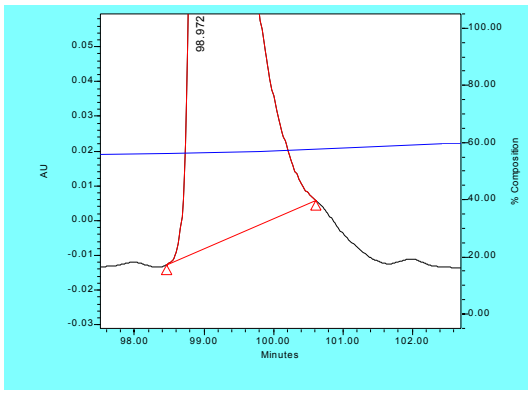
Reverse Horizontal by Peak (FHP) & Reverse Horizontal by Time (FHP)

The same as Forward but backward.

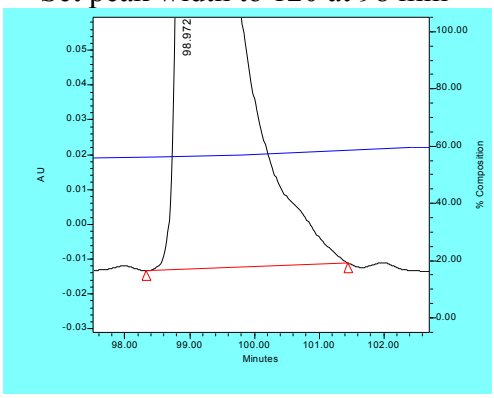
Set Peak Width

The peak width is a global integration event that appears at the Review window and in the processing method as the first parameter to set. However, it might be changing throughout the chromatogram either gradually broadening at isocratic conditions or variable according to the focusing effects of the gradient. Therefore, it can be changed at various time segments in the chromatogram.

Global event PW = 30



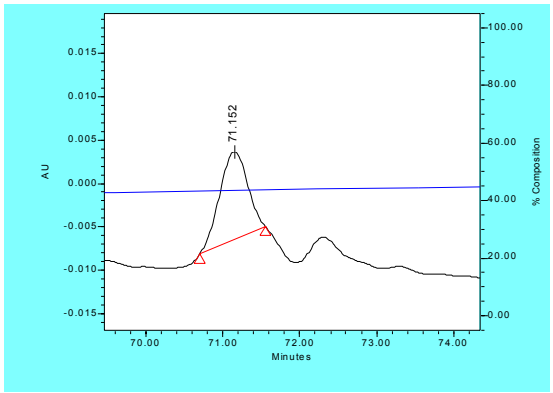
Set peak width to 120 at 98 min



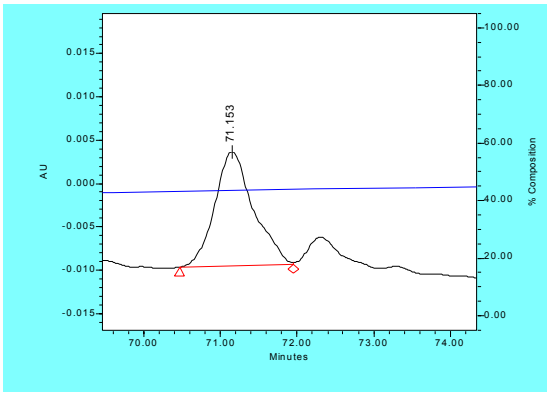
Set Liftoff & Set Touchdown

These two parameters come instead of the Threshold, which determines both peak liftoff and touchdown. If the global PT was not appropriate for a certain peak, it can be changed locally:

Threshold is at 300

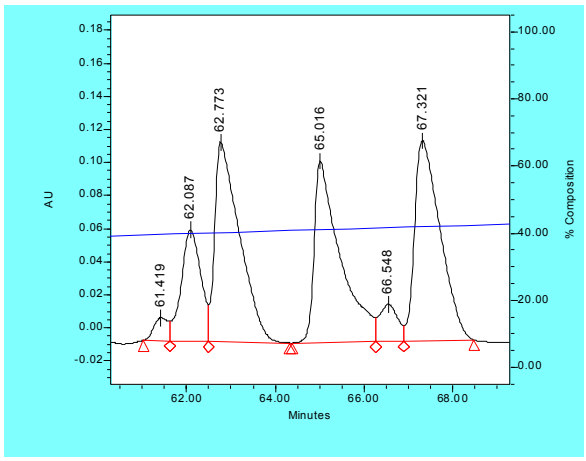


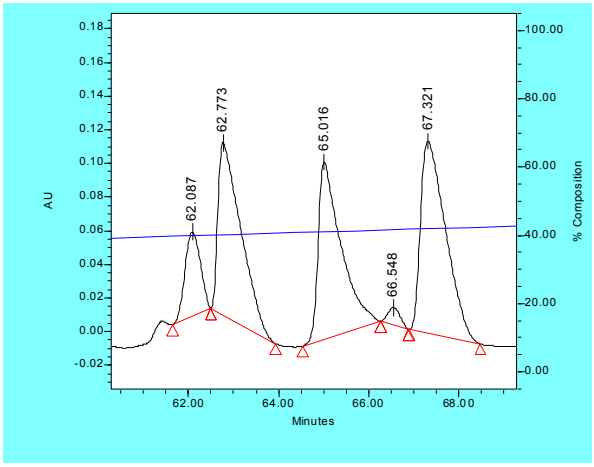
Set Liftoff and Touchdown at 50 from 70 min.
Remember to set it back to 300 at 74 min



Valley to Valley

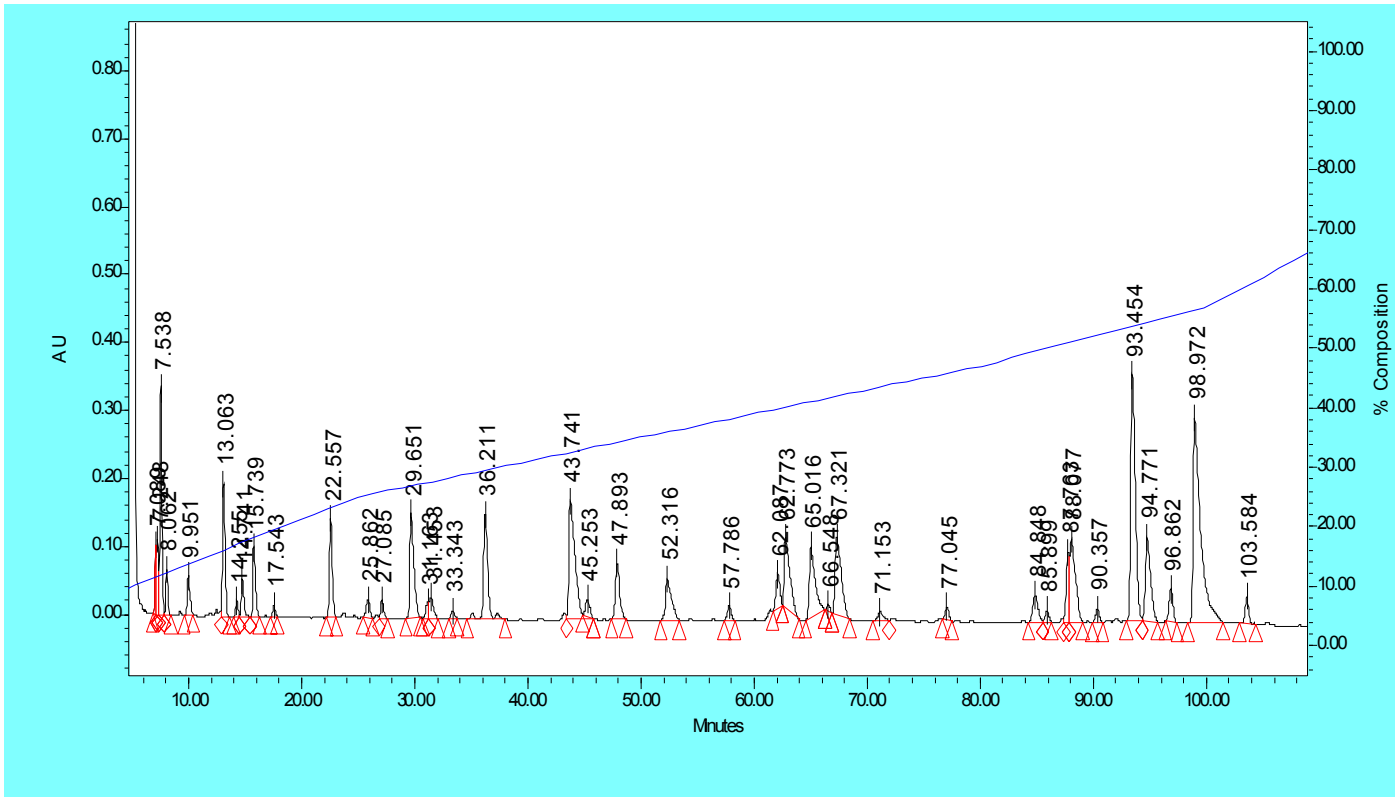
Sets the baseline at each valley start and end point in a fused peak (within the user-specified start and stop time window).
Note: Without this event, a common baseline is drawn for all fused peaks, with each peak separated by a drop line. When enabled, the Valley-to-Valley event reassigns the baseline at each peak start and end point. All peaks become baseline resolved and are labelled as BB in the Peaks table (Review Main window and Results window).





THE FINAL CHROMATOGRAM:

USING ALL THESE EVENTS:



And the Integration Events Table looks like that:

Peptide Mapping in Seminar as shula/Administrator - Review -

File Edit View Plot Process Navigate Options Window Help

Integration | Components | Default Amounts | Named Groups | Timed Groups | Suitability | Limits

Peak Width: 30.00 Threshold: 300.000

Minimum Area: 0 Minimum Height: 10000

	Time	Type	Value	Stop
1	0.000	Inhibit Integration		6.284
2	13.666	Forward Horizontal by Time		18.300
3	34.646	Force Peak		38.000
4	42.000	Tangential Skim	4.000	46.500
5	50.000	Force Baseline by Peak		59.400
6	60.400	Valley to Valley		69.000
7	70.000	Set Liftoff	50.000	
8	70.000	Set Touchdown	50.000	
9	74.000	Set Liftoff	300.000	
10	74.000	Set Touchdown	300.000	
11	98.000	Set Peak Width	120.000	
12	107.249	Inhibit Integration		

For Help, press F1 | Meth Set: Untitled | Proc Meth: Integration events proc | LC

The Report will look like that:

Current Date 13/10/99

Peptide Mapping Chromatogram

SampleName	Peptide Mapping
Vial	3
Injection	1
Injection Volume	100.00 ul
Channel	2487Channel 1
Run Time	130.0 Minutes

Sample Type	Standard
Date Acquired	10/06/99 2:21:56 PM
Acq Method Set	08PMQC1
Processing Method	Integration events proc
Date Processed	13/10/99 11:55:23 AM