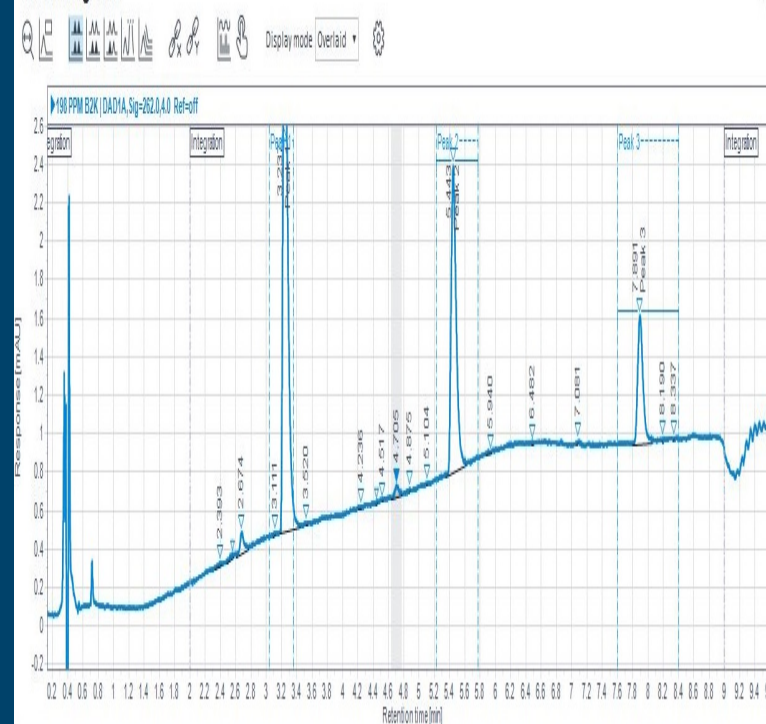


Integrate with Confidence

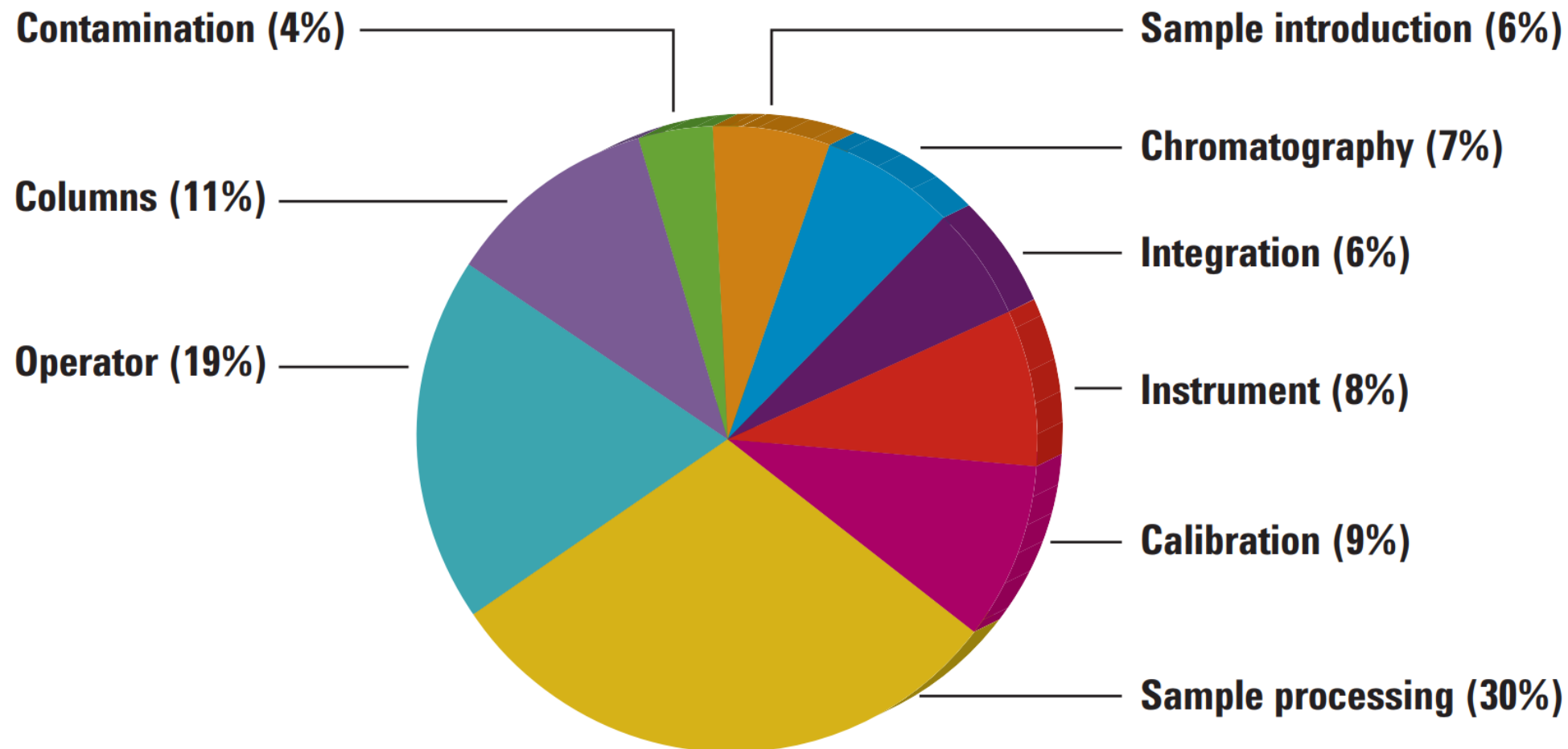
Gregory Hunlen
Applications Scientist, LC and LC/MS



Chromatograms

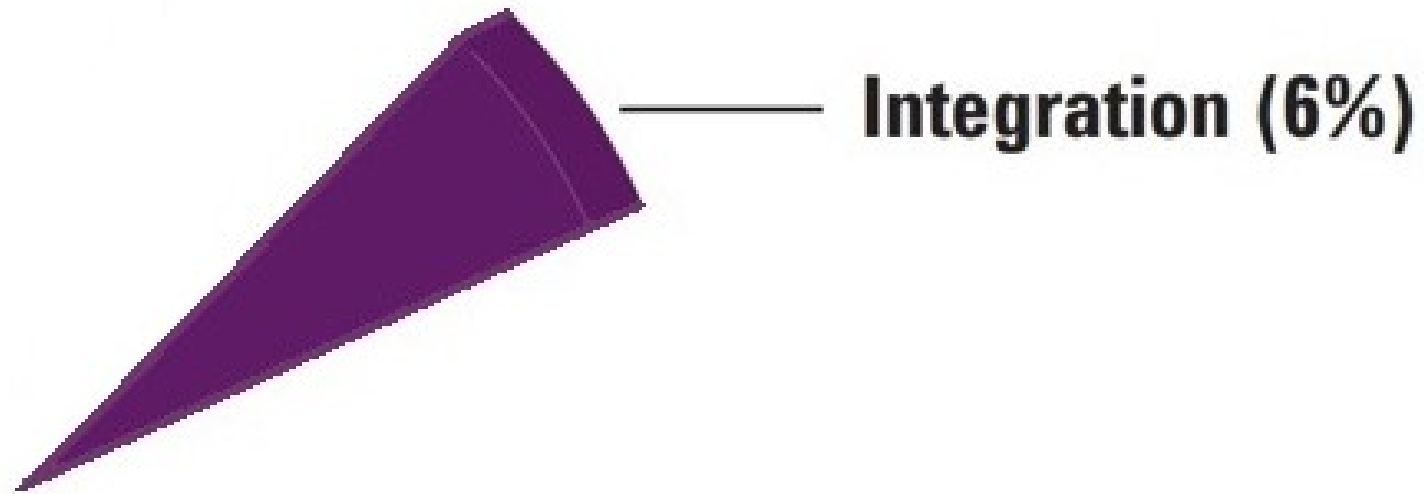


Sources of Error Generated During Chromatographic Analysis

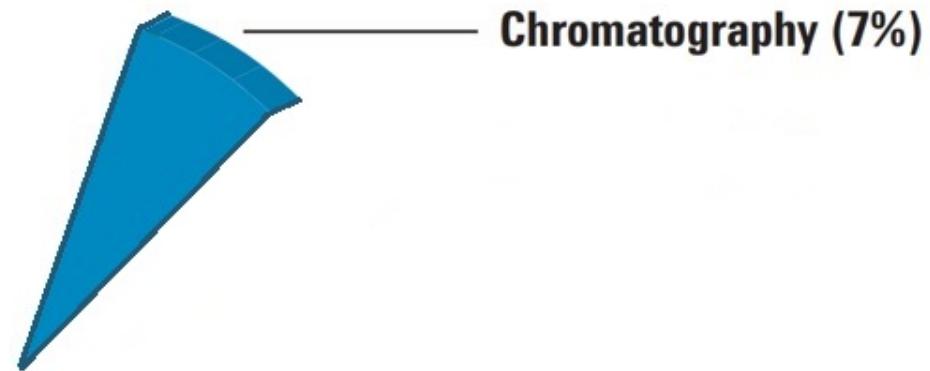


Data taken from Agilent Technologies survey

Sources of Error Generated During Chromatographic Analysis

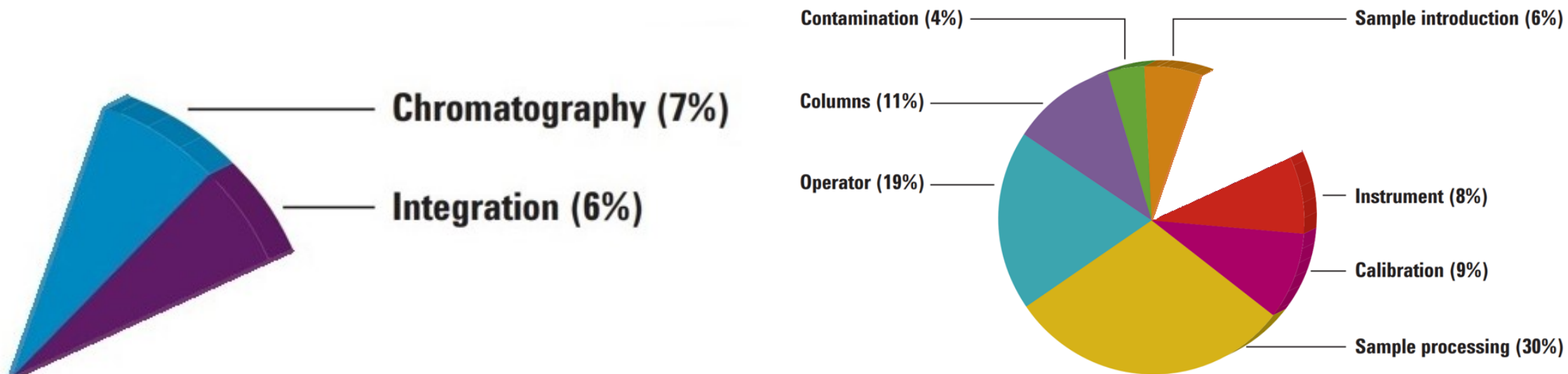


Sources of Error Generated During Chromatographic Analysis



Data taken from Agilent Technologies survey

Sources of Error Generated During Chromatographic Analysis



Data taken from Agilent Technologies survey

Session Overview

- **What is Integration**
- **Important integration events**
- **Manual Integration**
- **Integration Challenges**
- **UV setting affecting integration**

Integration Starts with Good Chromatography

Good Robust System preparation

Establish integration in procedure

Good robust auto integration

What is Integration?

Chromatographic peak integration defines the area under the chromatographic peak

- The measurement is like calculus. Splitting the peak into a large number of rectangles, which are then summed to provide an estimate of the total area under the peak.
- Two most important parameters for peak integration are peak threshold and sampling rate.
- Peak threshold allows the integrator to discriminate the start and end point of the peak from the baseline
- Integration of chromatographic peaks (determination of height, area, and retention time) is the first and most important step in the data analysis part of all chromatography-based analytical methods.

Integration Important Events

Peak Threshold

Peak Width

Integration (start and end)

Height Determination

Area Determination

Baseline Now

Slope Sensitive

Manual

Integration Important Events

Peak Threshold

Control the ability of the integrator to distinguish peak start and end.

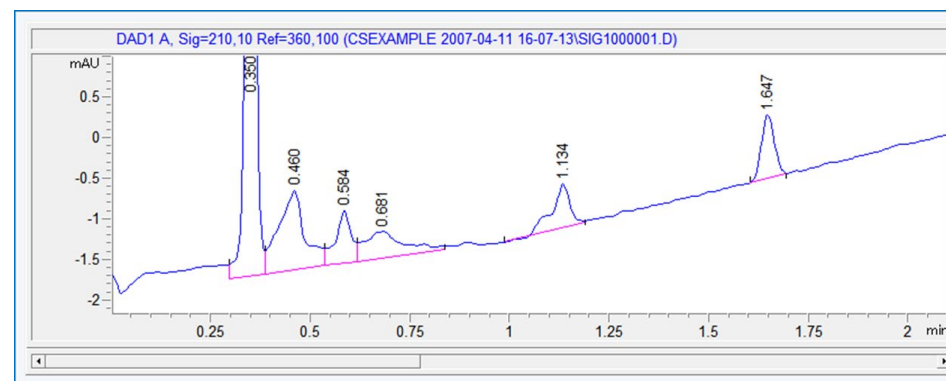
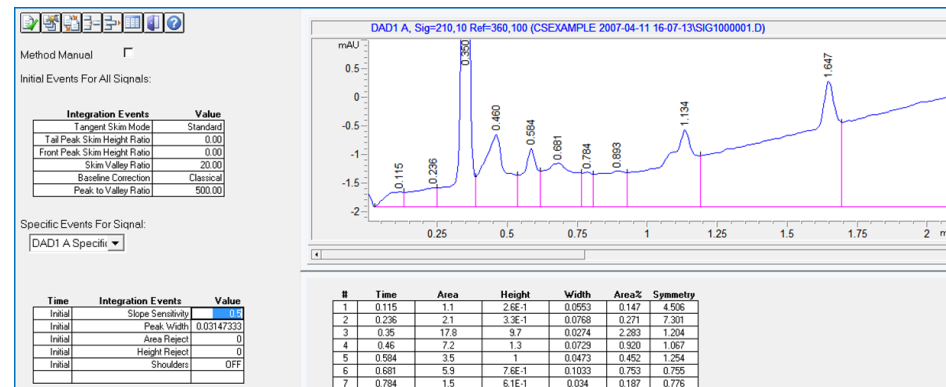
Works in tandem with peak width.

Integration Important Events

Slope Sensitivity

Decreasing slope sensitivity will result in detecting smaller and broader peaks.

Slope sensitivity to low

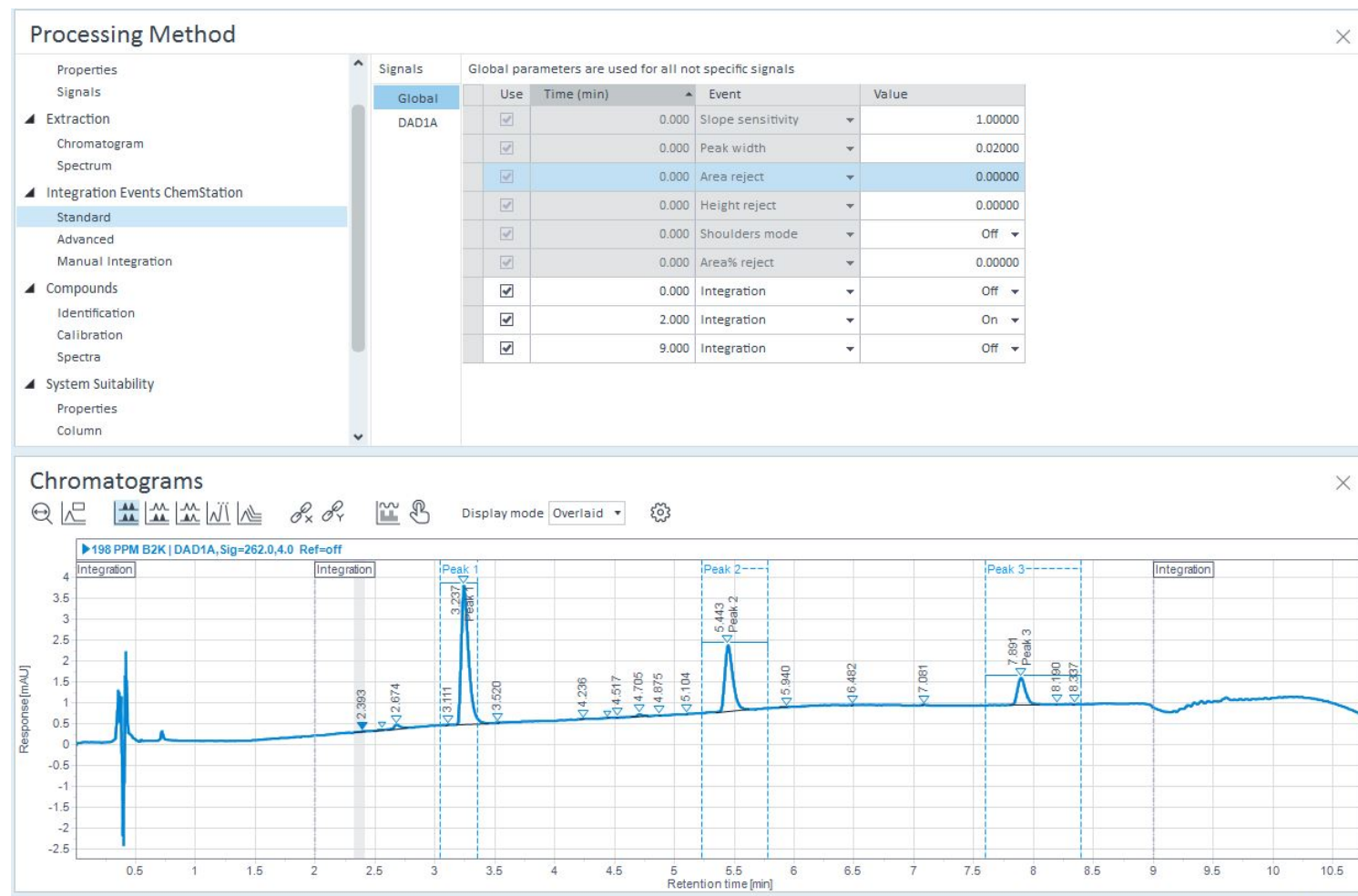


Integration Important Event

Peak Width

View for OpenLab 2.5

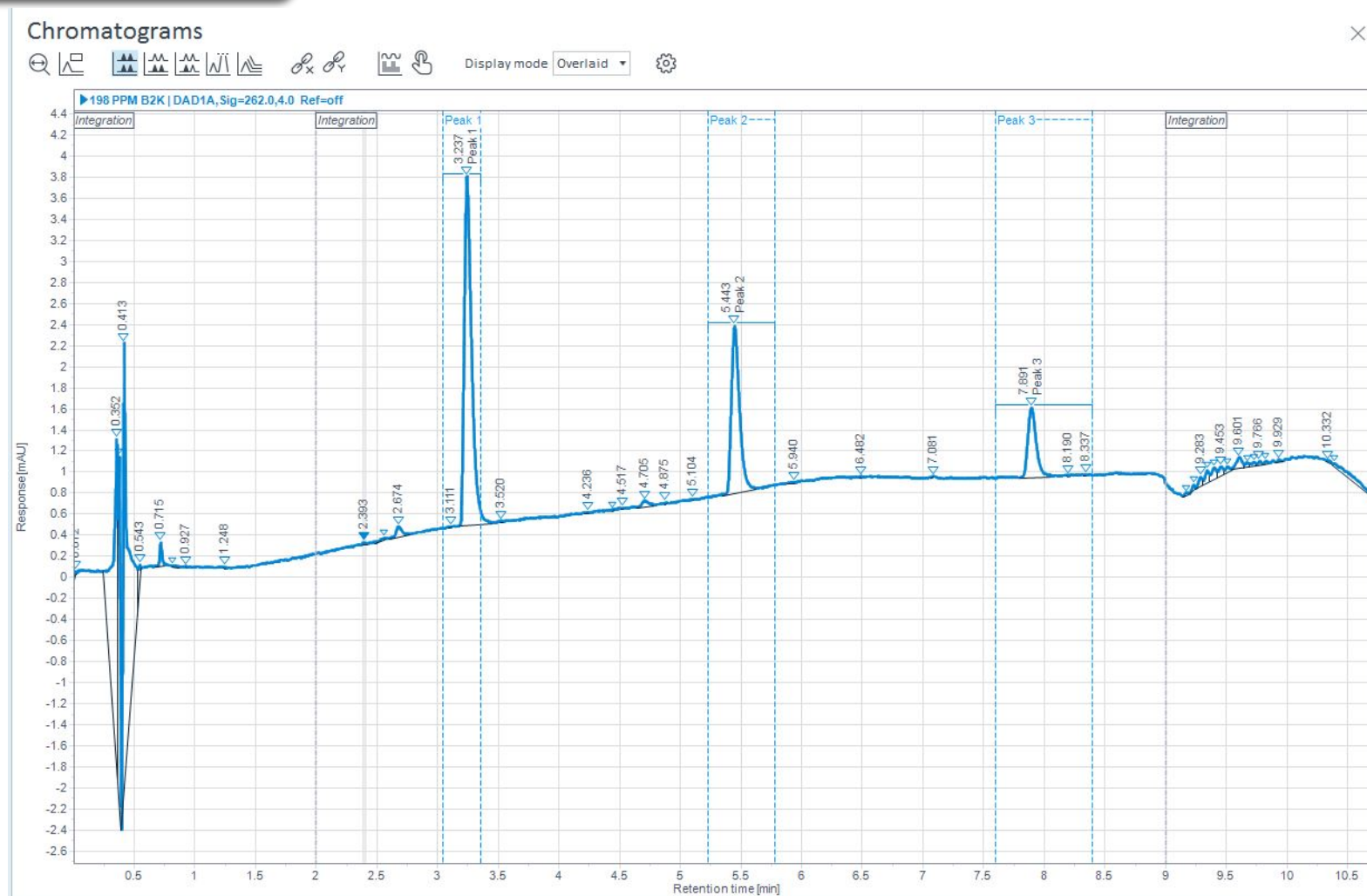
Set peak width according to our smallest peak of interest.



Integration Important Events

Integration “On” / “Off”

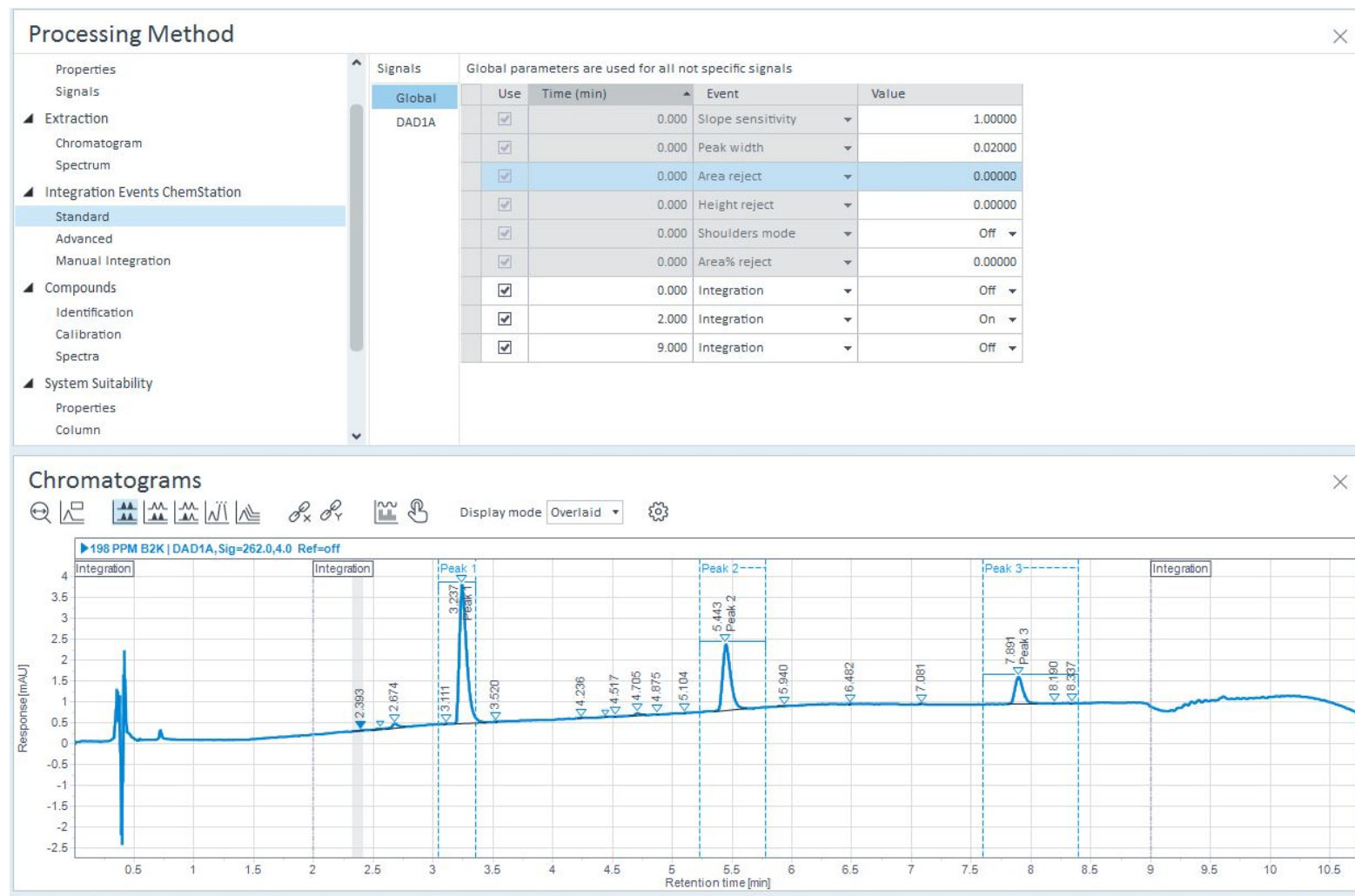
Remove undesirable peaks.
Matrix interferences



Integration Important Events

Integration “On” / “Off”

Remove undesirable peaks.
Matrix interferences



Integration Important Events

Peak Height

Peak Area

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.

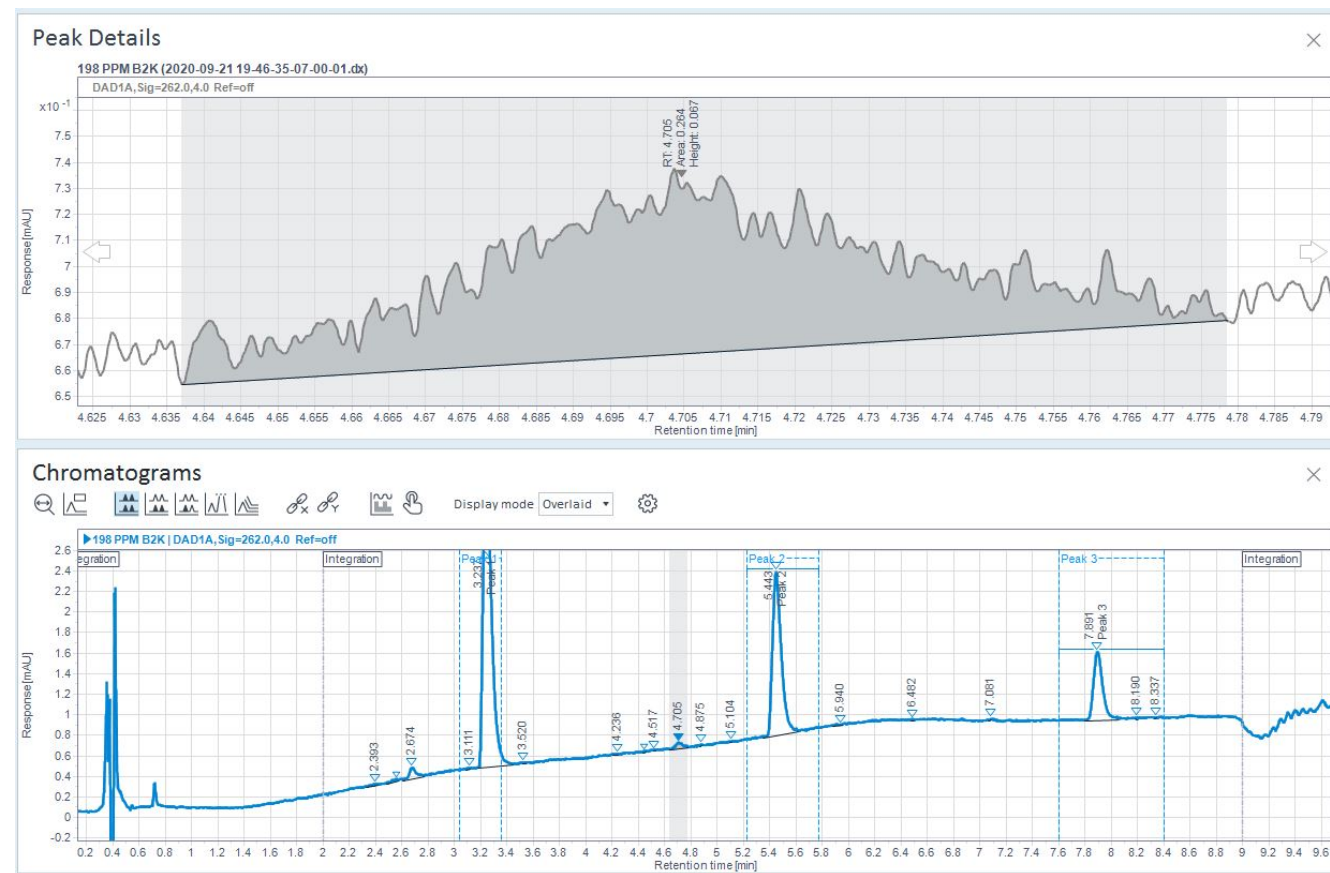
Integration Important Events

Peak Area Reject

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.

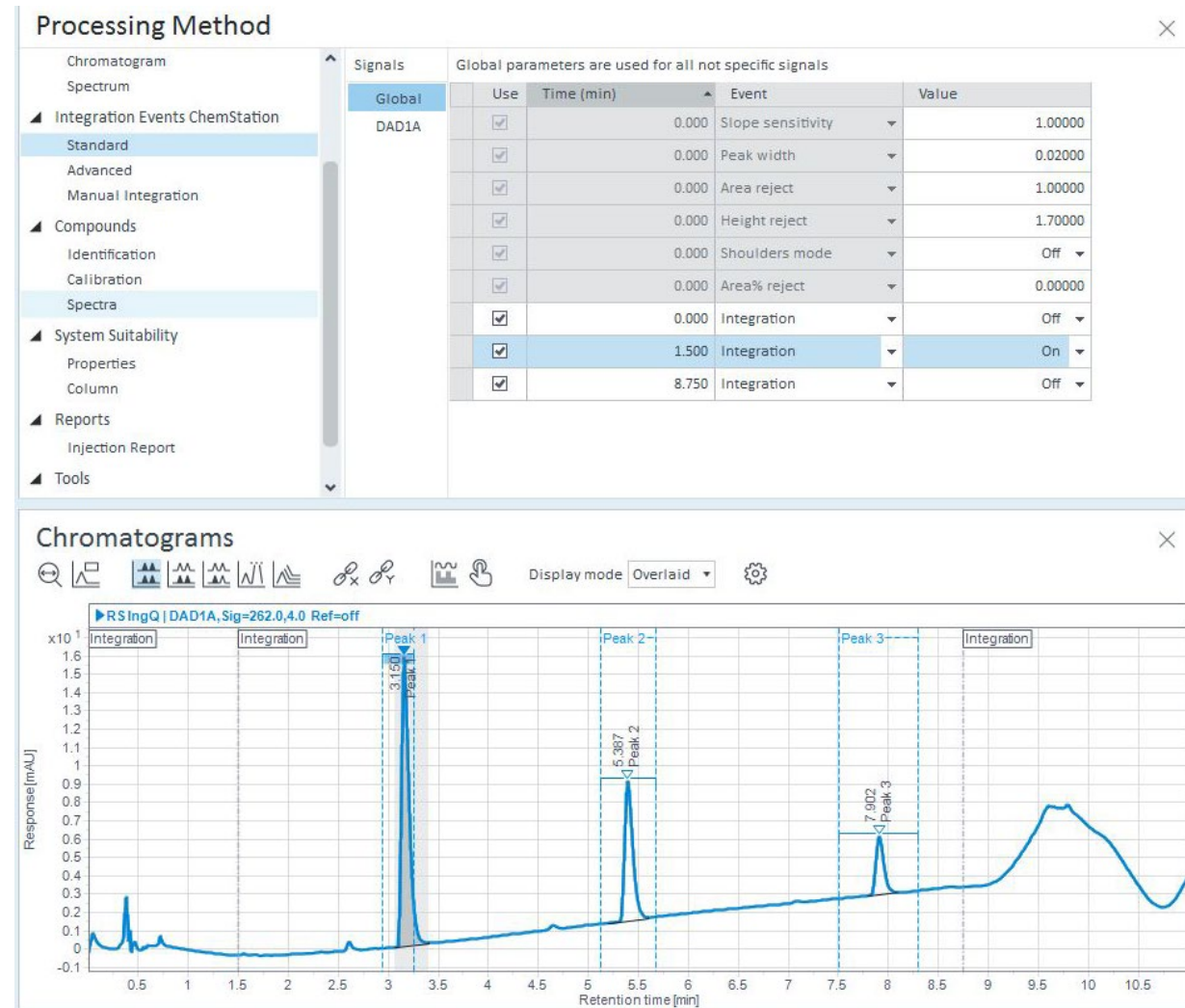


Integration Important Events

Height and Area Determination

Set initial height and area reject to zero

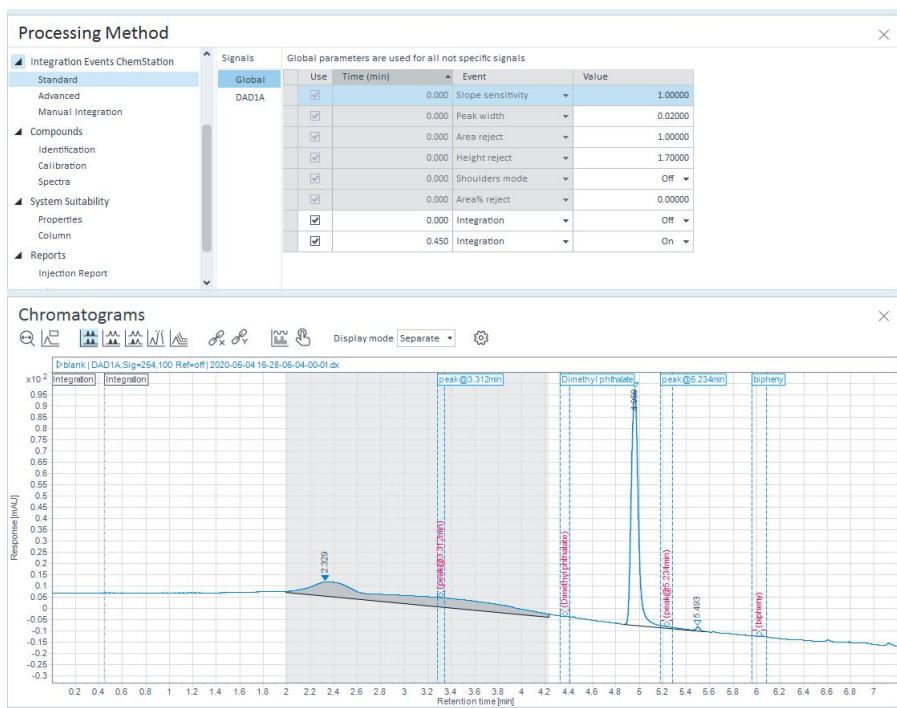
Remove undesired peaks with the height or area reject.



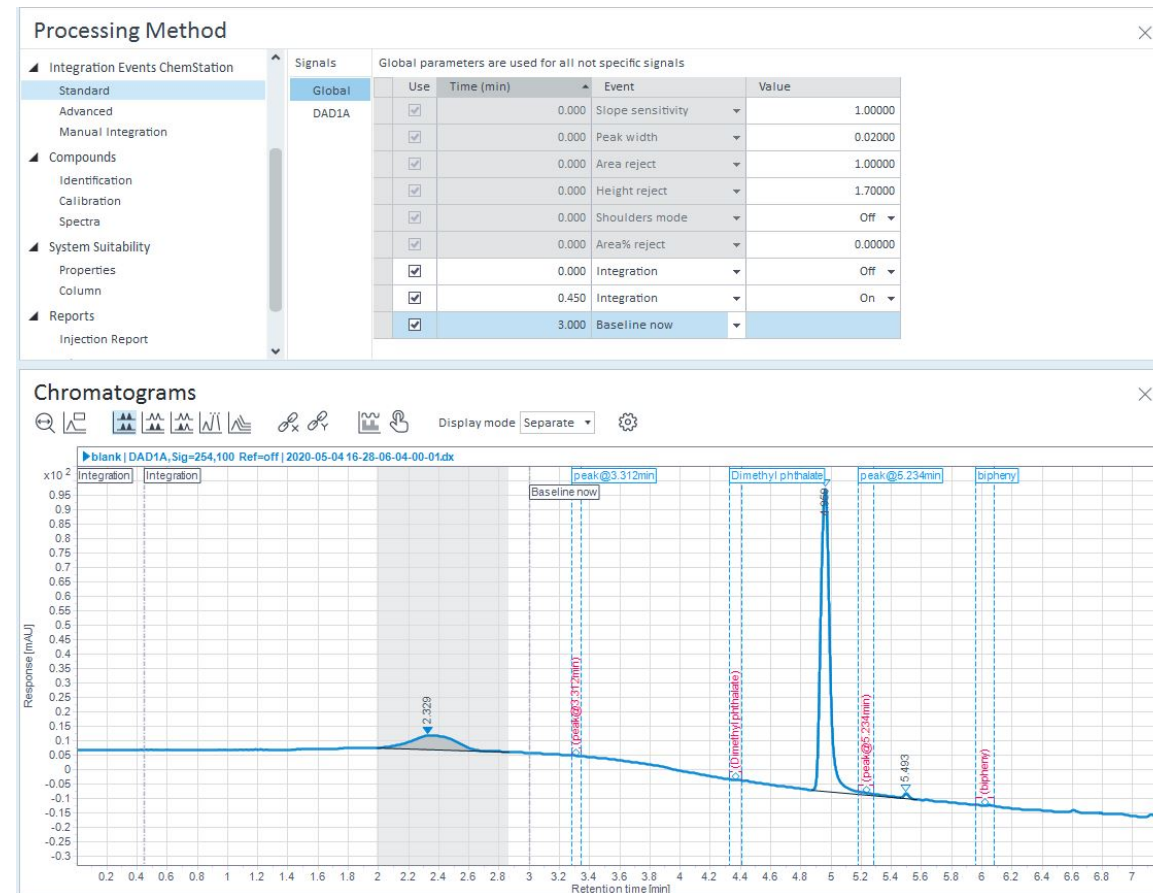
Integration Important Events

Baseline Now

Sets a point (time and signal) at which the integrator resets the baseline to the current data point.



Baseline Now!



Integration Important Events

Manual Integration

Manual integration is performed by the data user when auto integration performed by the software is not consist or not correct.

Why use Manual Integration

Split peaks

Shoulder peaks

Tailing peaks

Baseline noise

Matrix interference

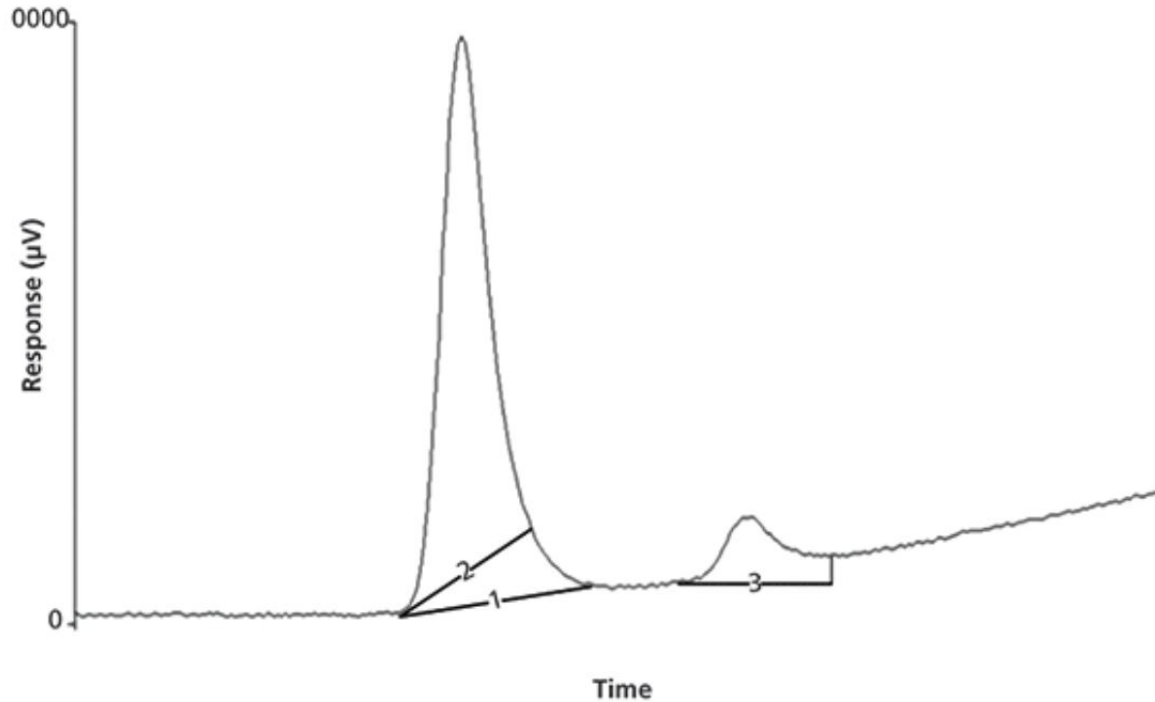
Spikes in the baseline

“Are You Controlling Peak Integration to Ensure Data Integrity?”

June 1, 2020, LCGC North America, volume 38, Issue 6, page number: 346–354

<https://www.chromatographyonline.com/view/are-you-controlling-peak-integration-ensure-data-integrity>

Manual Integration



<http://www.chromatographyonline.com/data-integrity-gxp-chromatography-laboratory-part-iii-integration-and-interpretation-data?pageID=1>

Manual Integration

Regulated Environment –SOP is a Must!

Split peaks

Shoulder peaks

Tailing peaks

Baseline noise

Matrix interference

Spikes in the baseline

Control

- Disallow manual integration at levels
- SOP describes the circumstance where it will be applied.

Manual Integration

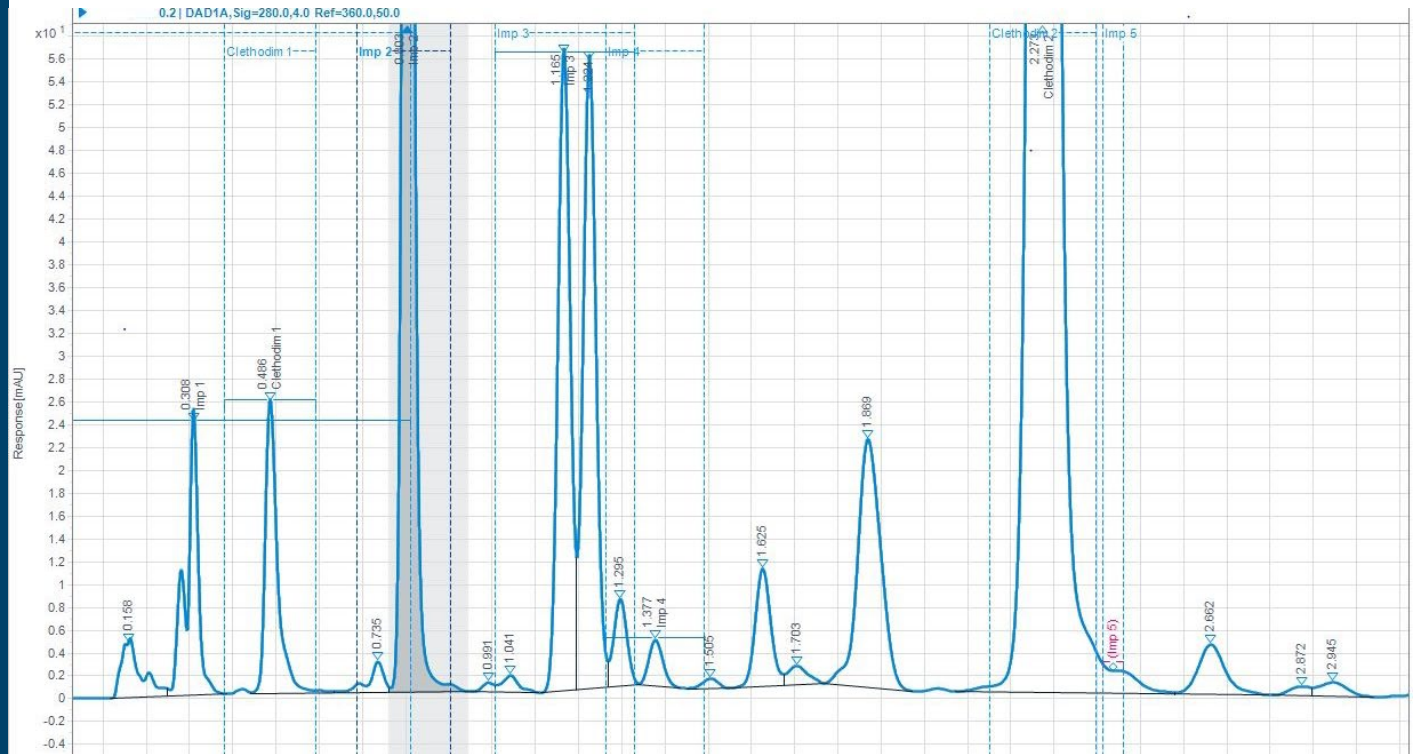
Scientific defensible

Consistent and appropriate integrations

Should not be performed to meet an acceptance criteria

Good idea to document justification for the manual integration.

Integration Challenges

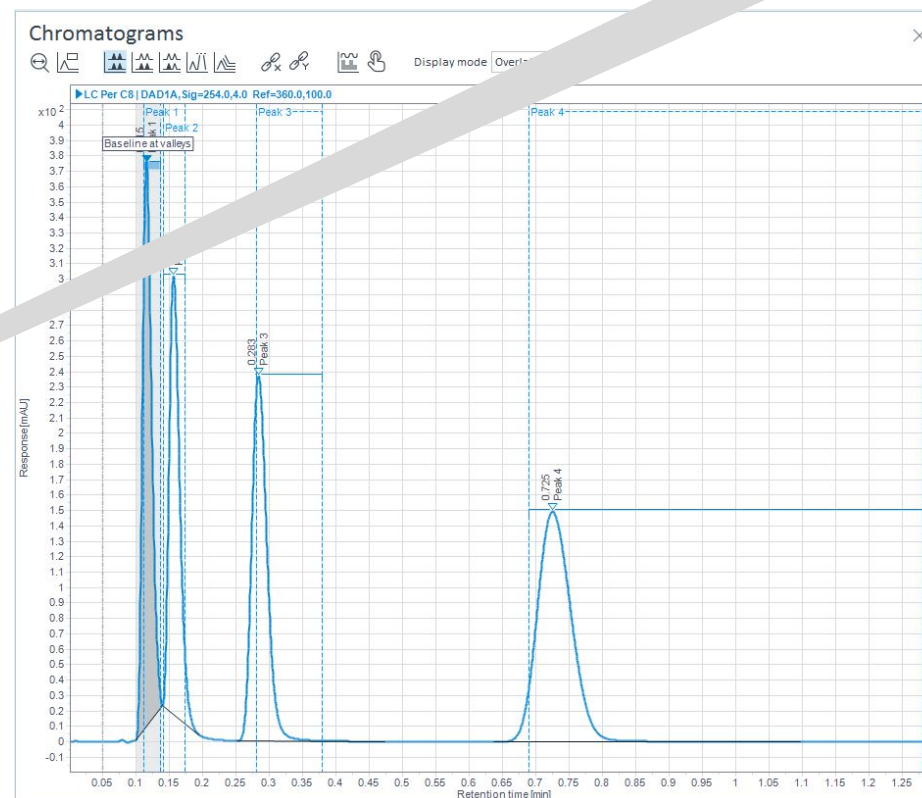
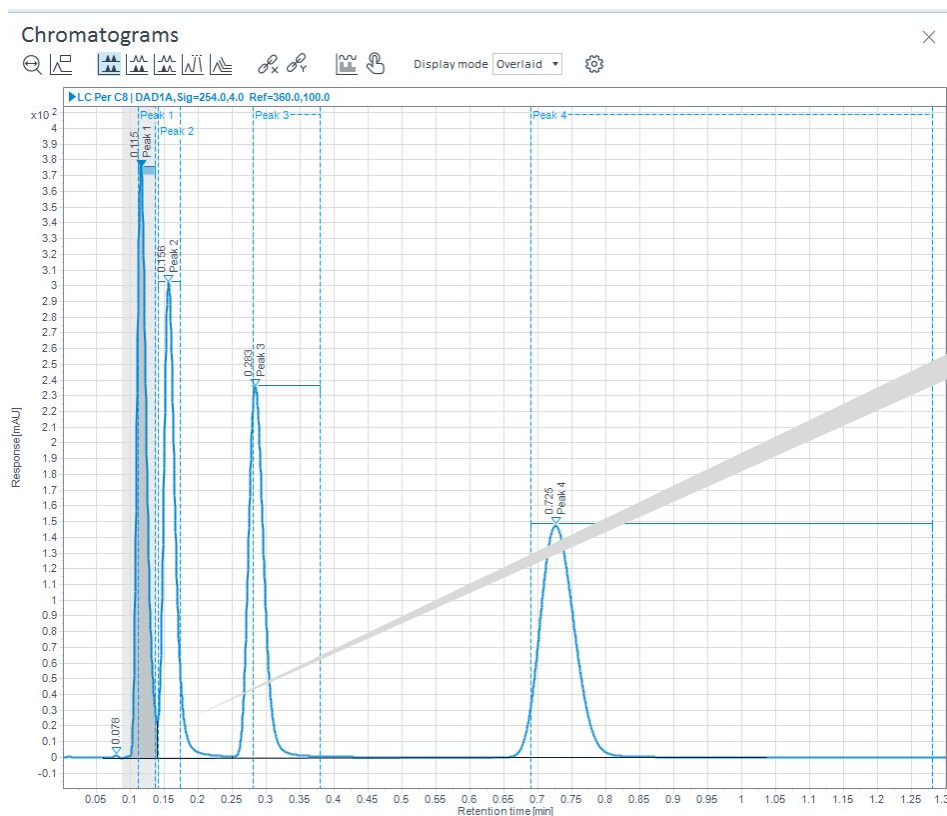


Integration Challenges

Drop Vertical (BV) Versus Valley to Base (VB)

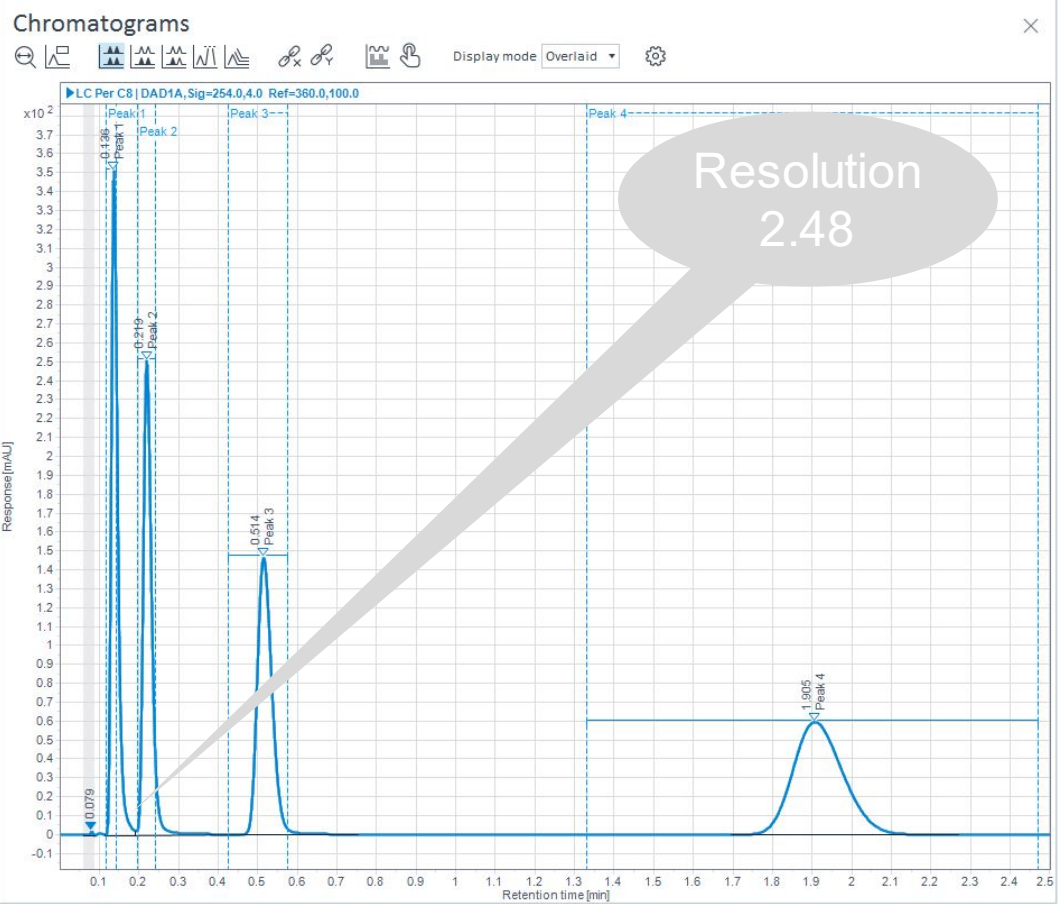
When the difference in peak size becomes larger, the errors for the smaller peak increase.

Resolution
1.48



Integration Challenges

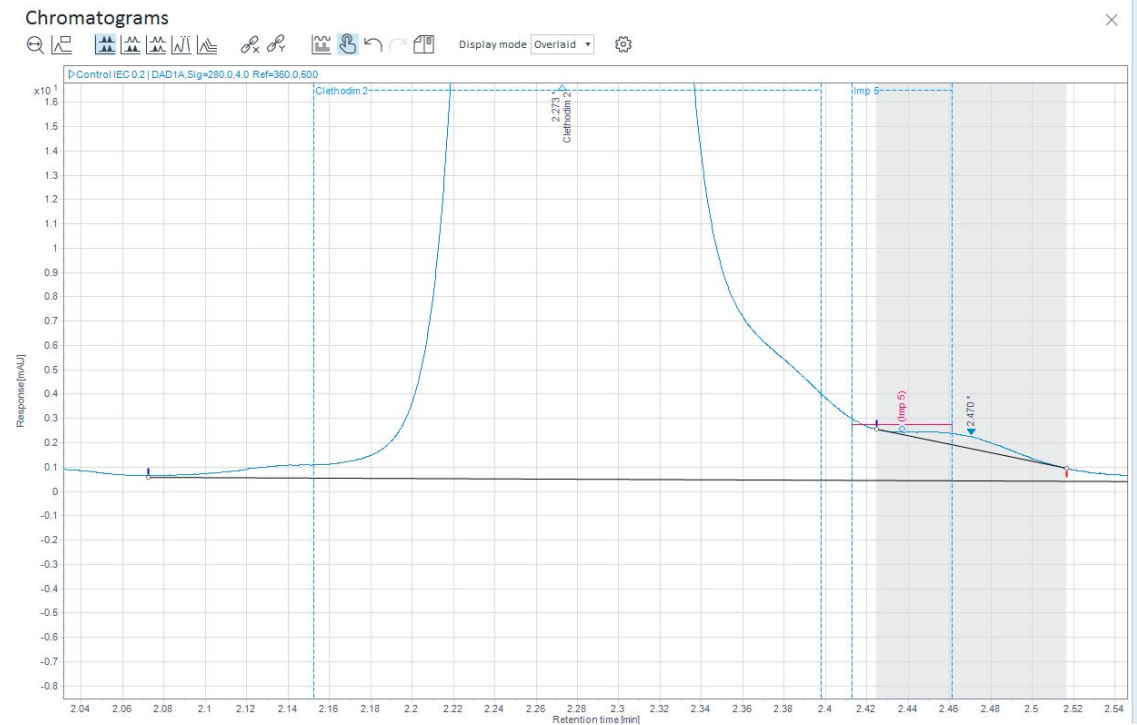
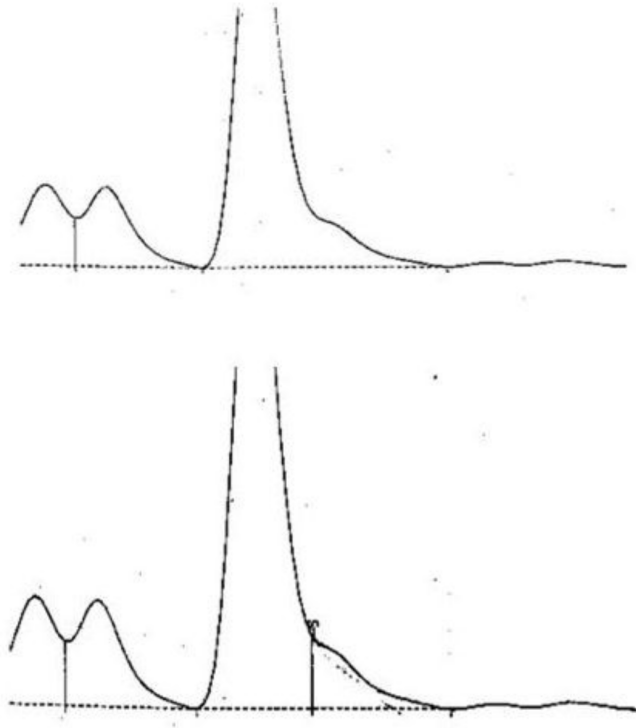
Drop Vertical Versus Valley to Base



Integ. Code	Peak 1		Peak 2		% Error	
	RT	Area	RT	Area	Peak 1	Peak 2
BV	0.115	381.1122	0.156	364.428	94.3	97.4
VB	0.115	351.3416	0.156	314.4827	86.9	84.0
BB	0.136	404.1422	0.218	374.1953		

Integration Challenges

Impurity is co-eluting of the tail of a parent peak

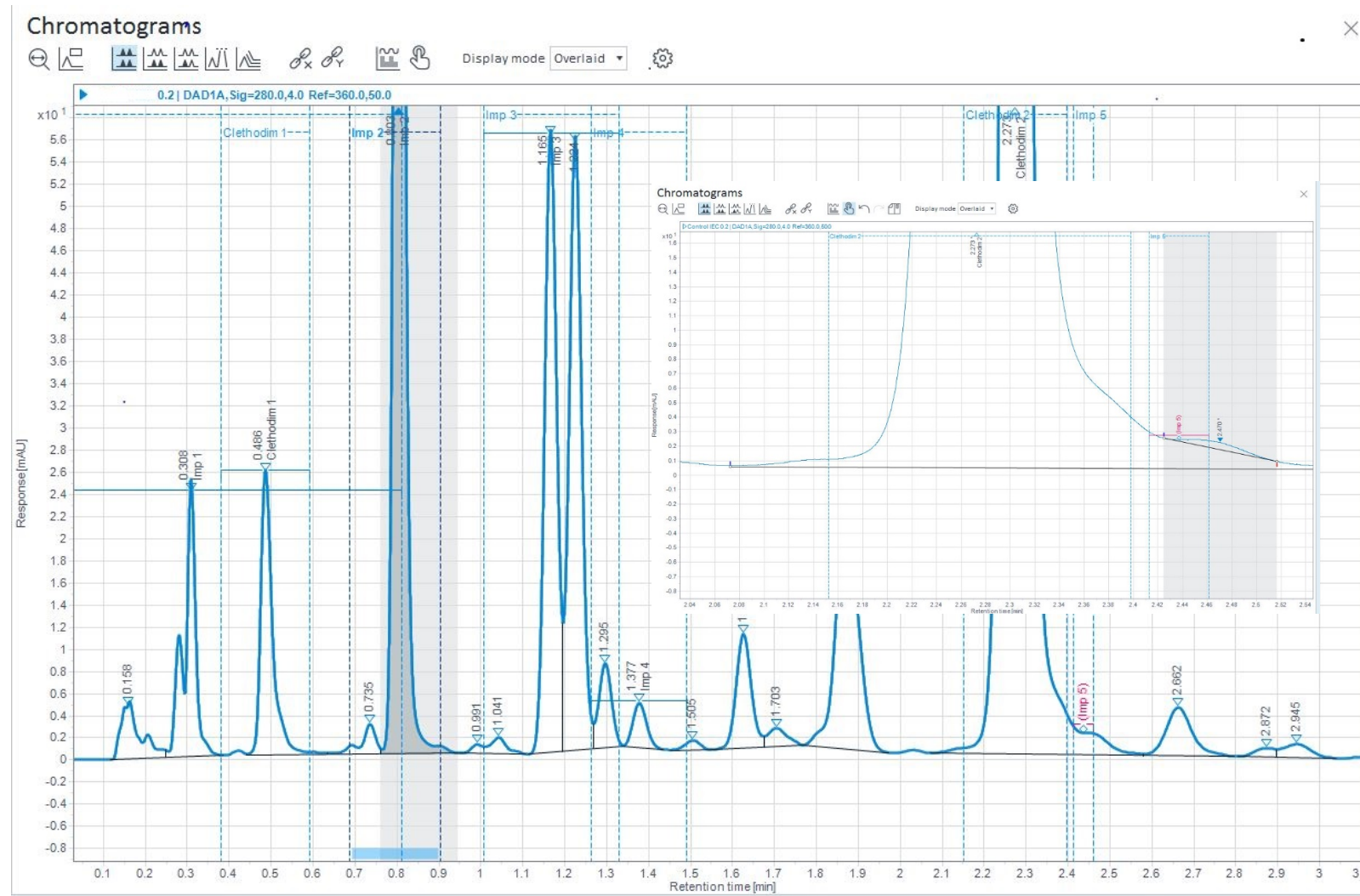


Integration Challenges

What % error
Complete separation

...the drop and Gaussian skim
methods produce the least error
in all situations.

<https://www.chromatographyonline.com/view/integration-errors-chromatographic-analysis-part-i-peaks-approximately-equal-size>

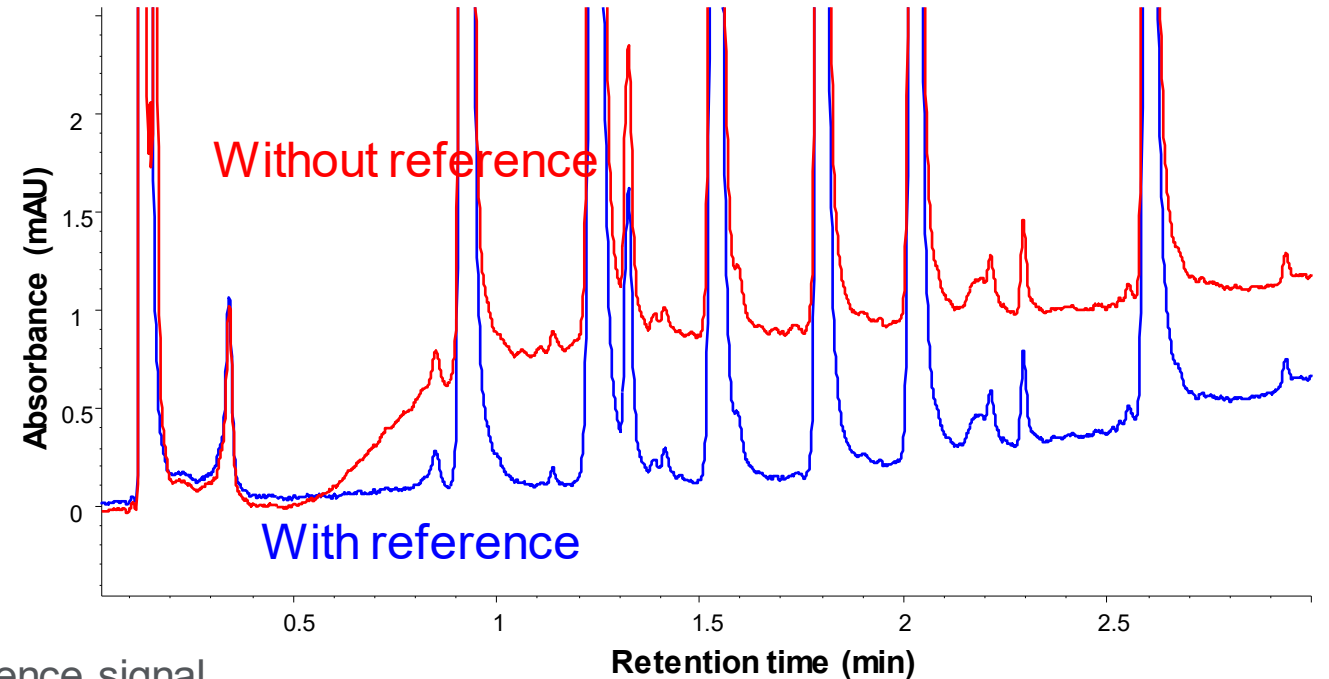
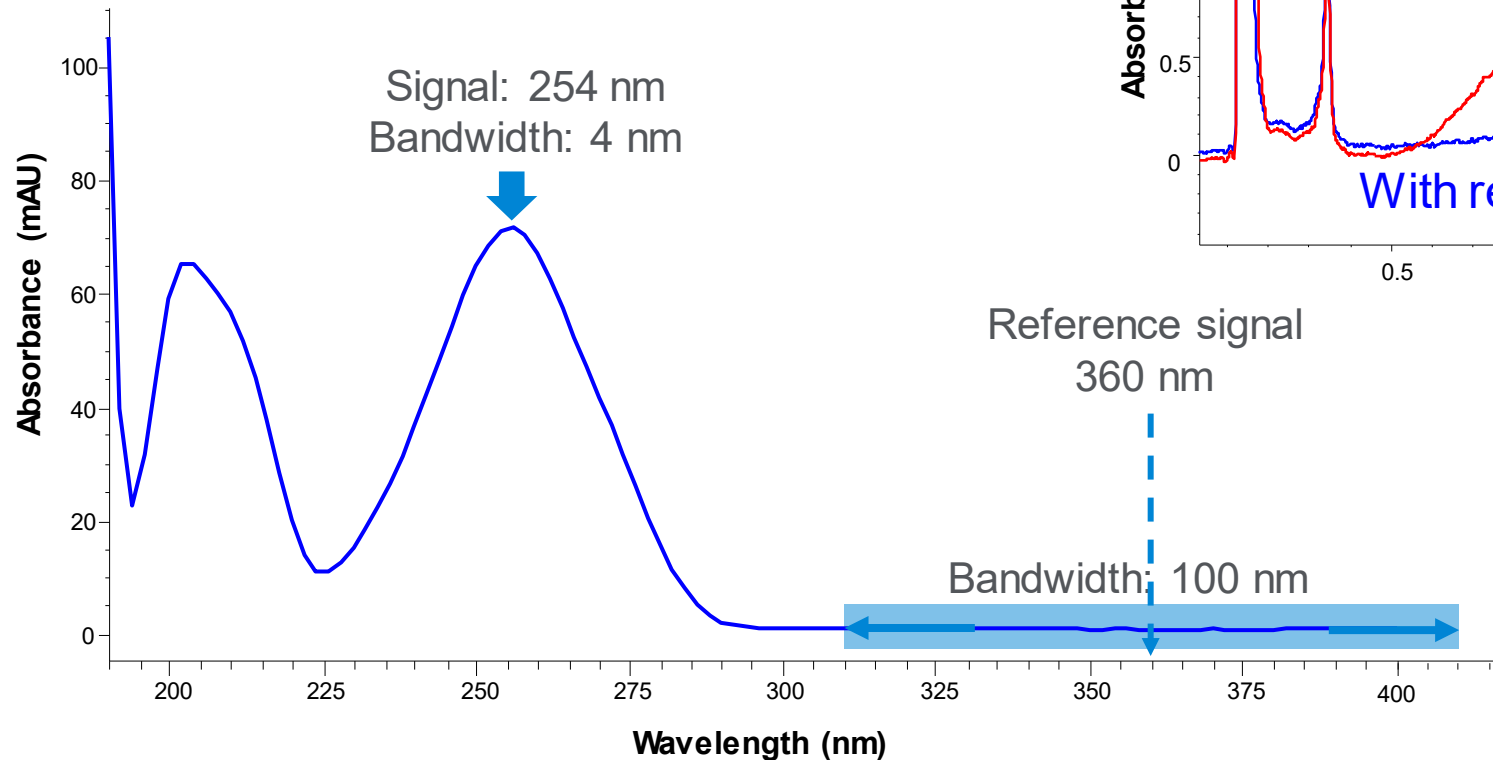


- **UV setting affecting integration**



DAD Setting — Choose the right signal and reference

Signals					
	Acquire	Wavelength	Bandwidth	Reference Wavelength	Reference Bandwidth
Signal A	☒	254.0	4.0	☒	360.0
Signal B	☒	254.0	4.0	☐	100.0

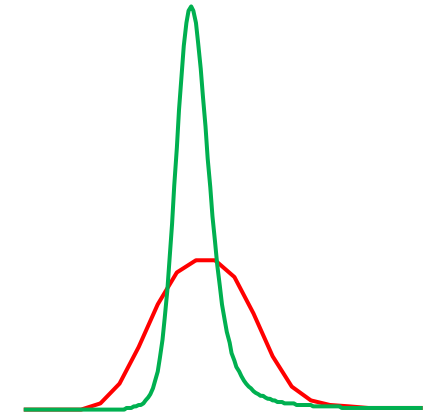
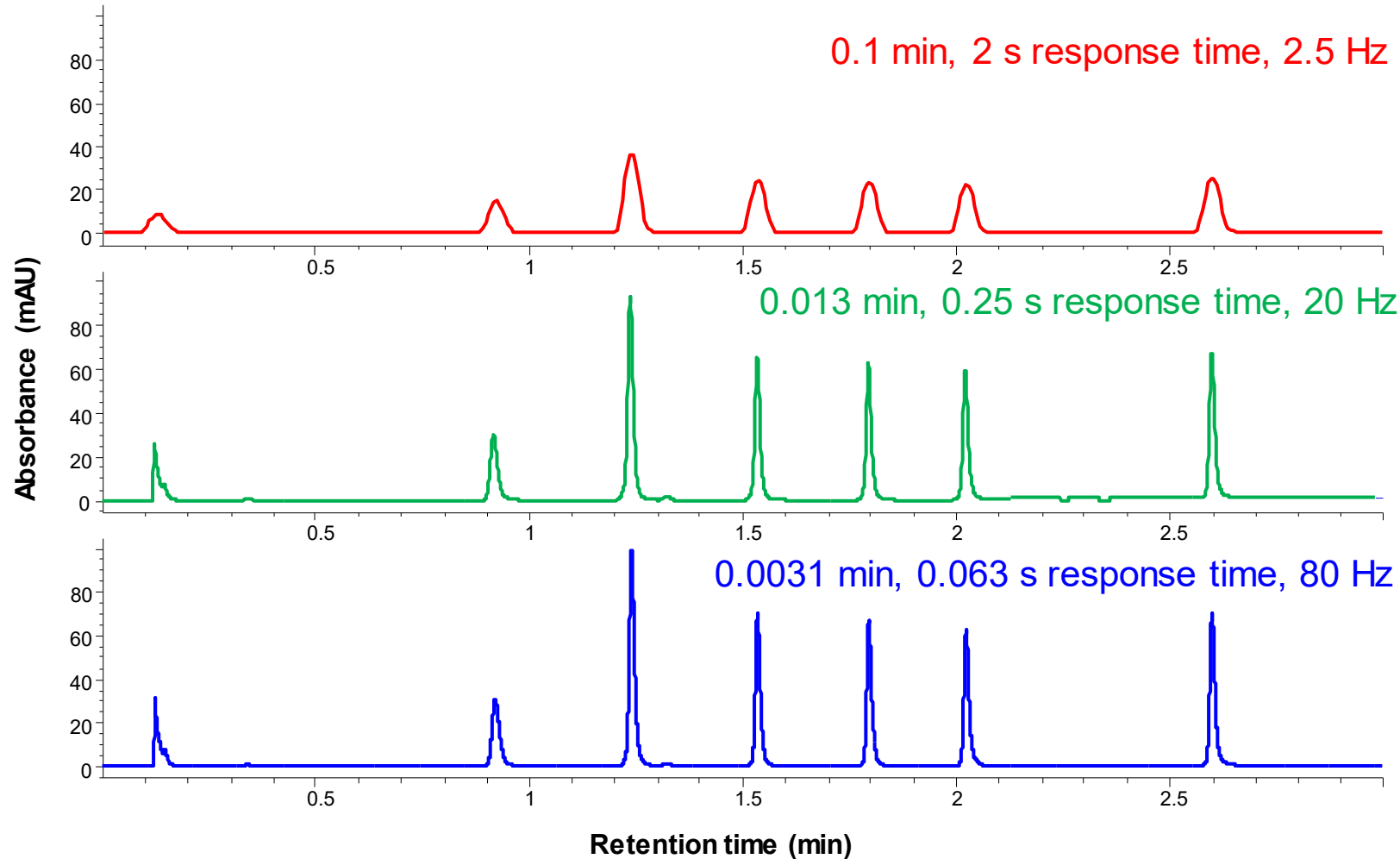


Reference signal
360 nm

Bandwidth: 100 nm

Gradient: 10-100% ACN in 3 min

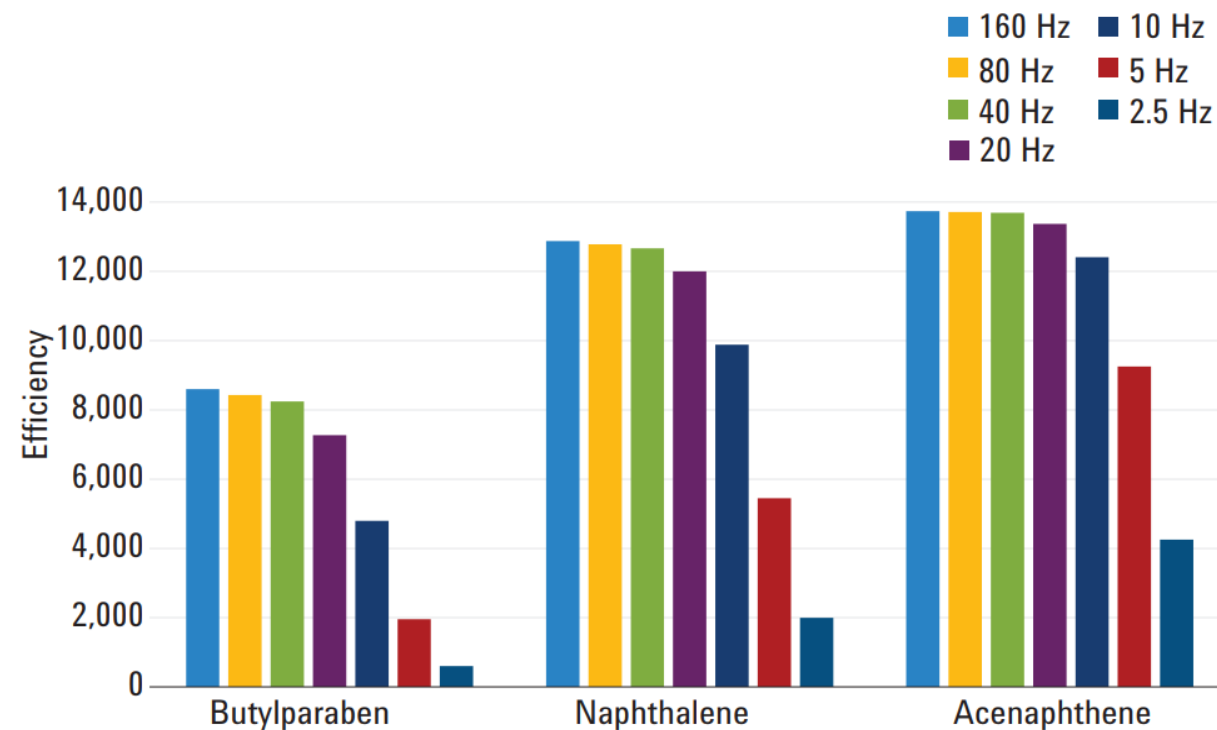
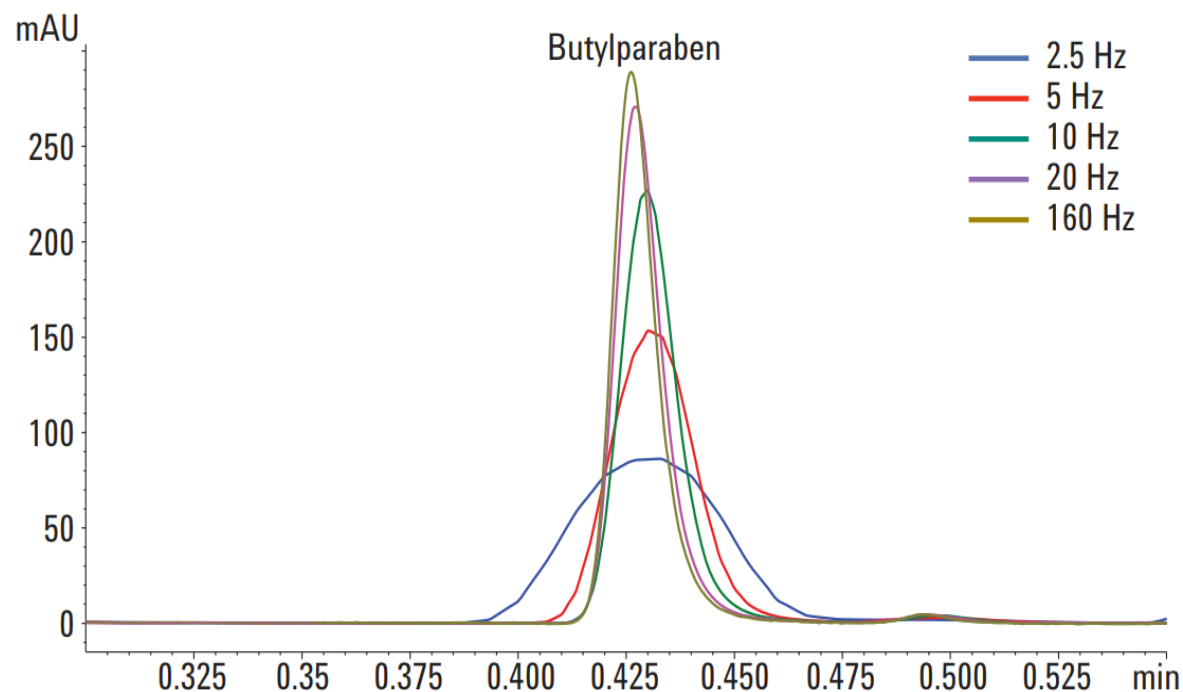
DAD Setting — Choose the right sampling rate



Changes in **Peak Width**
and **Resolution**

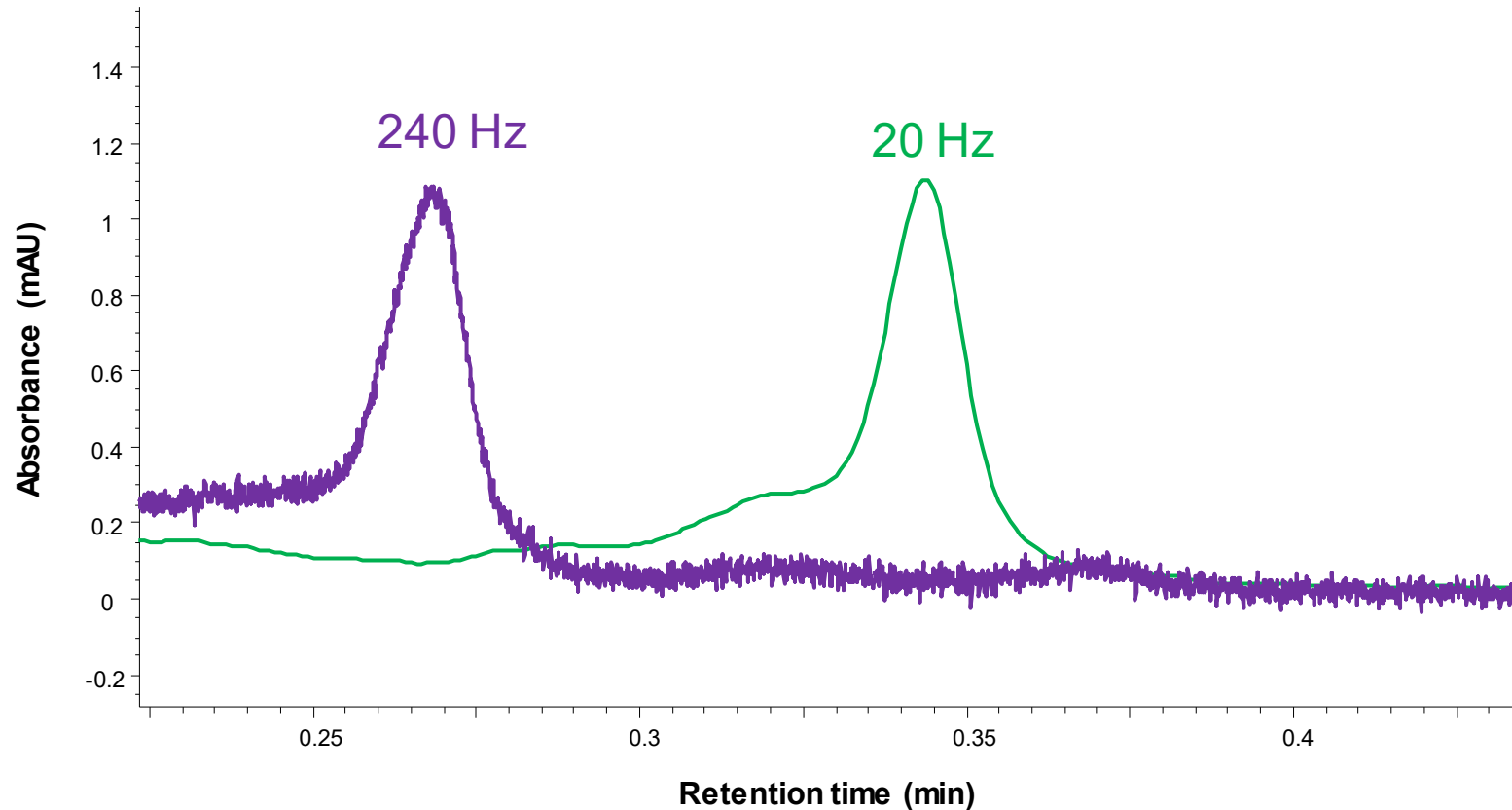
Column: ZORBOX Eclipse Plus C18, 2.1x50 mm, 1.8 μ m
Column temperature: 35 $^{\circ}$ C; Flow rate: 1 mL/min
Gradient: 10-100% ACN in 3 min
Signal: 254 nm, Bandwidth: 4 nm
Reference: 360 nm, Bandwidth: 100 nm

Data Rates May Impact Observed Resolution



Fast data collection rates must be used with Agilent InfinityLab Poroshell 1.9 μ m columns to accurately measure the efficiency of the column, especially for early eluting compounds such as butylparaben ($k' = 1.3$).

DAD Setting — Choose the right sampling rate



**Do Not Use Peakwidth
Smaller Than Necessary**

Peakwidth

Stop time

☒ As P ☐ min

> 0.013 min (0.25 s response time) (20 Hz)	▼
< 0.0008 min (0.008 s response time) (240 Hz)	
> 0.0008 min (0.016 s response time) (240 Hz)	
> 0.0016 min (0.031 s response time) (160 Hz)	
> 0.0031 min (0.063 s response time) (80 Hz)	
> 0.0063 min (0.13 s response time) (40 Hz)	
> 0.013 min (0.25 s response time) (20 Hz)	
> 0.025 min (0.5 s response time) (10 Hz)	
> 0.05 min (1 s response time) (5 Hz)	
> 0.1 min (2 s response time) (2.5 Hz)	
> 0.2 min (4 s response time) (1.25 Hz)	
> 0.4 min (8 s response time) (0.62 Hz)	
> 0.85 min (16 s response time) (0.31 Hz)	

Column: ZORBOX Eclipse Plus C18, 2.1x50 mm, 1.8 μ m
Column temperature: 35 $^{\circ}$ C; Flow rate: 1 mL/min
Gradient: 10-100% ACN in 3 min
Signal: 254 nm, Bandwidth: 4 nm
Reference: 360 nm, Bandwidth: 100 nm

Conclusion

- Integrate and view the results.
- If all peaks of interest were not integrated, lower the slope sensitivity until all real peaks are integrated.
- If there are still peaks that cannot be integrated, lower the peak width setting
- Use timed events if necessary.
- Remove undesired peaks with the height or area reject.
- Use manual Integration

Conclusion

Integration in a Regulated Environment

Robust Chromatographic Integration SOP

The contents of an SOP on integration should include the following items:

1. Definitions: automatic integration, manual intervention, manual integration, analysis, method, test, sample, inhibit, and processing method.
2. These are among many terms that must be defined for clarity in interpretation among personnel. This need becomes more critical as the number of users and laboratory sites is increased.

Resources — Primers

[5990-7595EN](#)

The LC Handbook

Guide to LC Columns and Method Development

[5991-2359EN](#)

Two Dimensional Liquid Chromatography

[5990-3777EN](#)

High Performance Capillary Electrophoresis

[5991-5509EN](#)

Supercritical Fluid Chromatography

[5989-6639EN](#)

Principles in Preparative HPLC

[5991-3326EN](#)

Sample Preparation Fundamentals for Chromatography

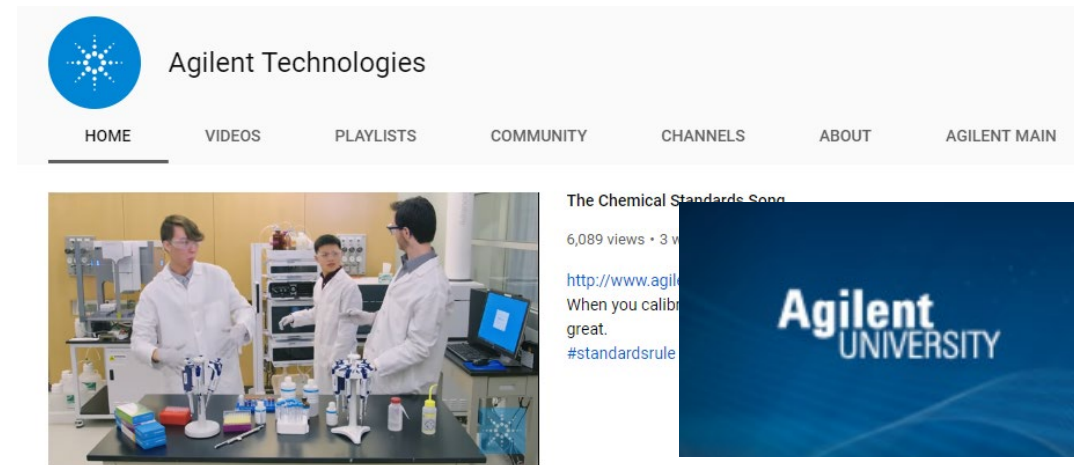
[5980-1397EN](#)

Fundamentals of UV-visible Spectroscopy



Resources for Support

- Collection of LC resources:
https://community.agilent.com/docs/DOC-1852-lc-insights-to-go#jive_content_id_LC_Troubleshooting
- Agilent support resources:
<https://community.agilent.com/community/resources>
- Agilent University: <http://www.agilent.com/crosslab/university>
- Agilent resource center:
<http://www.agilent.com/chem/agilentresources>
- InfinityLab Supplies Catalog ([5991-8031EN](#))
- Your local FSE and Specialists
- Youtube – [Agilent Channel](#)
- Sales and support phone assistance (US and Canada):
1-800-227-9770 [Phone Tree Navigation Assistance](#)



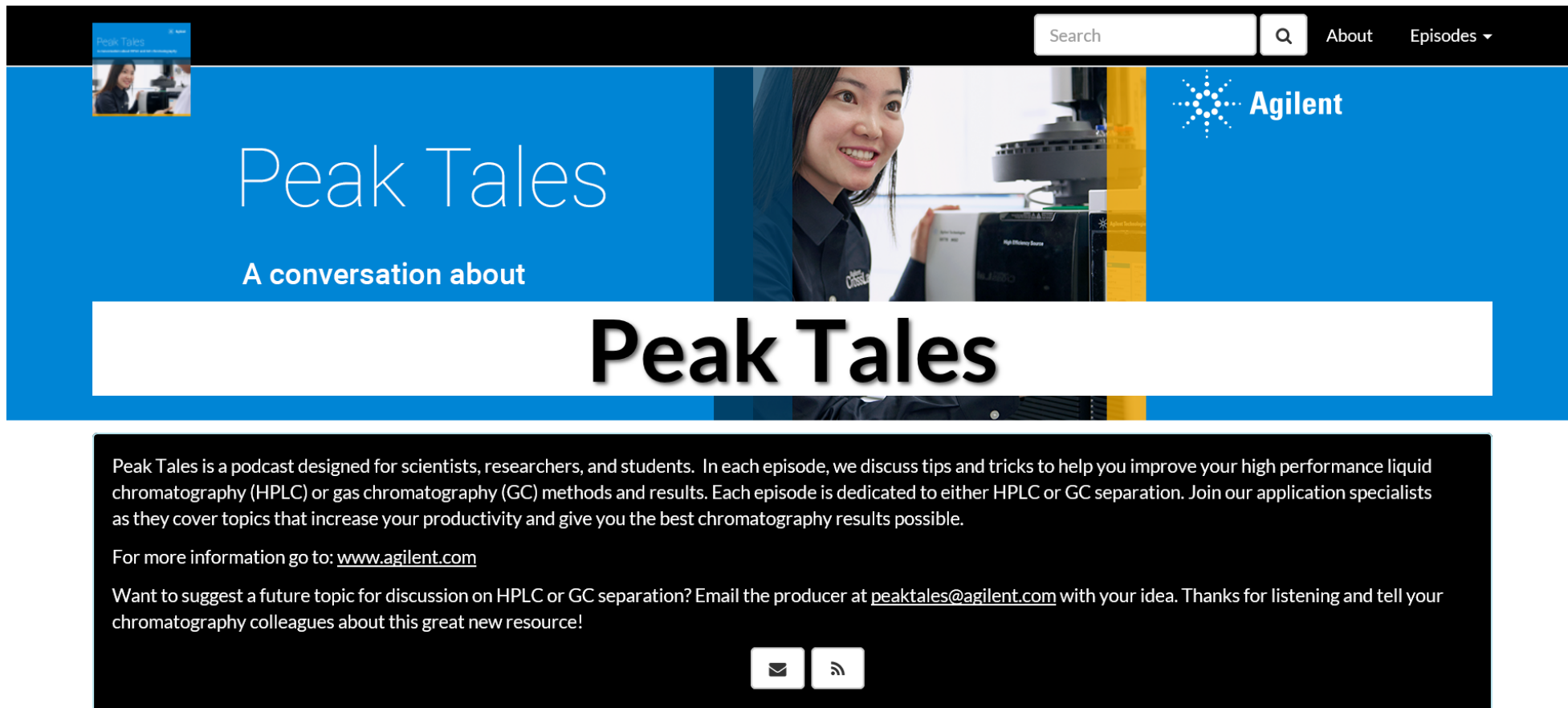
gc-column-support@agilent.com
lc-column-support@agilent.com
spp-support@agilent.com
spectro-supplies-support@agilent.com



Resources for Support

- Collection of LC resources: https://community.agilent.com/docs/DOC-1852-lc-insights-to-go#jive_content_id_LC_Troubleshooting
- Agilent support resources: <https://community.agilent.com/community/resources>
- Agilent University: <http://www.agilent.com/crosslab/university>
- Agilent resource center: <http://www.agilent.com/chem/agilentresources>
- InfinityLab Supplies Catalog ([5991-8031EN](#))
- Your local FSE and Specialists
- <http://www.chromatographyonline.com/integration-errors-chromatographic-analysis-part-i-peaks-approximately-equal-size?id=&sk=&date=&pageID=7>
- <https://www.chromacademy.com/chromatography-Integration-Parameters-1.html>
- <https://www.accta.com/integrate.html>
- [Integration Errors in Chromatographic Analysis, Part I: Peaks of Approximately Equal Size](#)
- [Apr 01, 2006, Merlin K.L. Bickin, LCGC North America, Volume 24, Issue 4, pg 402–414](#)

For more troubleshooting trick and information
check out Agilent Peak Tales



The screenshot shows the top section of the Agilent Peak Tales website. At the top is a black navigation bar with a search box, a magnifying glass icon, and links for 'About' and 'Episodes'. Below this is a blue banner. On the left, the text 'Peak Tales' is written in large white font, with 'A conversation about' in smaller white font below it. In the center, there is a photo of a smiling woman in a lab coat standing next to a piece of laboratory equipment. On the right, the Agilent logo is displayed. Below the banner is a white box containing the text 'Peak Tales' in large black font. At the bottom of the page, there is a black box with white text providing information about the podcast, a link to the website, and contact information for suggestions. At the very bottom of this black box are icons for email and RSS feed.

Peak Tales

A conversation about

Peak Tales

Peak Tales is a podcast designed for scientists, researchers, and students. In each episode, we discuss tips and tricks to help you improve your high performance liquid chromatography (HPLC) or gas chromatography (GC) methods and results. Each episode is dedicated to either HPLC or GC separation. Join our application specialists as they cover topics that increase your productivity and give you the best chromatography results possible.

For more information go to: www.agilent.com

Want to suggest a future topic for discussion on HPLC or GC separation? Email the producer at peaktales@agilent.com with your idea. Thanks for listening and tell your chromatography colleagues about this great new resource!

Thanks for your attention!

*Thanks for attending today.
Greg & Sue*

