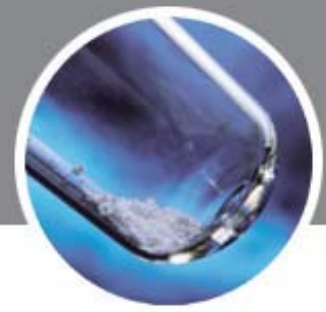
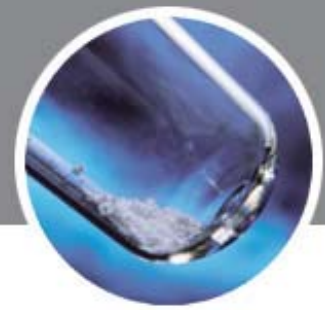


# Sophisticated peak-based fraction collection – working with up and down slope



Presented by

***Christophe Luigi***  
**LC Solutions Product Specialist**



- **Introduction**

- What is slope?

- Up and down slope triggering algorithm

- Triggering decisions

- Upper threshold

*Hints and Tips!*

- **Applications 1**

- Separation of steep peaks from shallow peaks

- Separation of non-baseline separated peaks

- **Applications 2**

- Chromatograms with drifting baseline

- Highly overloaded peaks



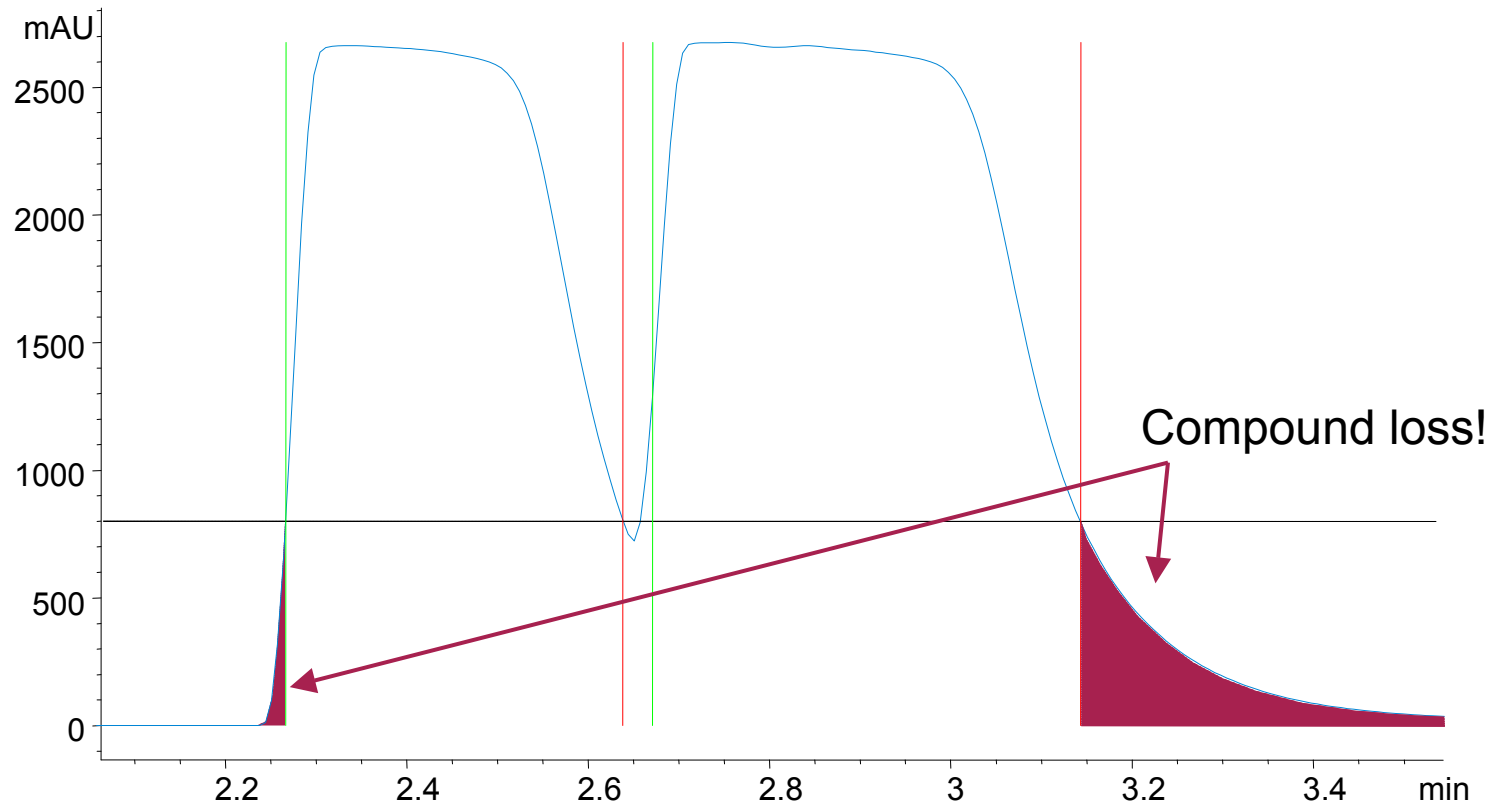
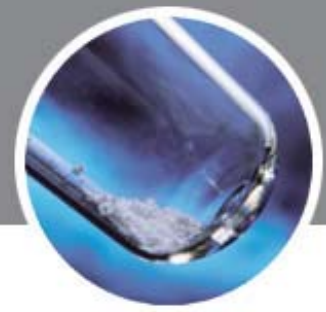


## Peak-based vs. mass-based fraction collection

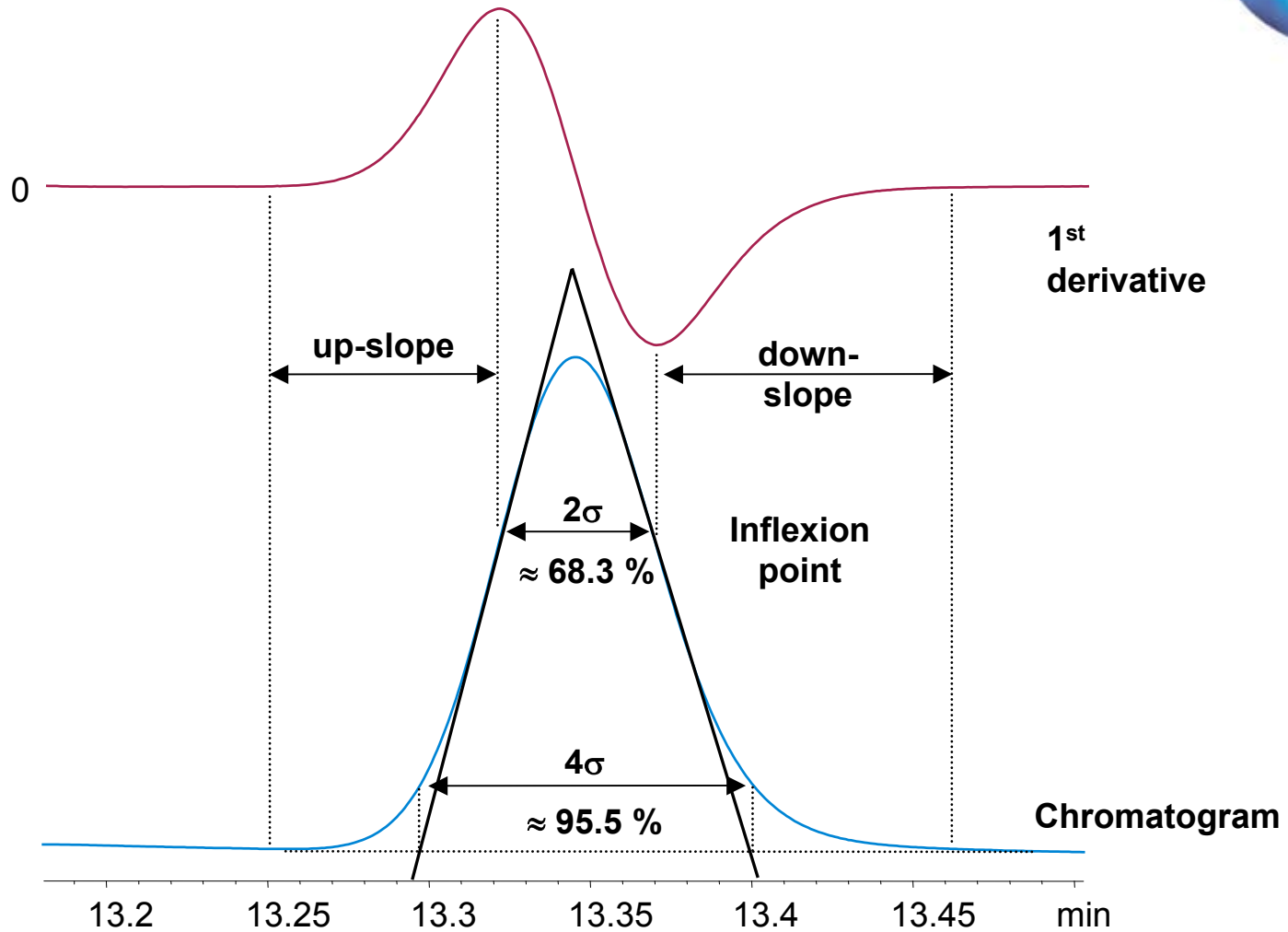
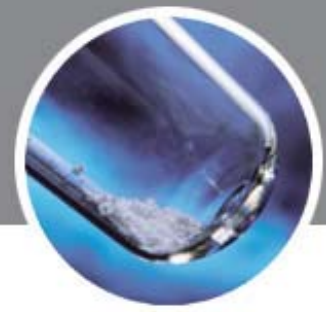
- Highest selectivity
- Less fractions
- Molecular mass or structure must be known
- Compound must ionize
- Restrictions in mobile phase (buffers, DMSO, etc.)
- Easier system set up (no splitter required, easier trigger timing)
- Presence of desired compound in fraction already confirmed
- MSD is expensive



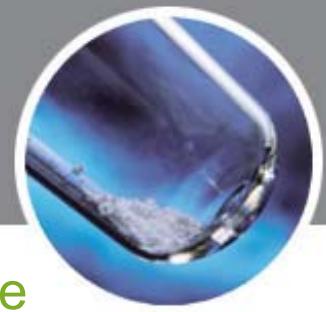
# Peak-based fraction collection on threshold only



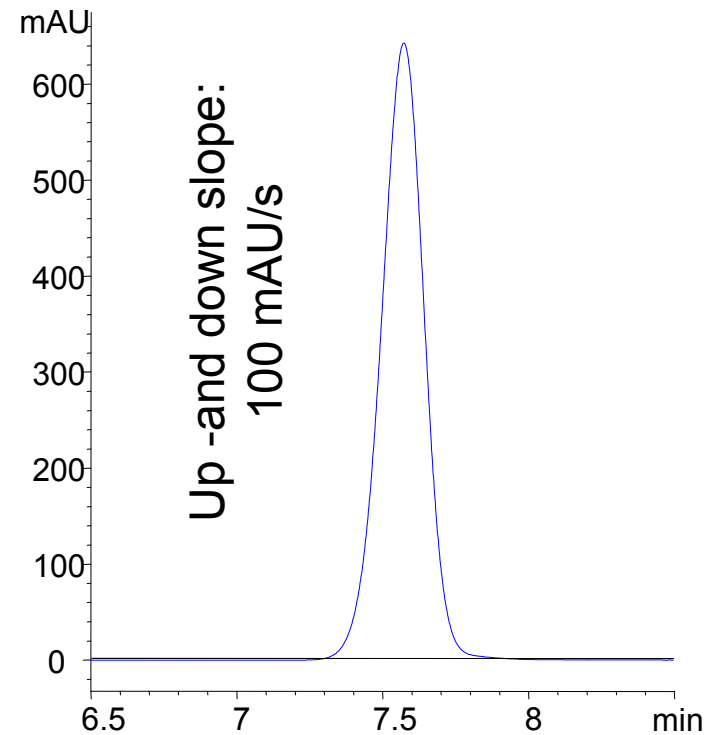
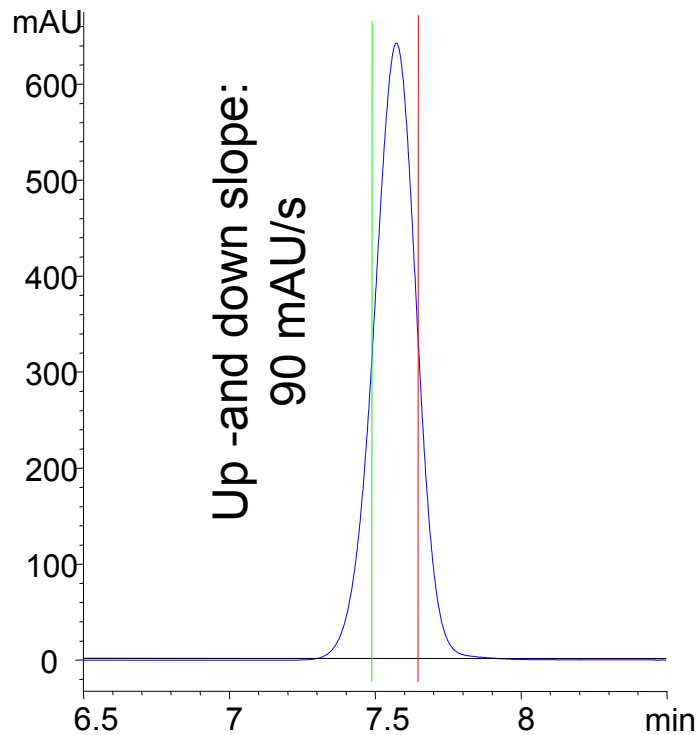
# What is slope?



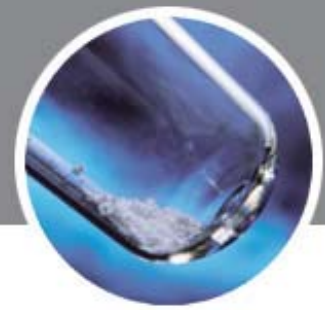
# Hints and Tips



Start and Stop can only be triggered using up and down slope between the baseline and the inflexion point. At the inflexion point the slope reaches its maximum.

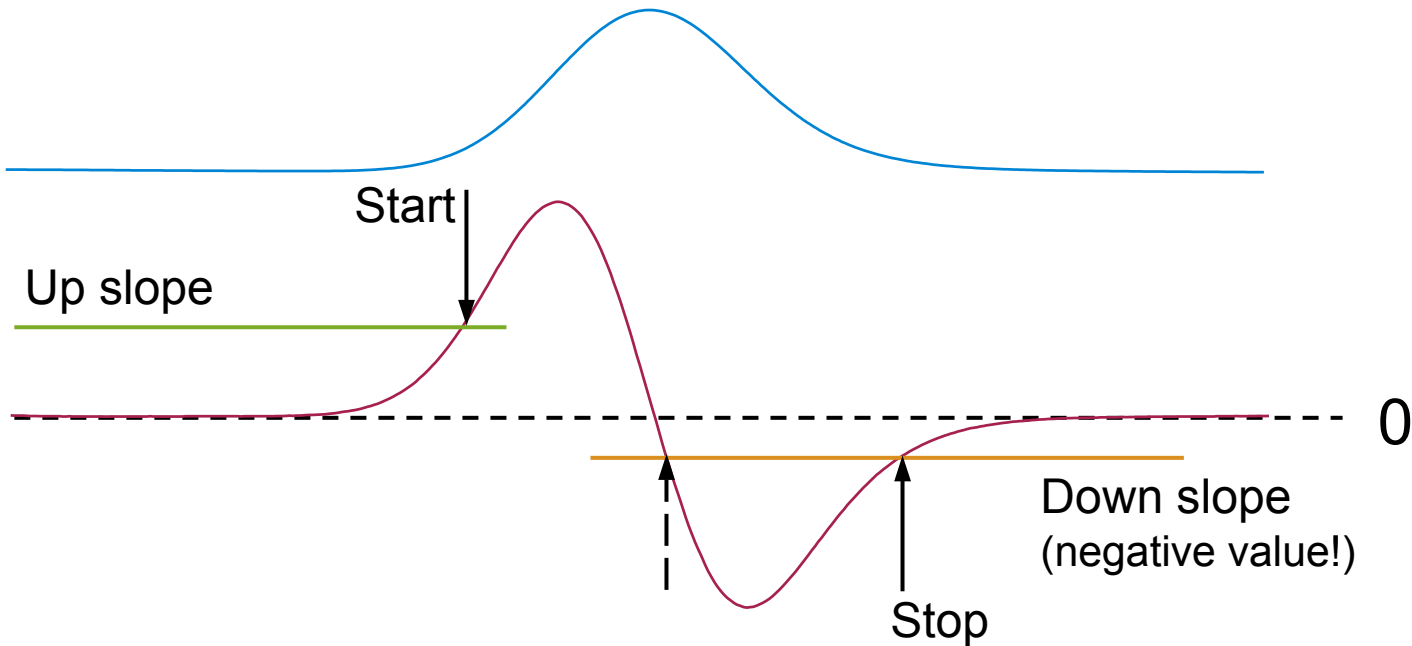


# Up and down slope triggering algorithm



## Peak Detectors

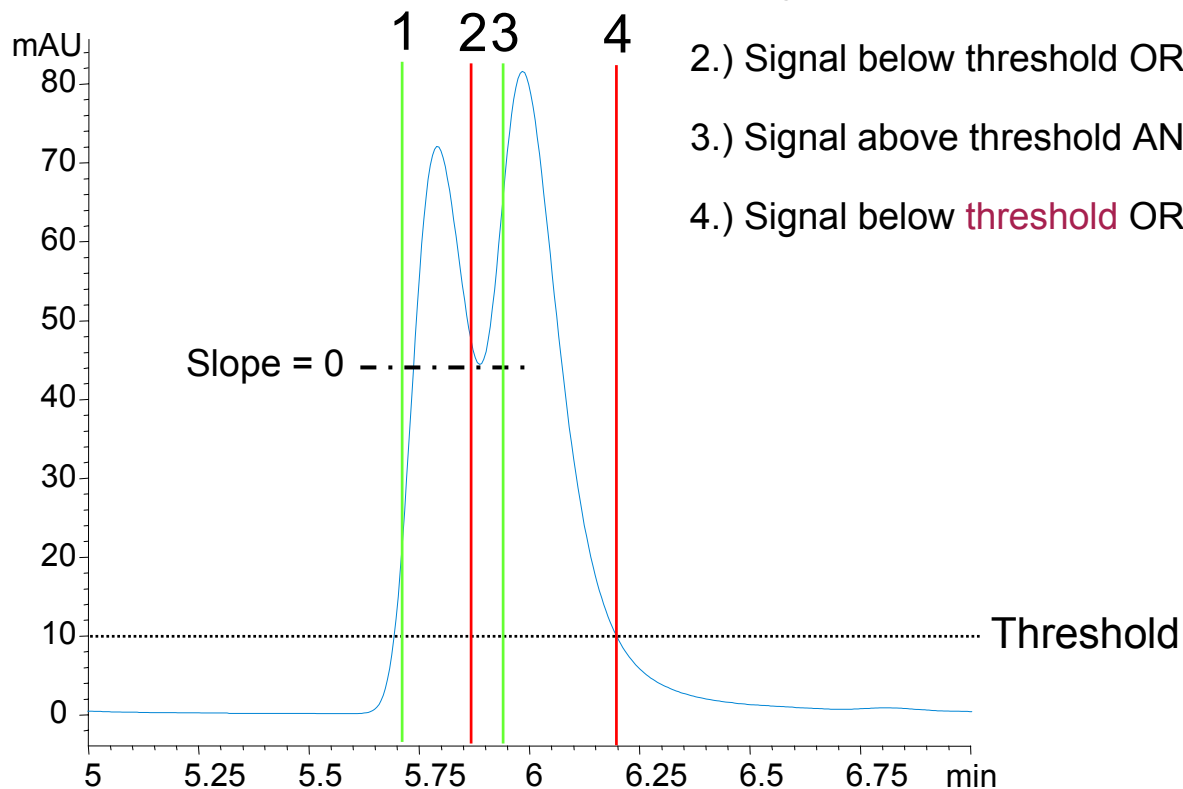
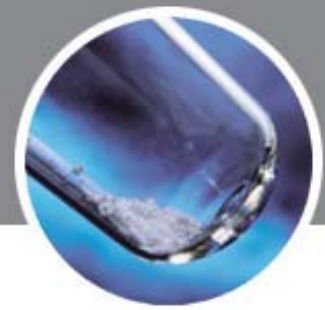
Detector	Working Mode	Up Slope [Unit/s]	Down Slope [Unit/s]	Threshold [Unit]	Upper Threshold [Unit]	Unit
1	DAD1	15.00	5.00	10.000	3000.000	mAU
2	UIB	0.01	0.01	5.000	3000.000	mV



Start: Slope rises above up-slope value  
Stop: Slope falls below down-slope value  
then rises above down-slope value



# Triggering decisions when using threshold and up and down slope



- 1.) Signal above threshold AND up slope  $\Rightarrow$  Start collection
- 2.) Signal below threshold OR **down slope**  $\Rightarrow$  Stop collection
- 3.) Signal above threshold AND up slope  $\Rightarrow$  Start collection
- 4.) Signal below **threshold** OR down slope  $\Rightarrow$  Stop collection





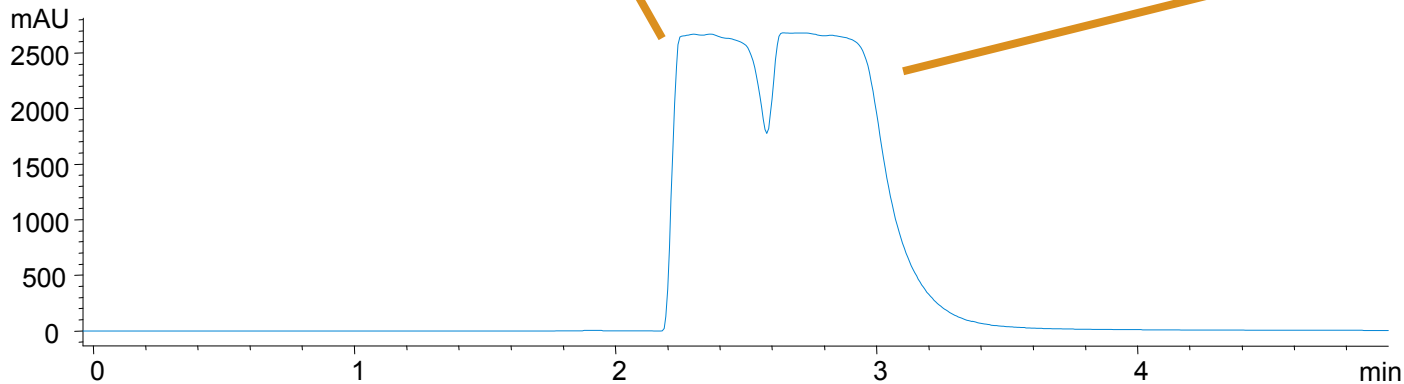
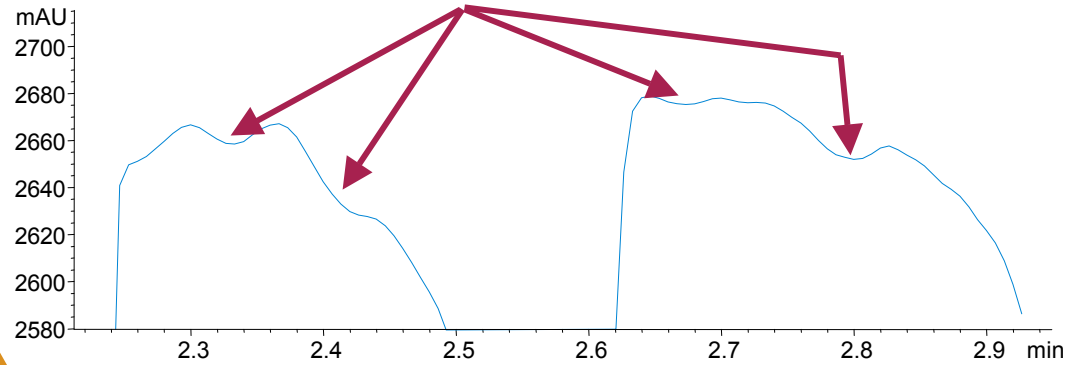
# Upper threshold



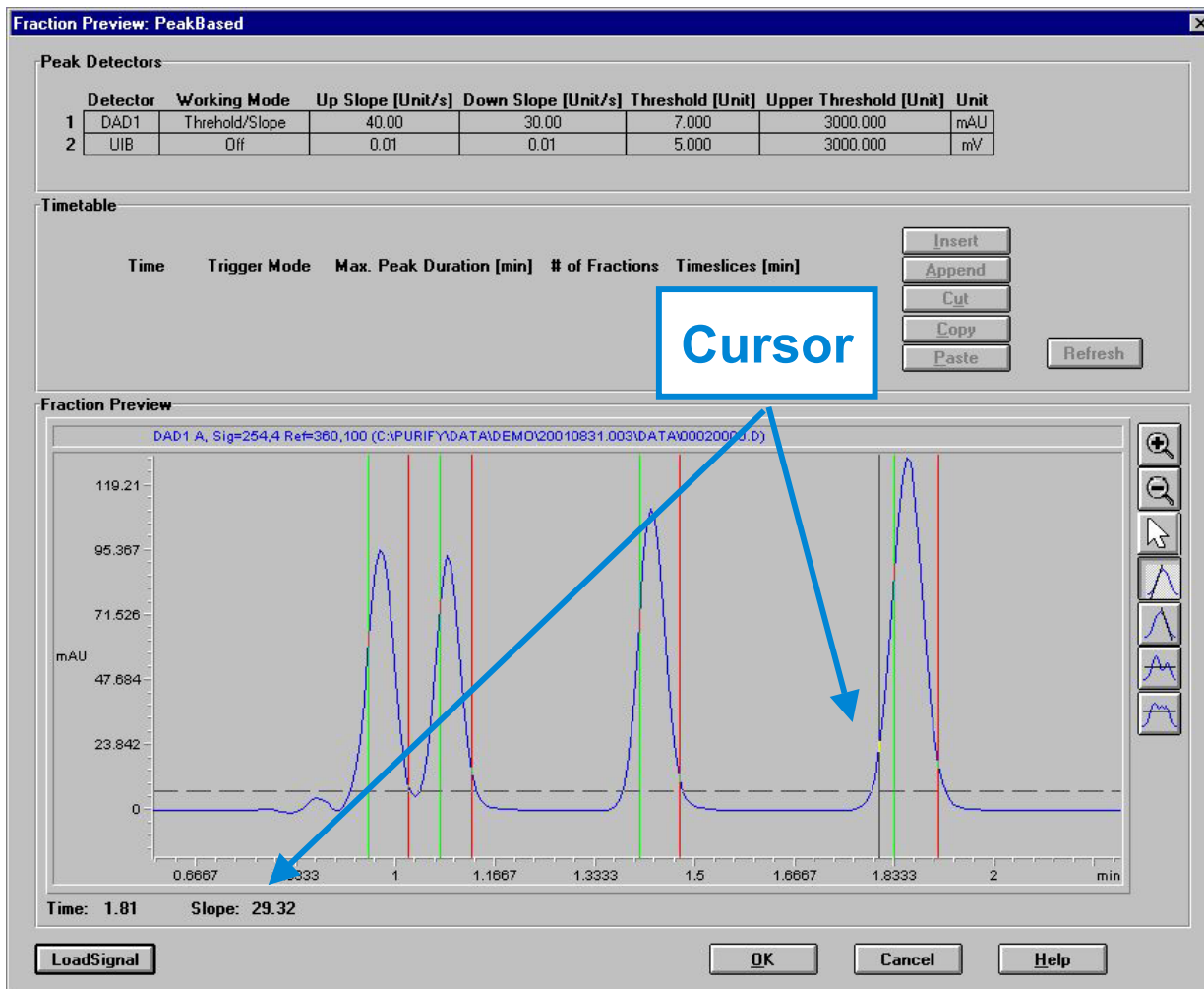
[Unit]	Upper Threshold [Unit]	Unit
	3000.000	mAU
	3000.000	mV

If upper threshold is exceeded fraction is collected regardless of up and down slope.

Electronic noise leads to triggering of unwanted fractions!



# Hints and Tips



There are **NO** generic settings for threshold and up and down slope that can be applied to all fractionation problems!





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Question for Presenter:

There are no questions pending.

## Question & Answer Session

Please type your question into the Question Box at any time during the presentation.

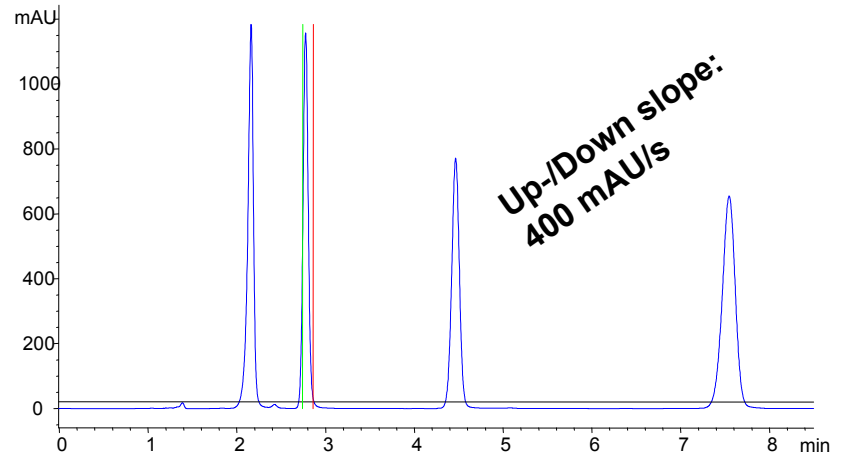
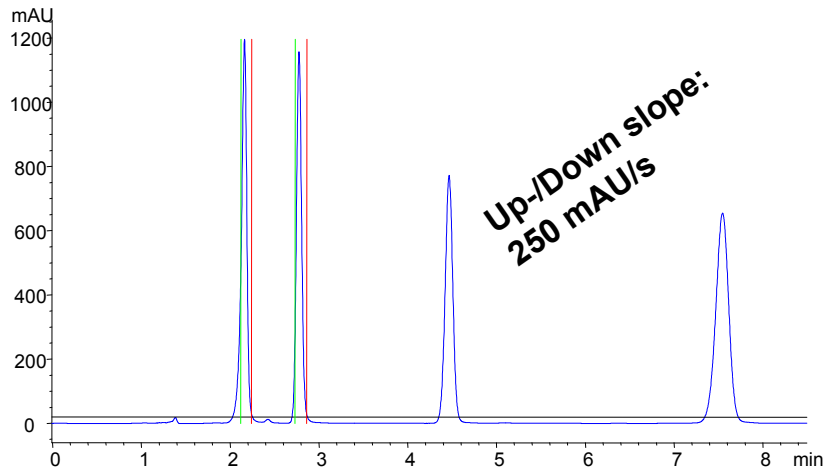
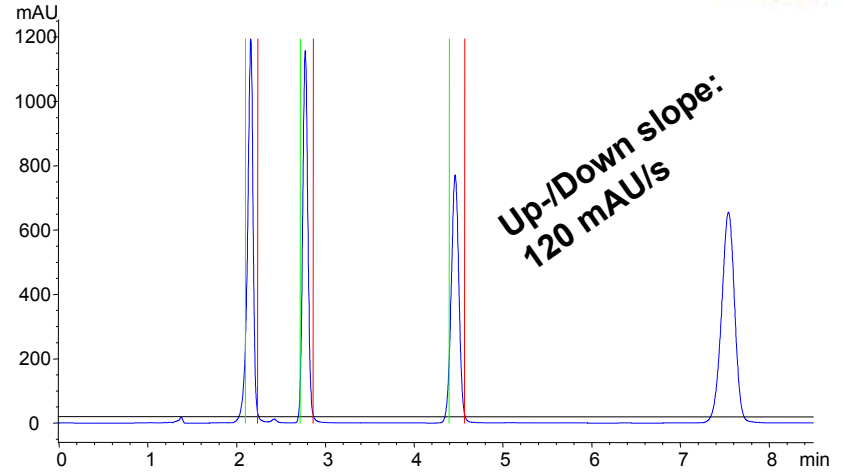
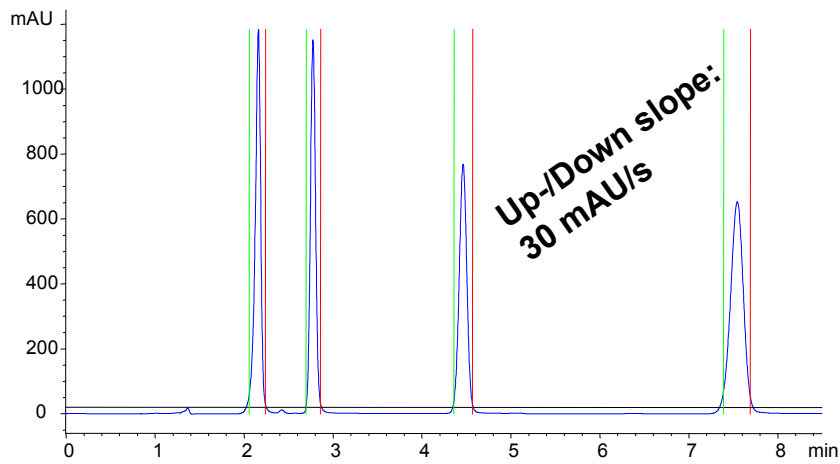
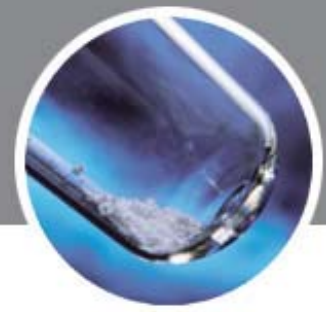
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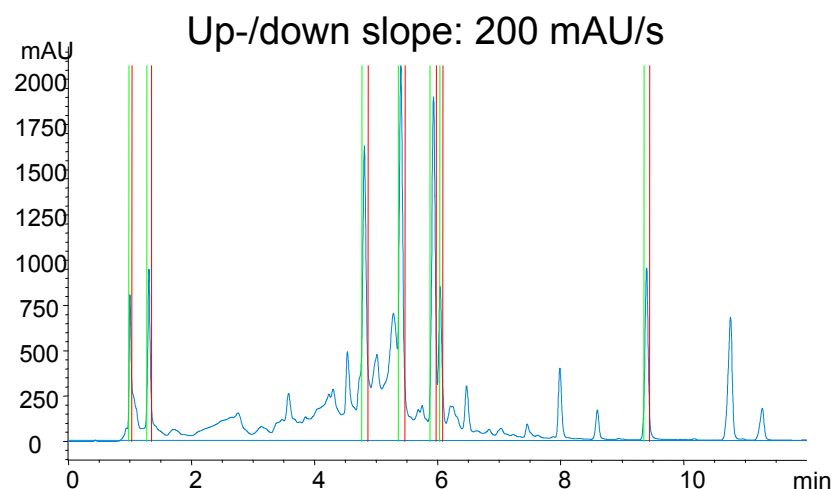
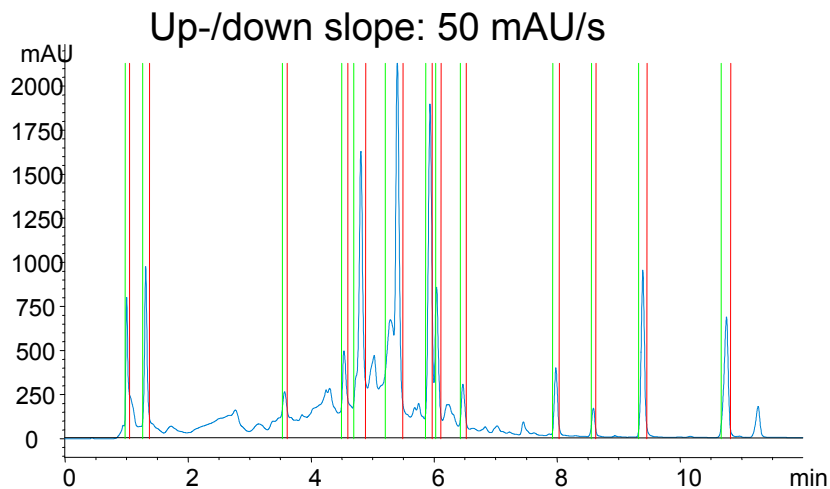
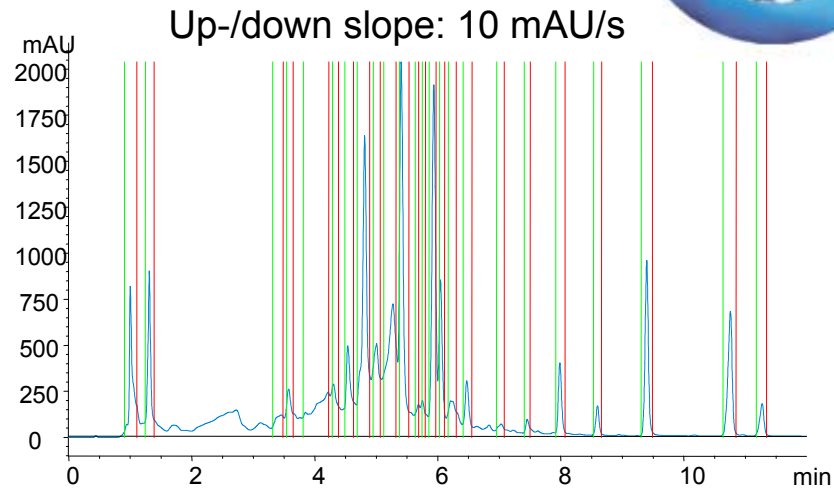
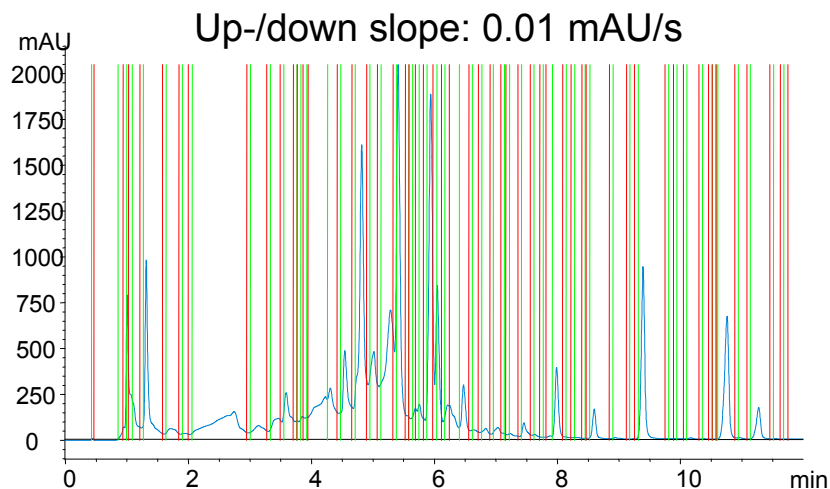


- Separation of steep from shallow peaks in a complex matrix
- Separation of non-baseline separated peaks

# Separation of steep from shallow peaks



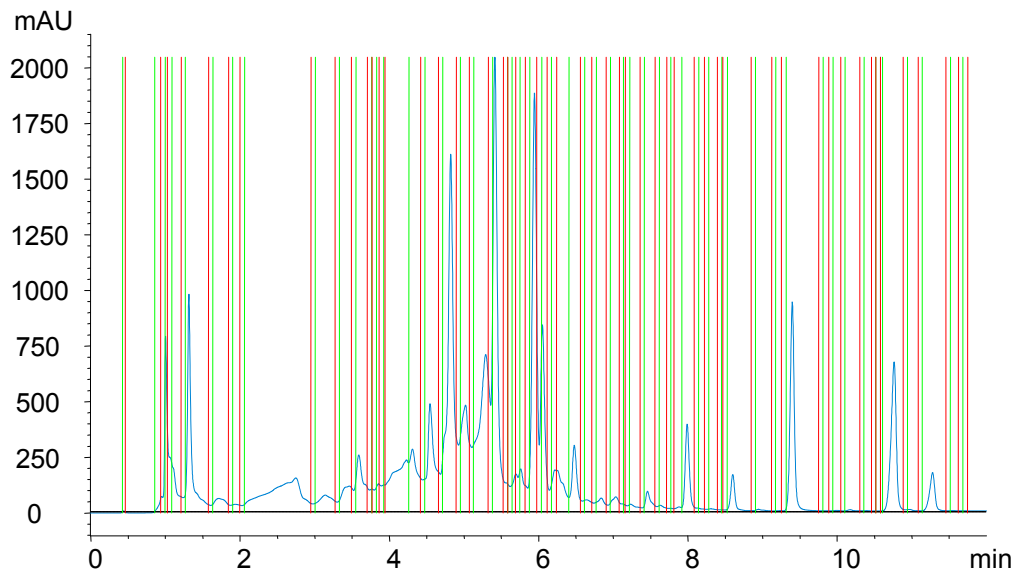
# Separation of steep from shallow peaks



# Hints and Tips



Reliable fraction collection using up and down slope can not be expected with slope settings less than  $\sim 10$  mAU/s. At lower settings chromatography is not precise enough to give reproducible results.



Up and down slope:  
0.01 mAU/s

Number of collected fractions:

- |         |    |
|---------|----|
| 1. Run: | 54 |
| 2. Run: | 56 |
| 3. Run: | 60 |

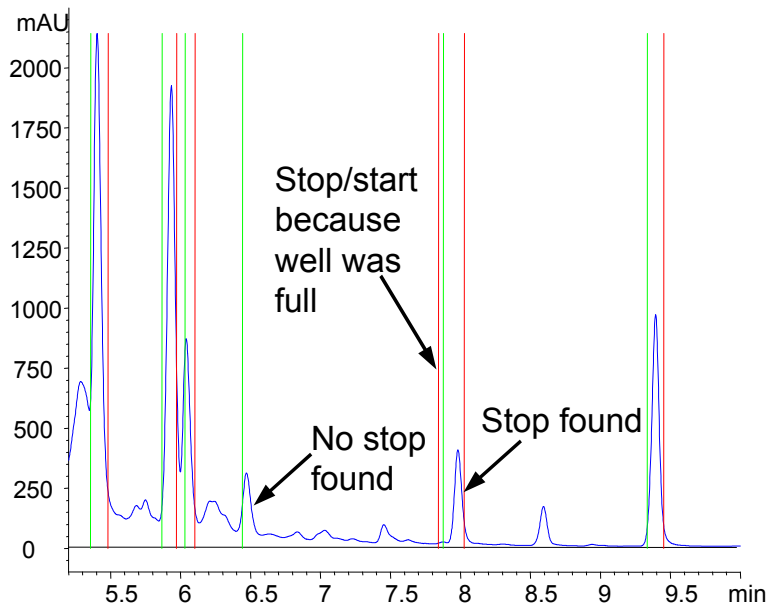


# Hints and Tips

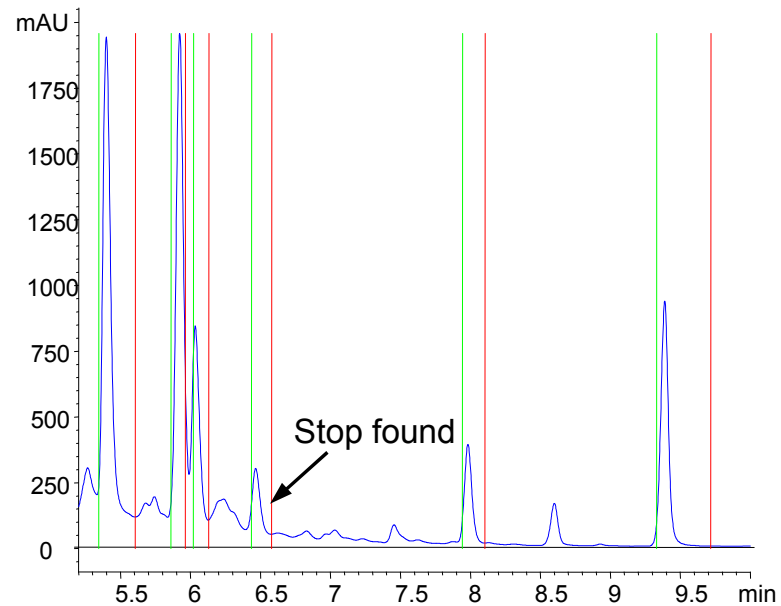


The down slope value should always be set to a lower value than the up slope. Most peaks are tailing and with identical up and down slope values sometimes a peak stop cannot be found.

Up-/down slope: 100 mAU/s

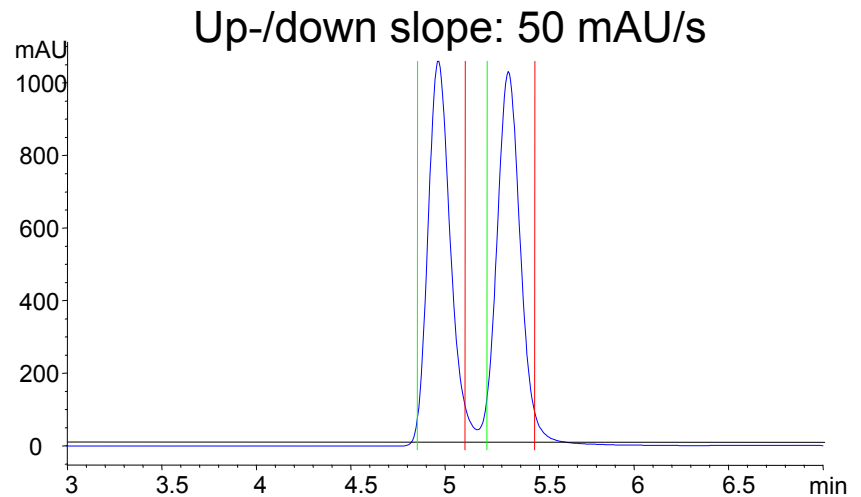
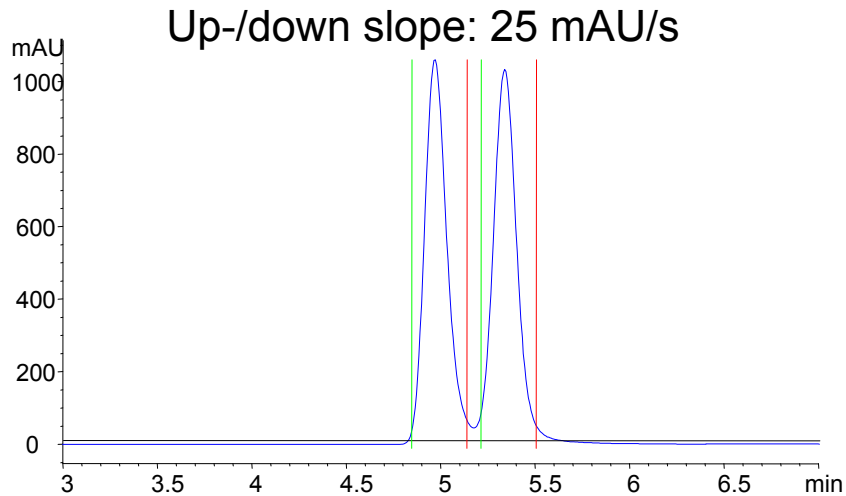
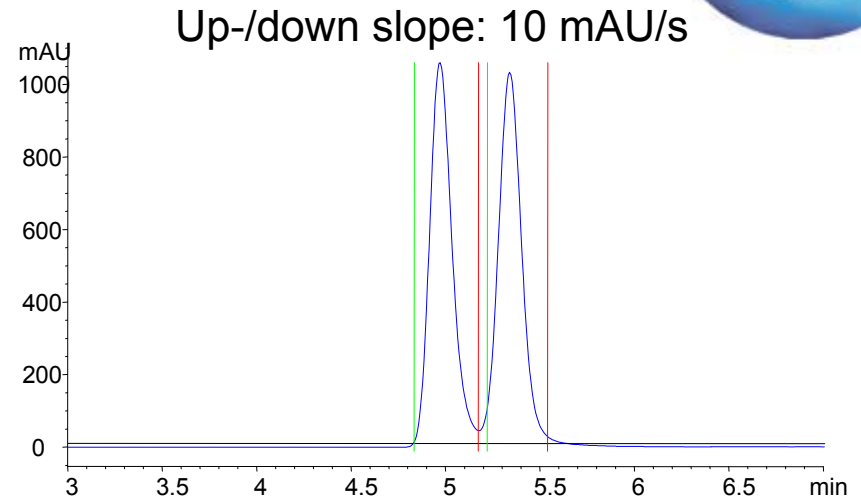
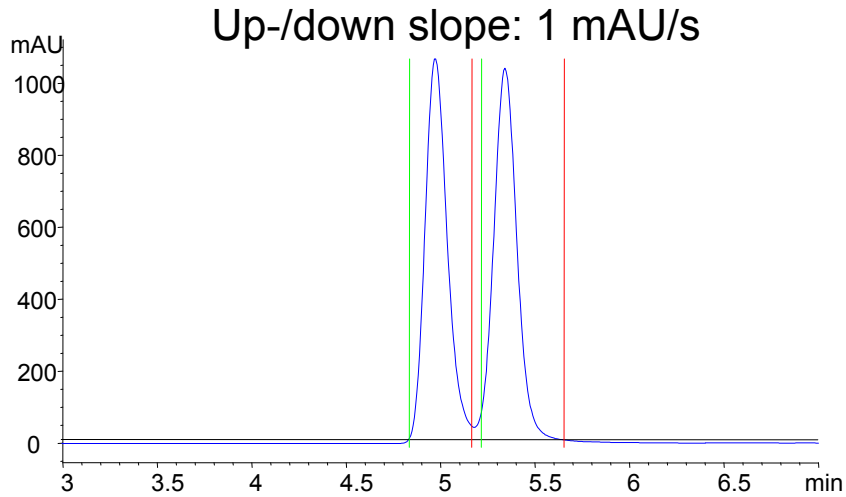
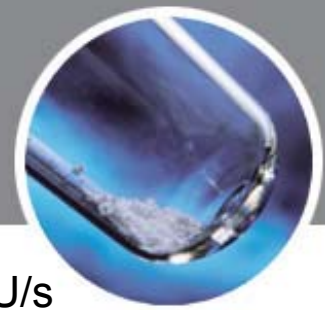


Up slope: 100 mAU/s  
Down slope: 1 mAU/s





# Separation of non-baseline separated peaks

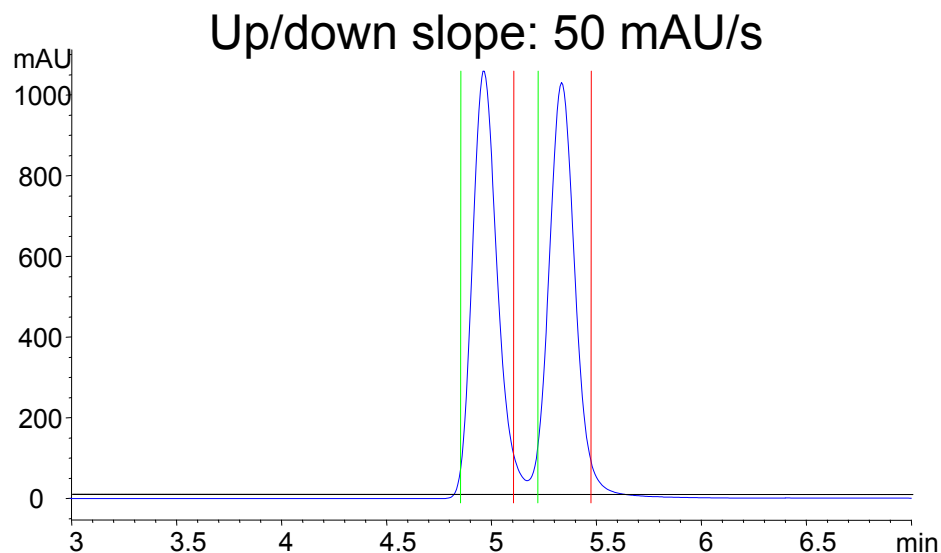


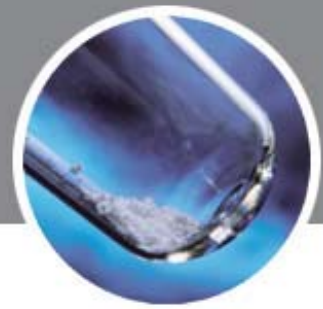
# Hints and Tips



Basically the two peaks can be separated by using the minimal slope value of 0.01 mAU/s. The gap between the start and stop tick-mark is due to the movement of the needle to the next fraction container.

Higher slope settings, e.g. 10 mAU/s give better purity with slightly lower recovery of compounds.





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Question for Presenter:

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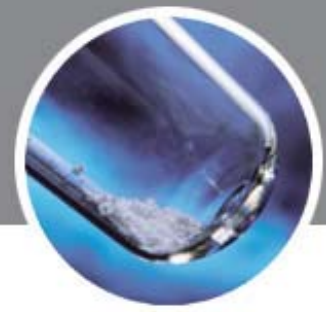
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# Applications 2

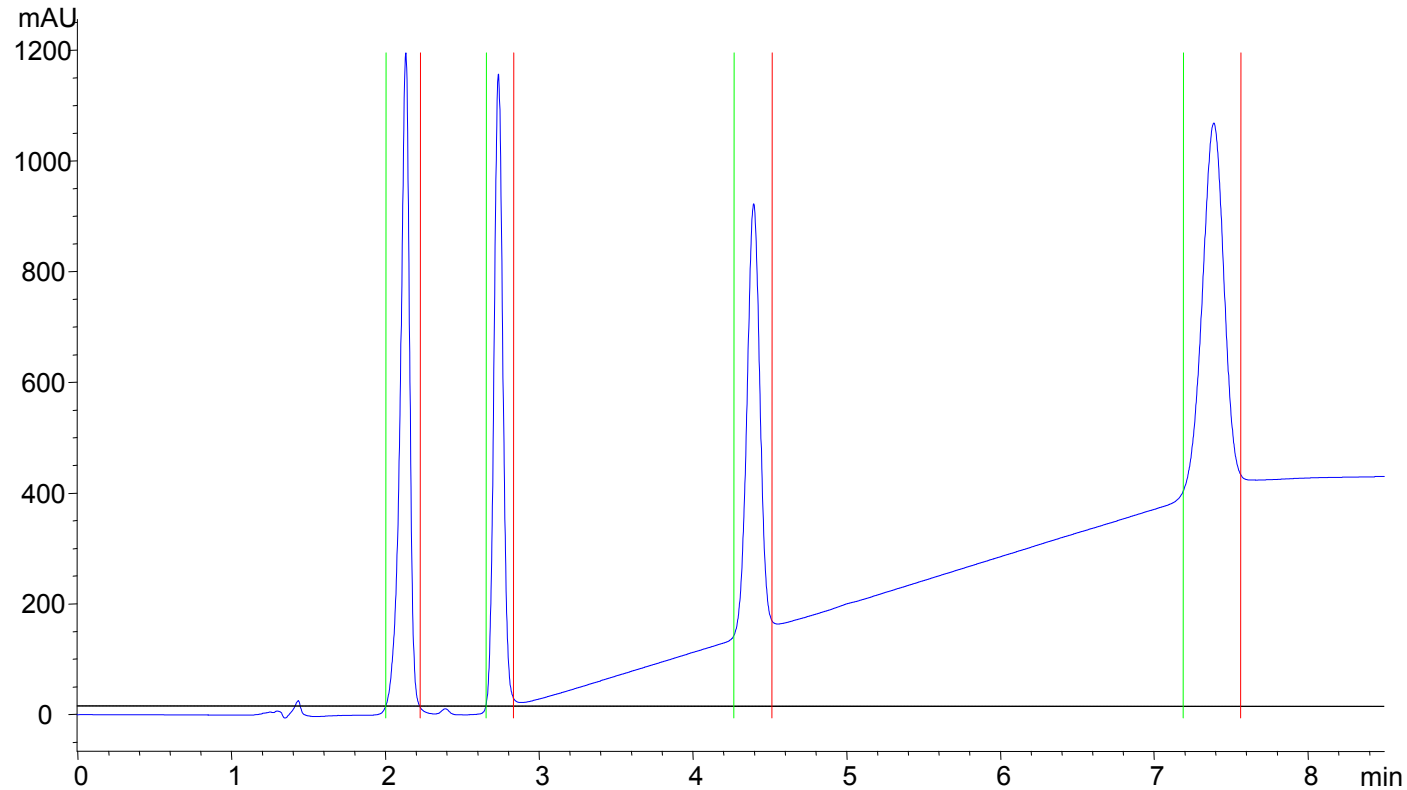


- Chromatograms with drifting baseline
- Highly overloaded peaks

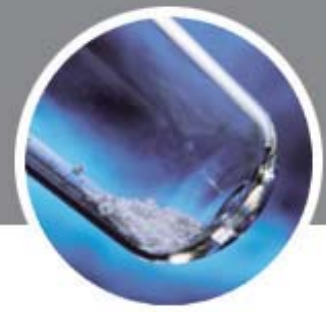
# Chromatograms with drifting baseline



Threshold: 15 mAU  
Up/down slope: 10 mAU/s

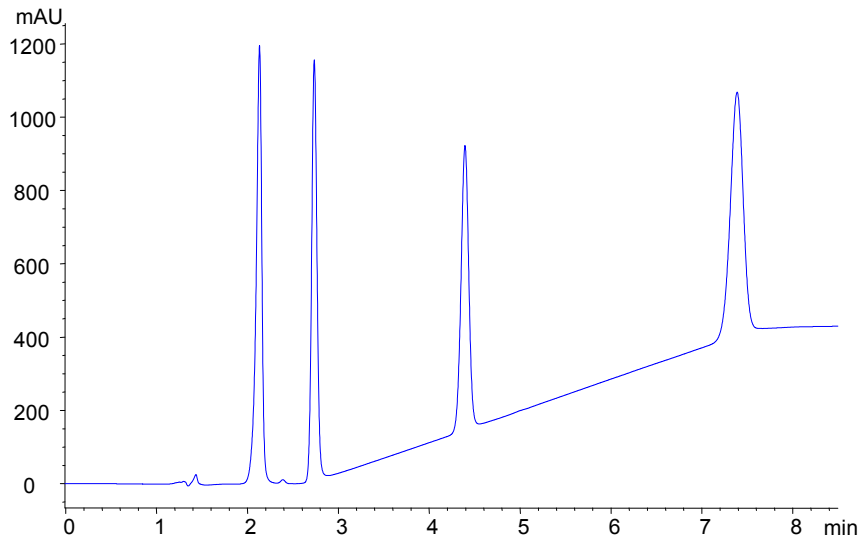


# Hints and Tips

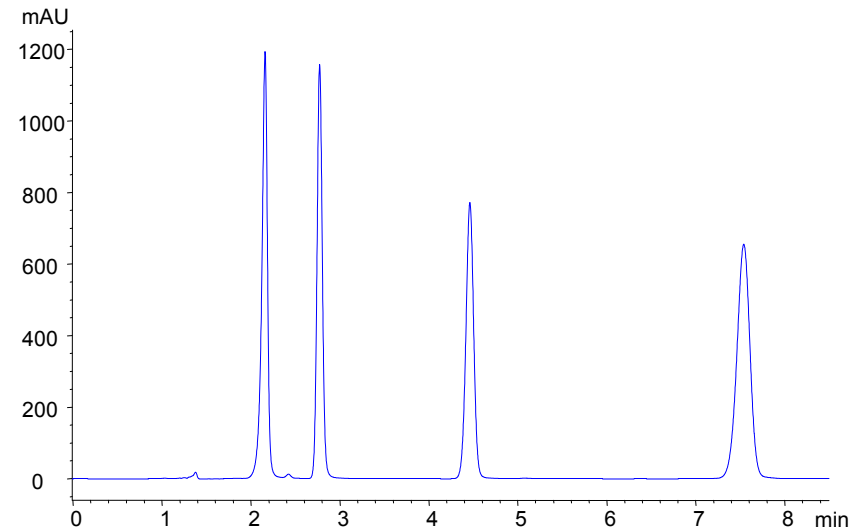


Drifting baseline can be avoided in many cases by using a proper reference wavelength.

w/o reference wavelength



with reference wavelength

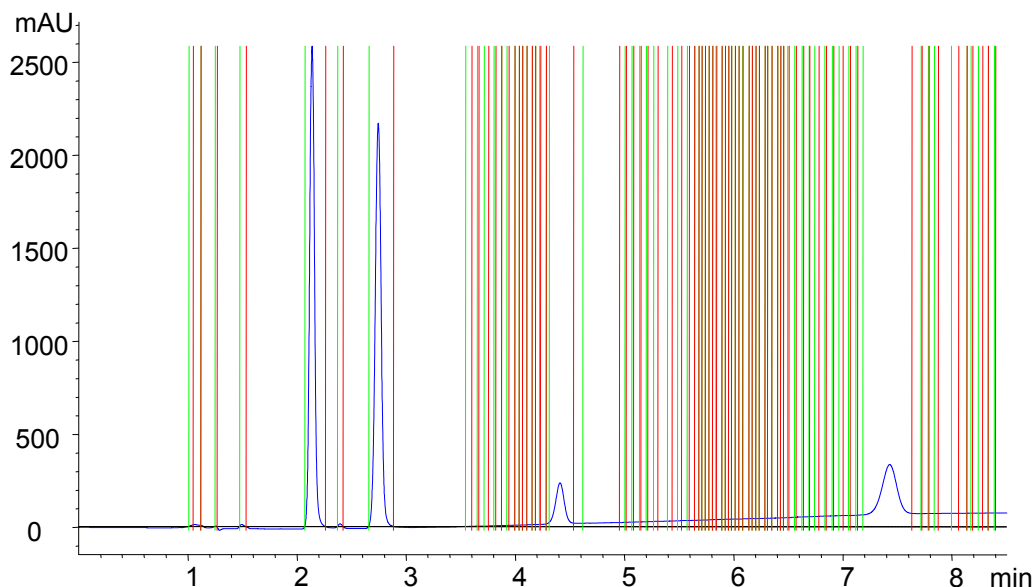


# Hints and Tips



In a chromatogram with rising baseline the threshold will be exceeded constantly. The peaks can only be separated from the baseline using slope.

Too much noise in the baseline can lead to too many fractions.

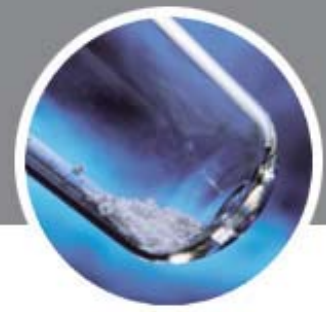


Avoid noise:

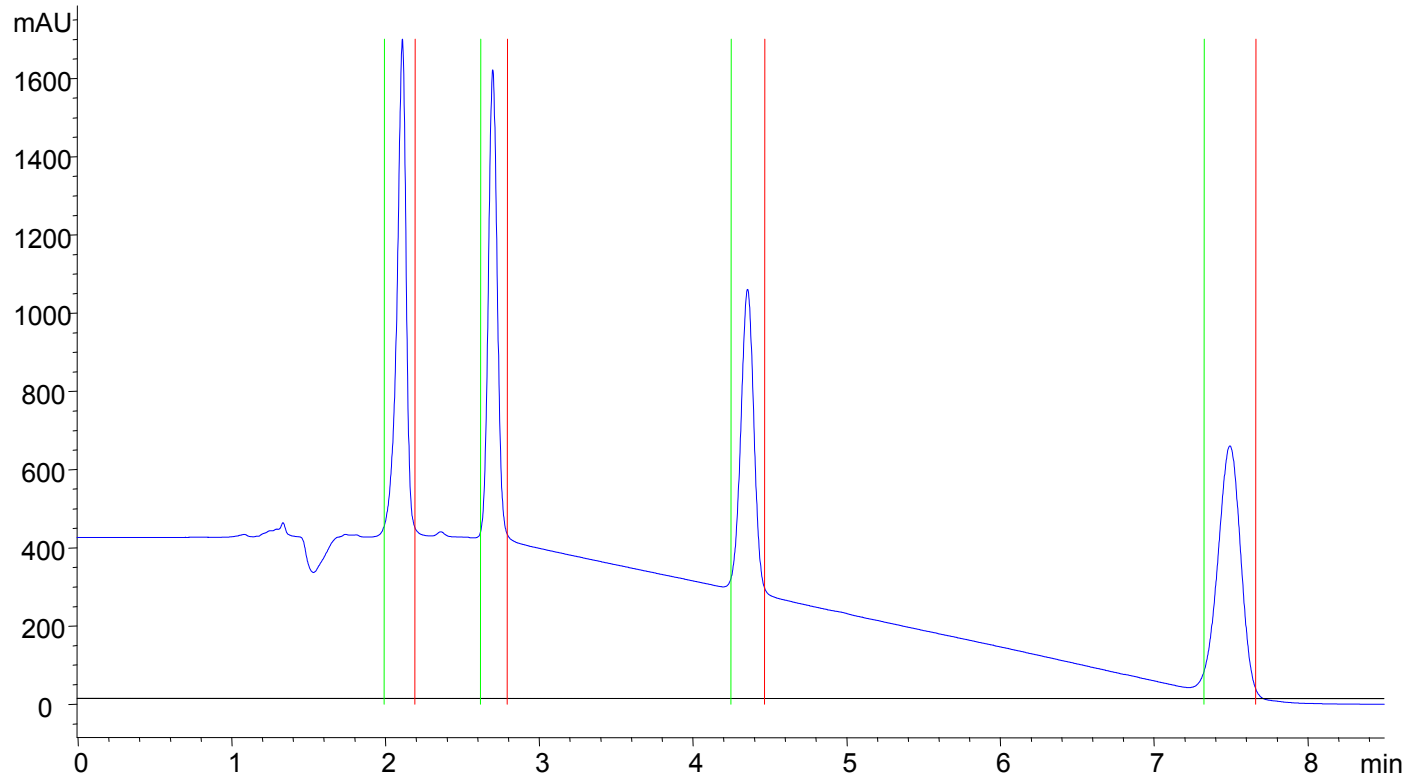
- Slope settings not too low ( $\geq 10$  mAU/s)
- Wavelength high enough ( $\geq 220$  nm)
- Proper compressibility settings for the solvents



# Chromatograms with drifting baseline



Threshold: 15 mAU  
Up/down slope: 20 mAU/s

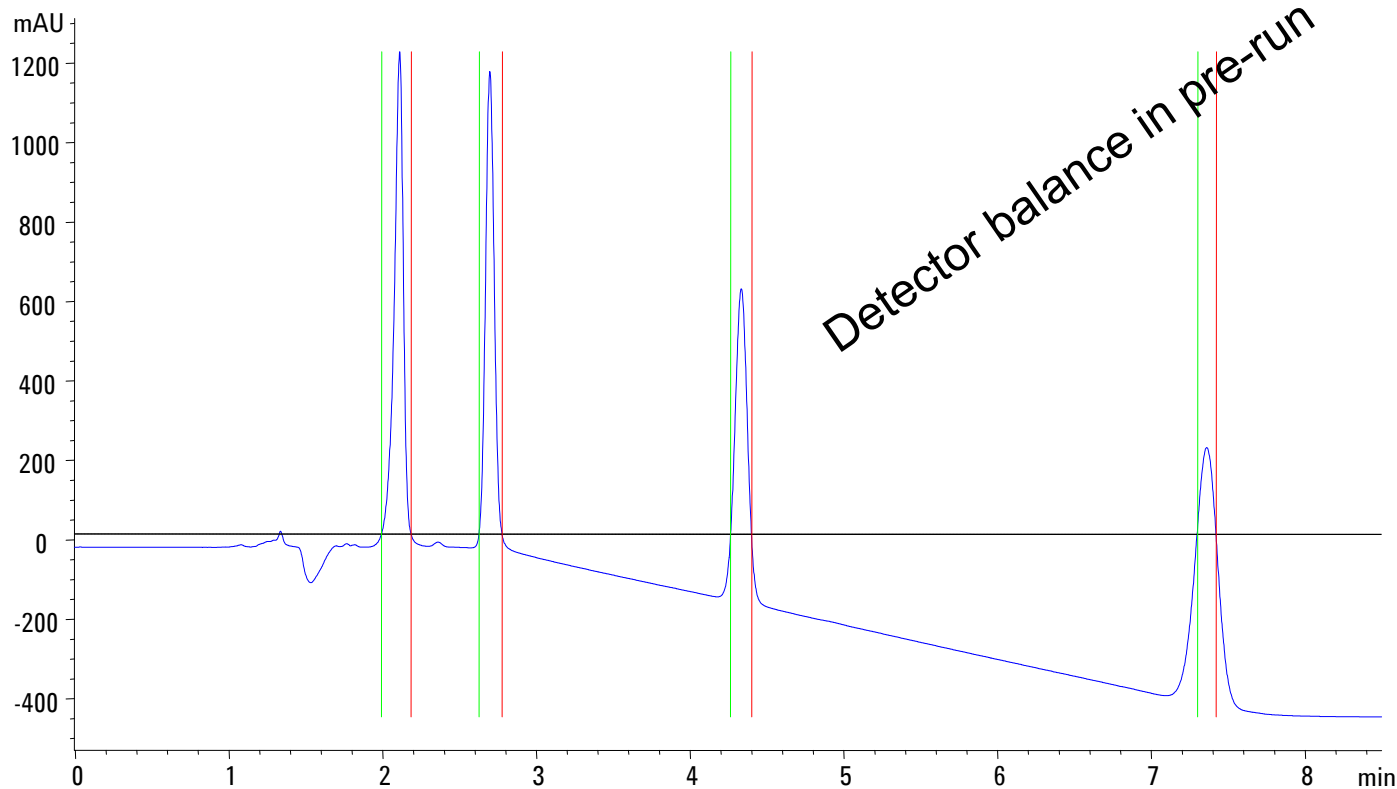




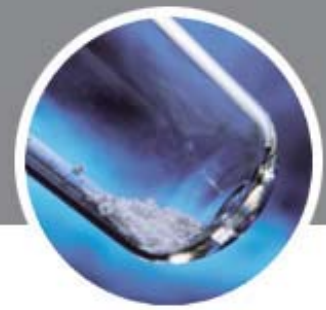
# Hints and Tips



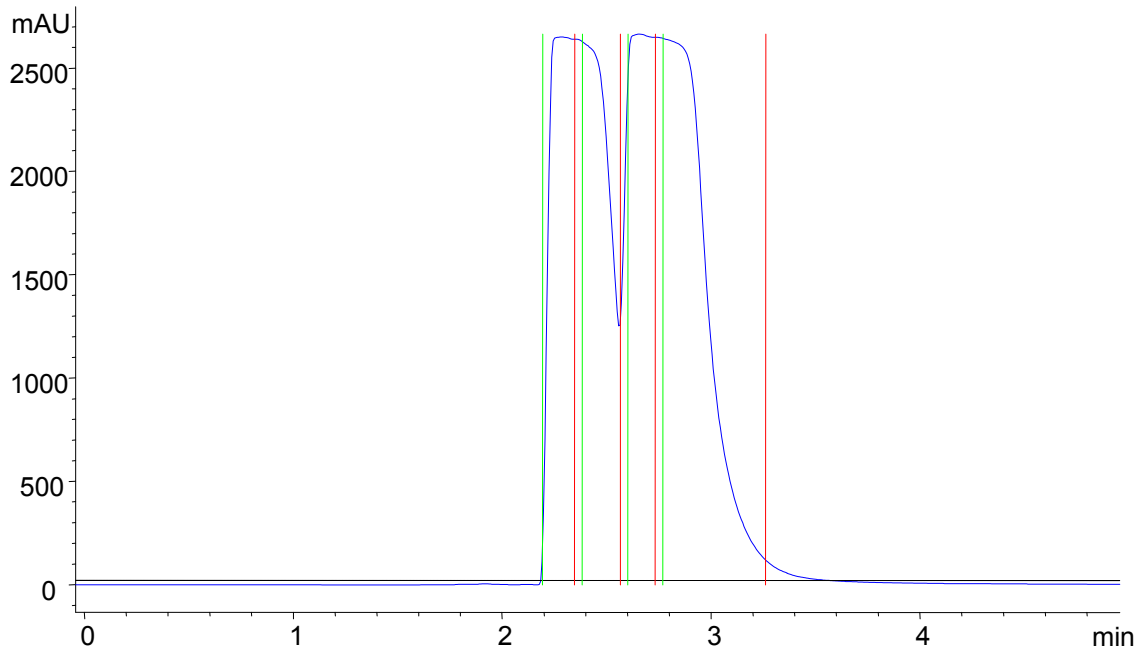
To avoid that the baseline drifts to negative values the detector balance should be performed in the post-run.



# Highly overloaded peaks



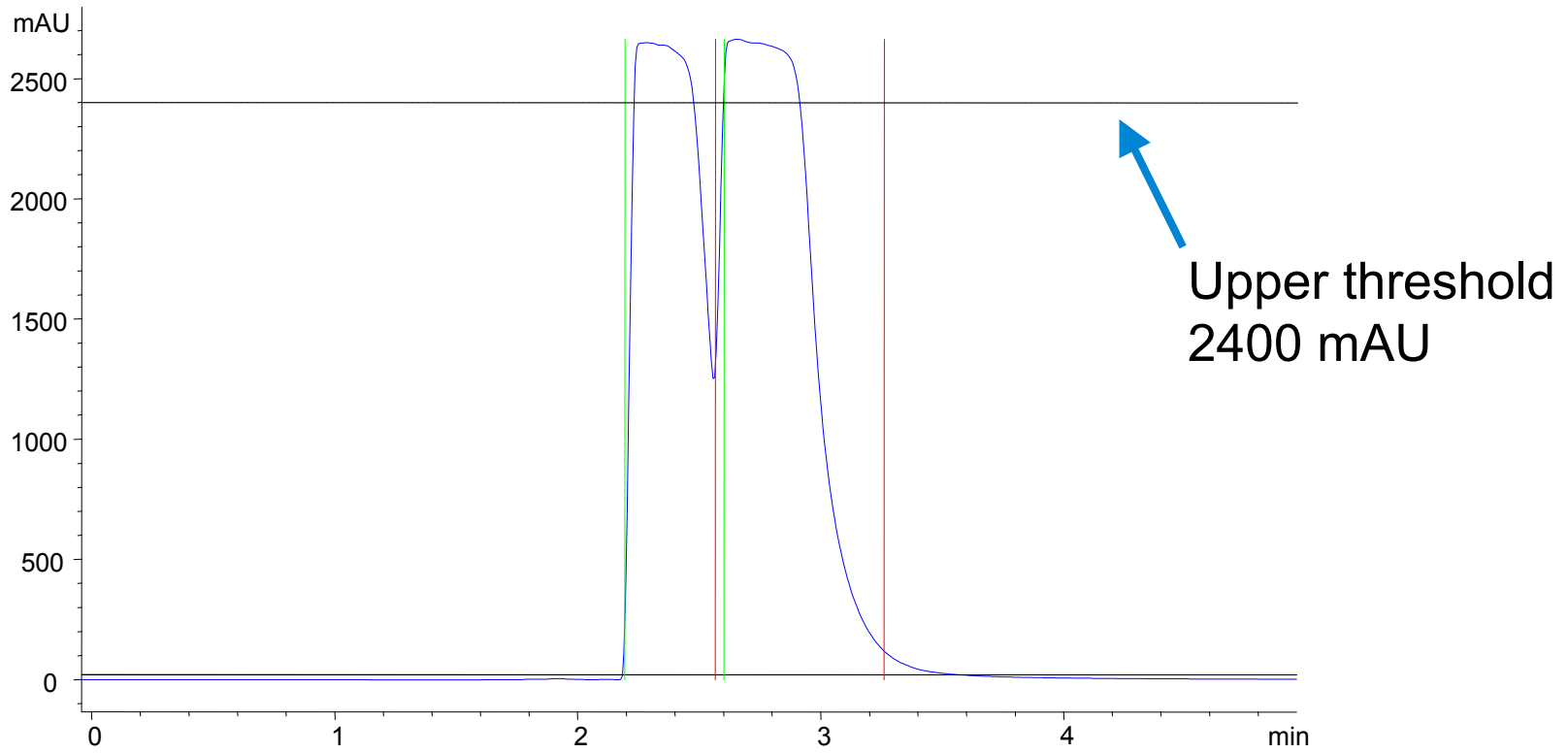
Highly overloaded peaks can lead to triggering of unwanted fractions due to electronic noise during detector saturation. Valve switching/needle movement results in lower recovery.



# Highly overloaded peaks



Usage of upper threshold: If upper threshold is exceeded fraction is collected regardless if up or down slope criteria is met or not.



# Sophisticated peak-based fraction collection – working with up and down slope



- **Introduction**

What is slope, up and down slope triggering algorithm and triggering decisions.

- **Applications requiring the usage of up and down slope**

Separation of steep from shallow peaks in complex matrix

Separation of non-baseline separated peaks

Chromatograms with drifting baseline

Highly overloaded peaks

