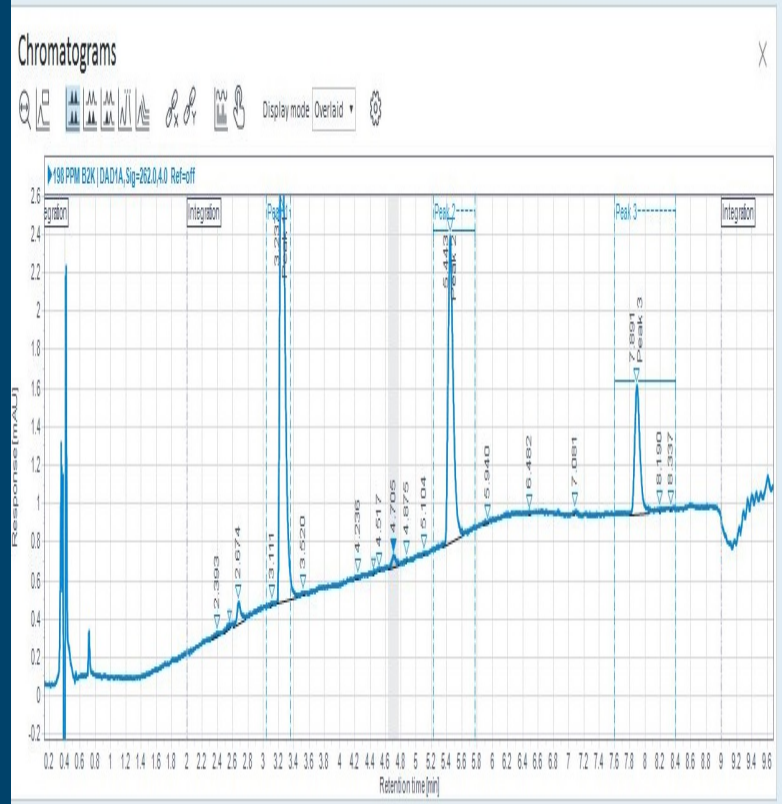
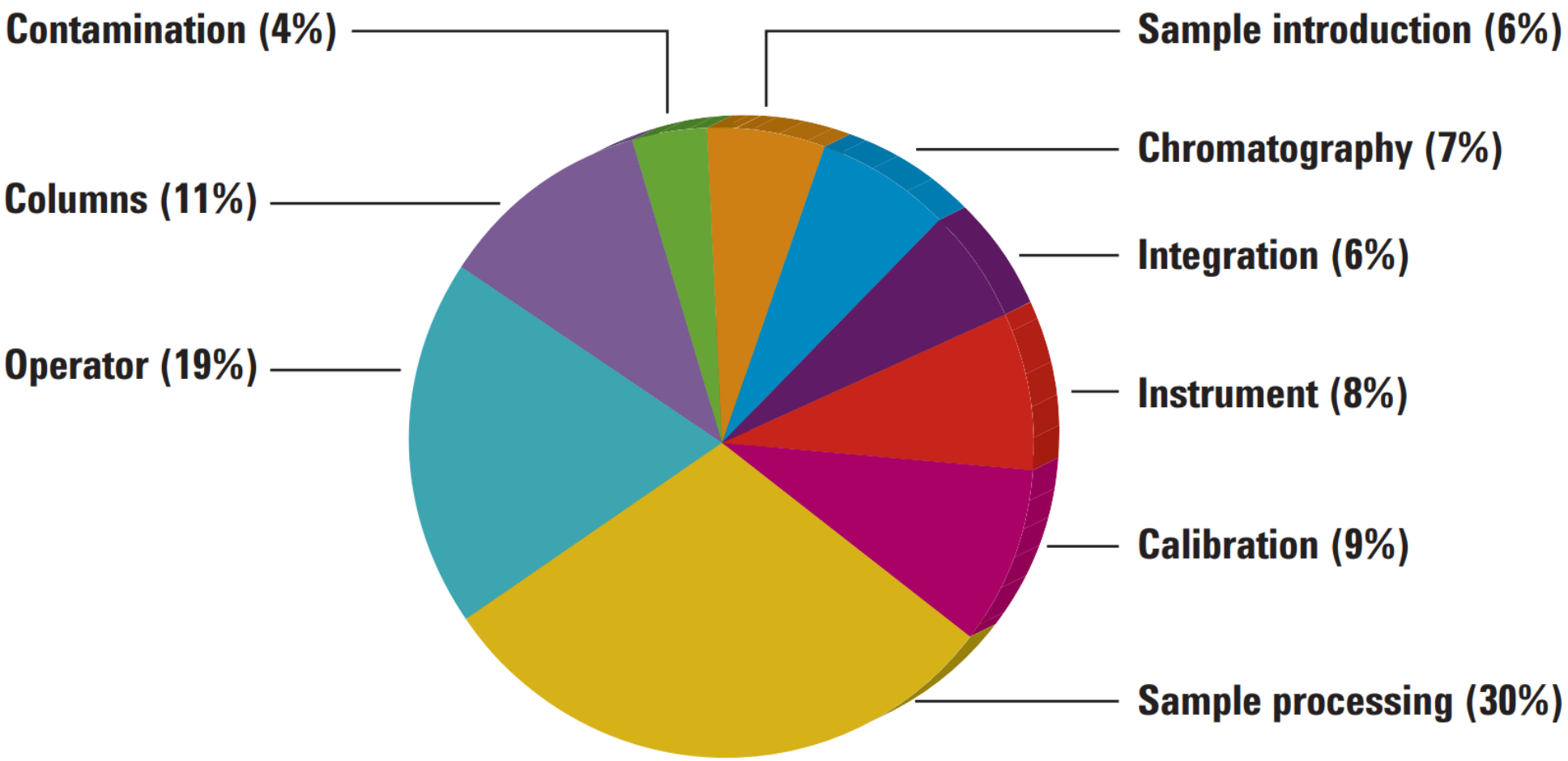
Integrate with Confidence

Gregory Hunlen
Applications Scientist, LC and LC/MS

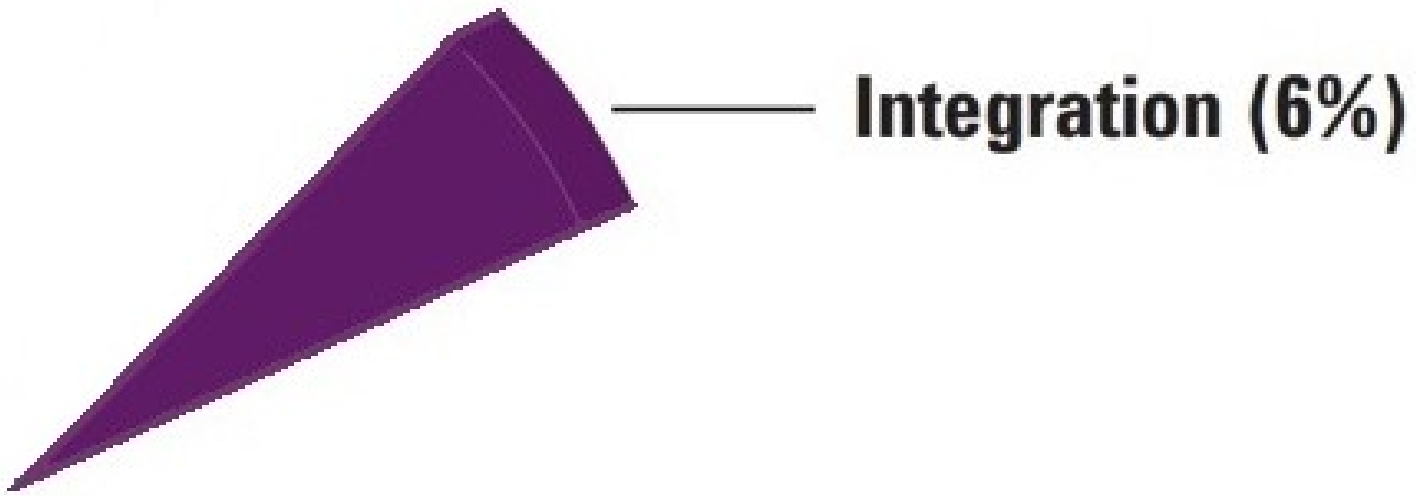


Sources of Error Generated During Chromatographic Analysis



Data taken from Agilent Technologies survey

Sources of Error Generated During Chromatographic Analysis



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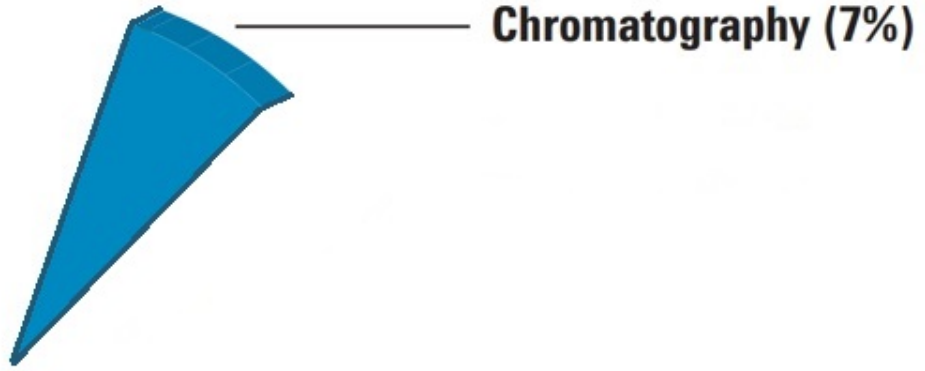
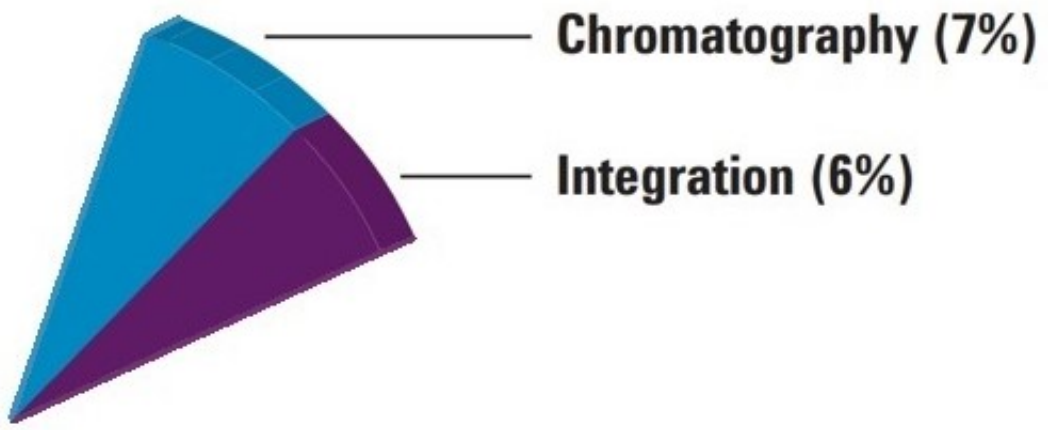
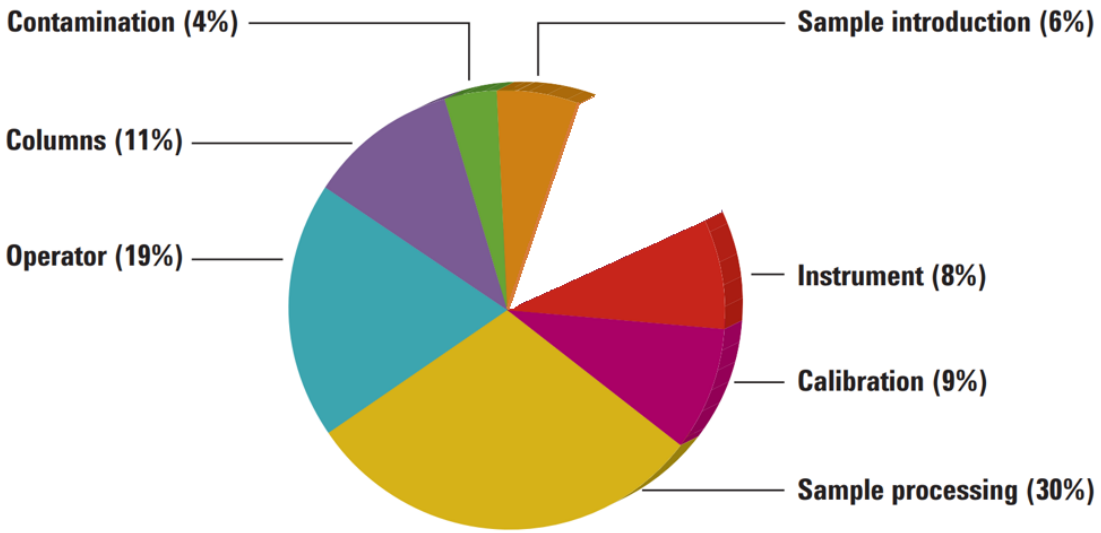


Data taken from Agilent Technologies survey

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.

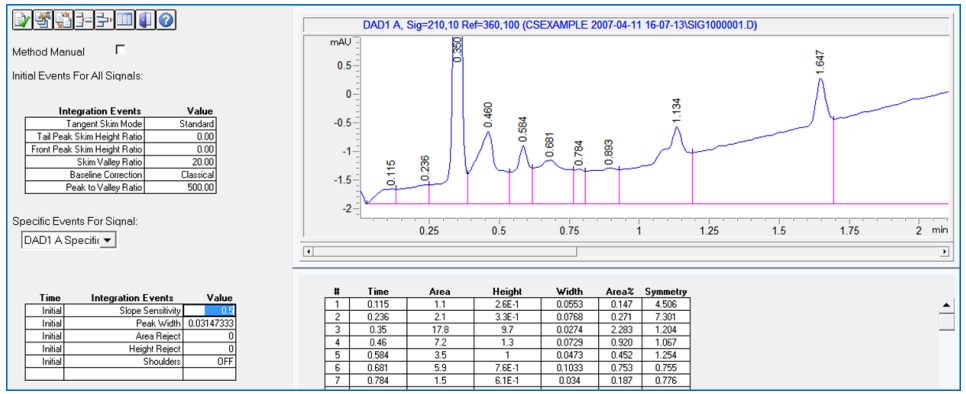
Works in tandem with peak width.

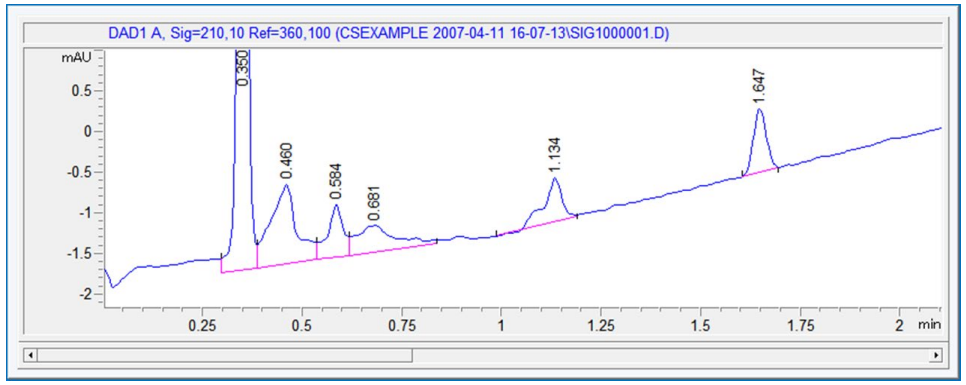
Integration Important Events

Slope Sensitivity

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

Slope sensitivity to low



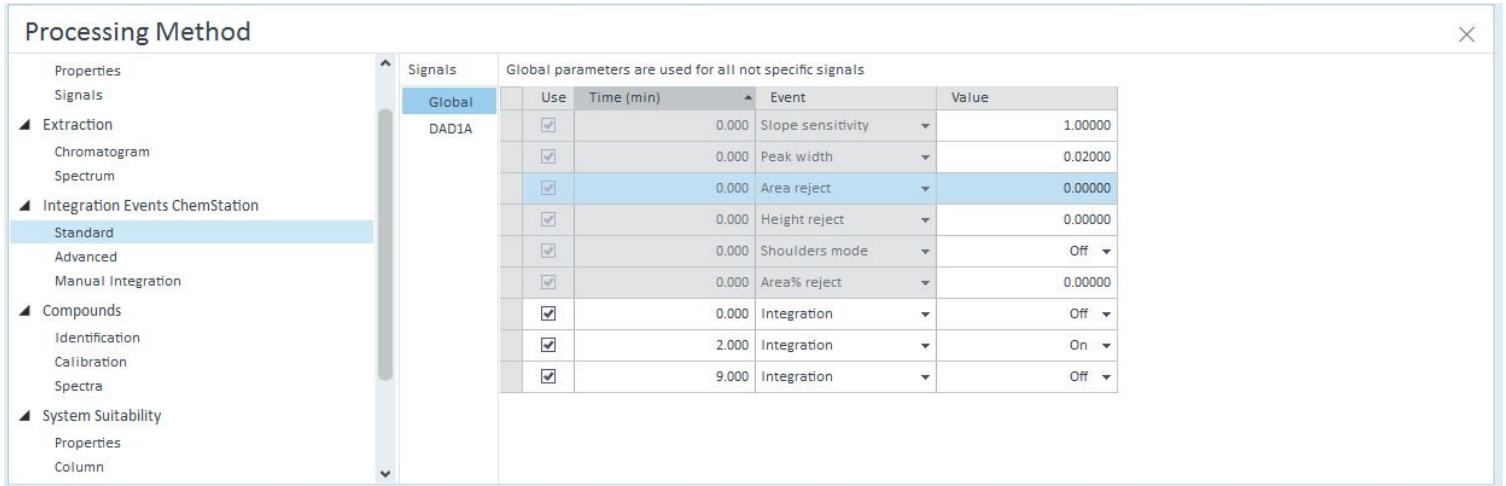


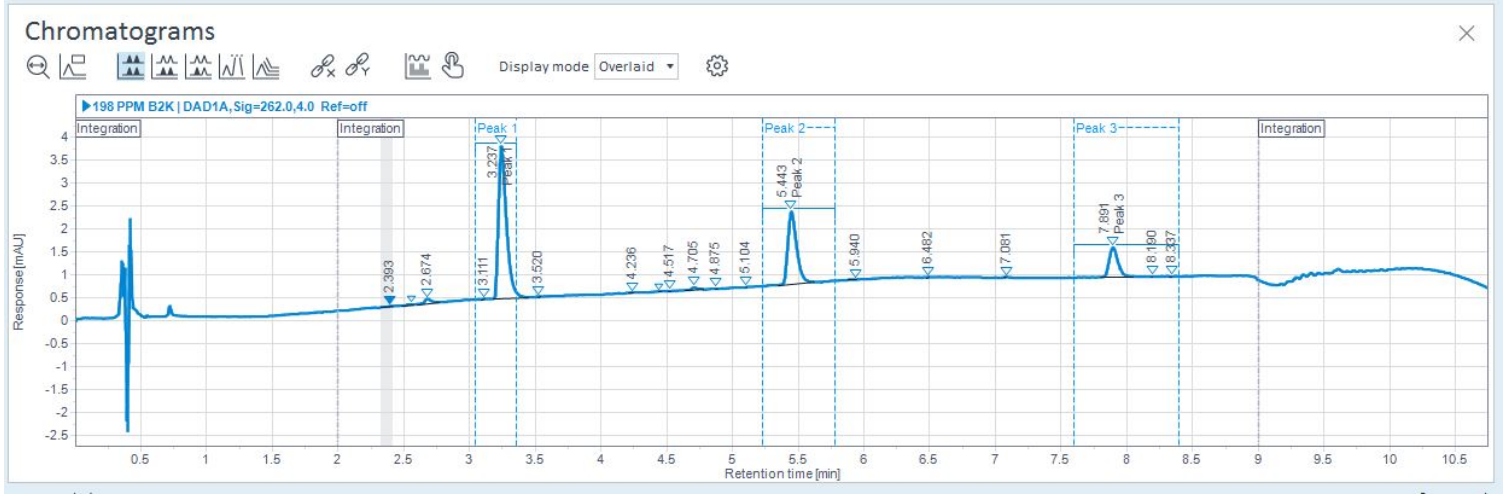
Integration Important Event

Peak Width

View for OpenLab 2.5

Set peak width according to our smallest peak of interest.



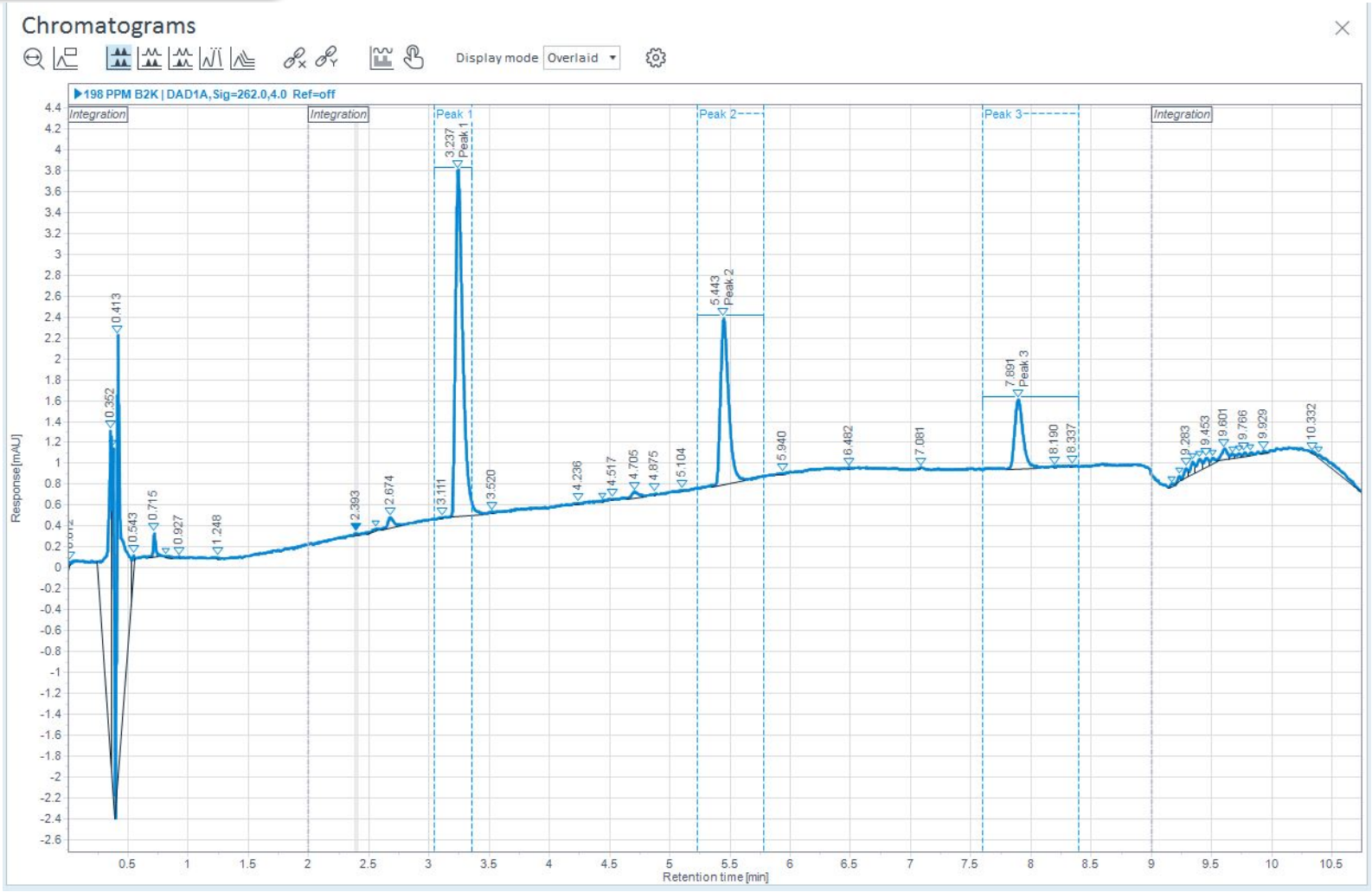


Integration Important Events

Integration “On” / “Off”

Remove undesirable peaks.

Matrix interferences



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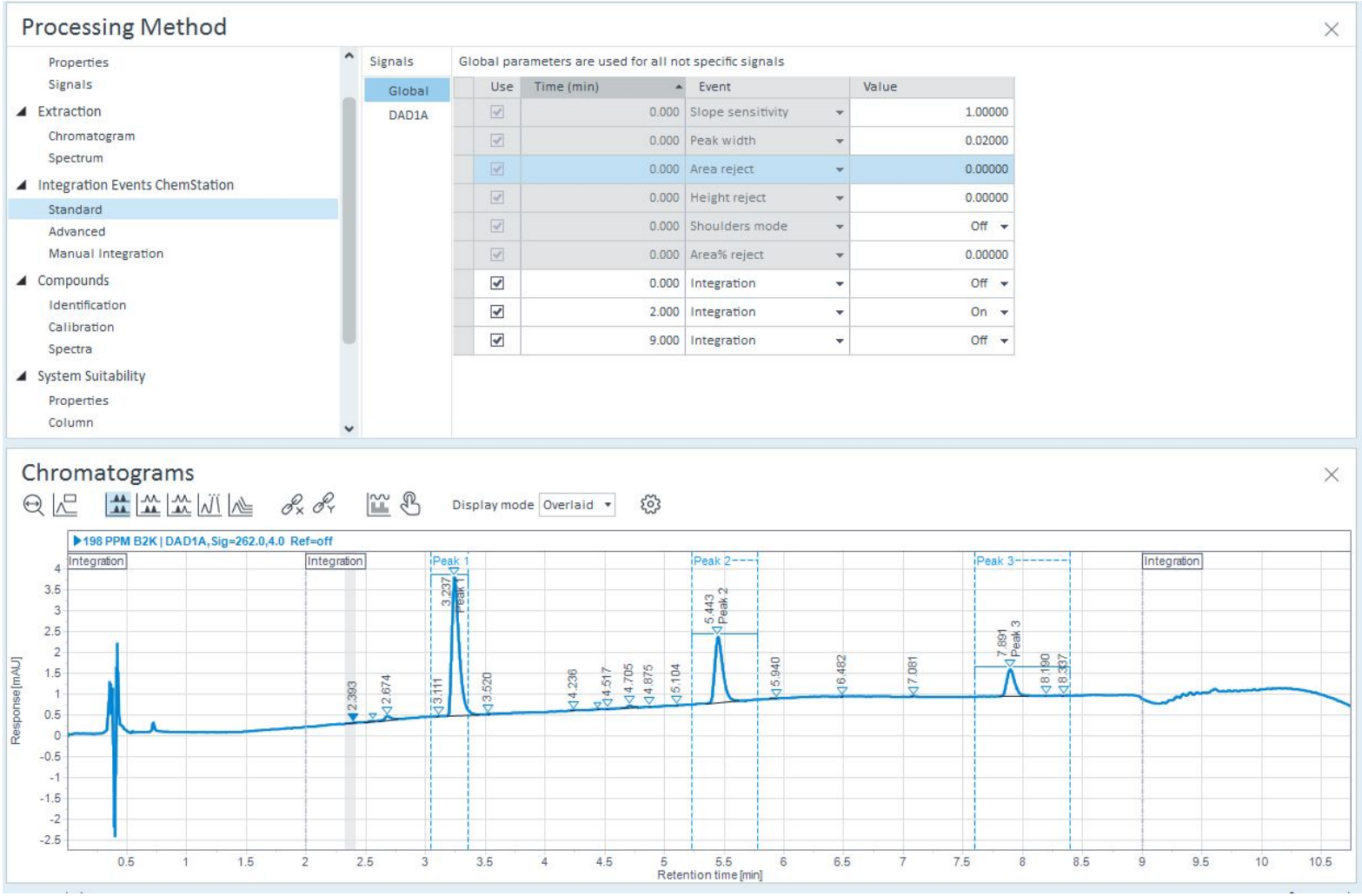


Integration Important Events

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.

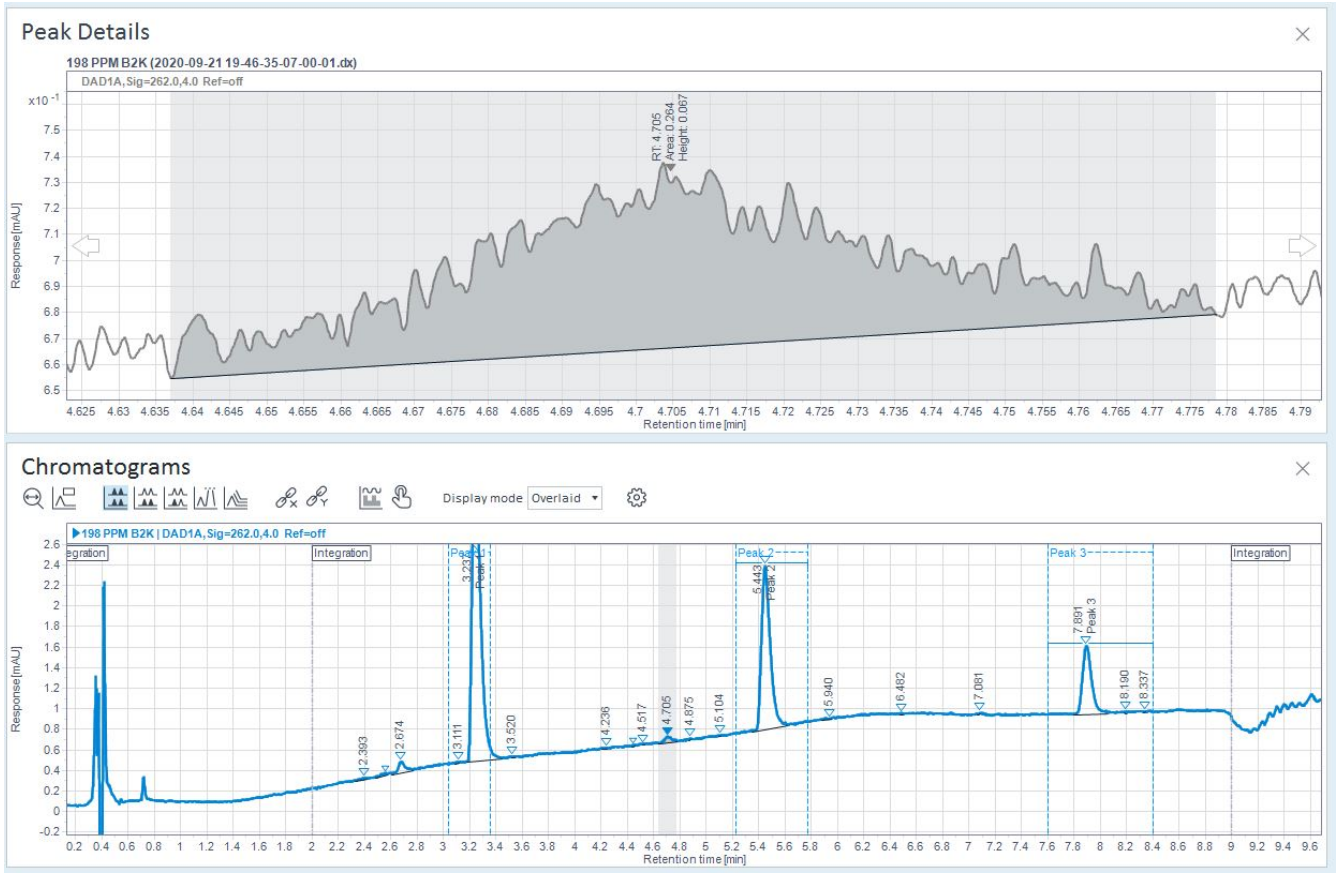
Integration Important Events

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.

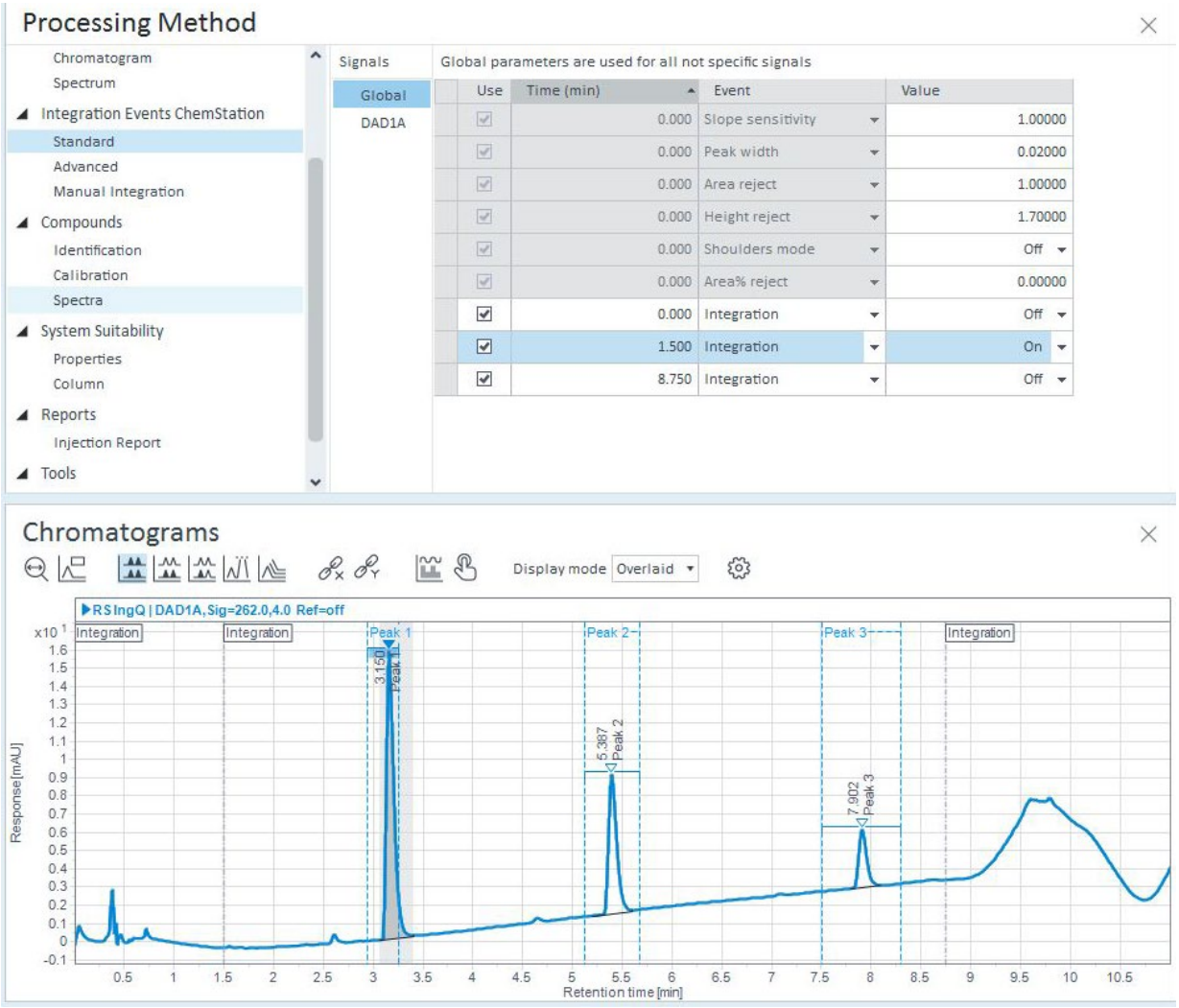


Integration Important Events

Height and Area Determination

Set initial height and area reject to zero

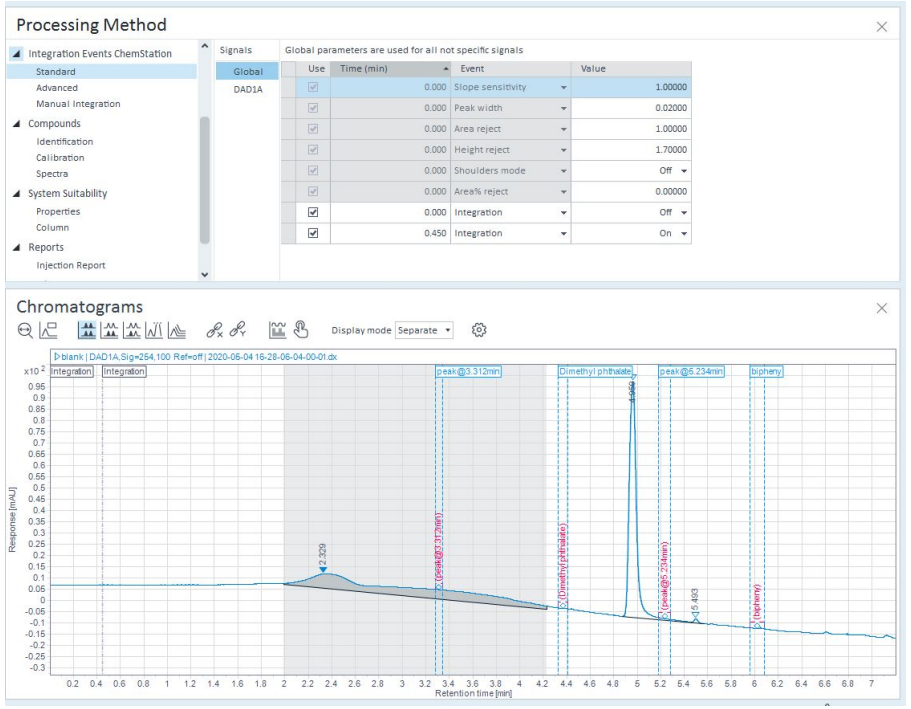
Remove undesired peaks with the height or area reject.



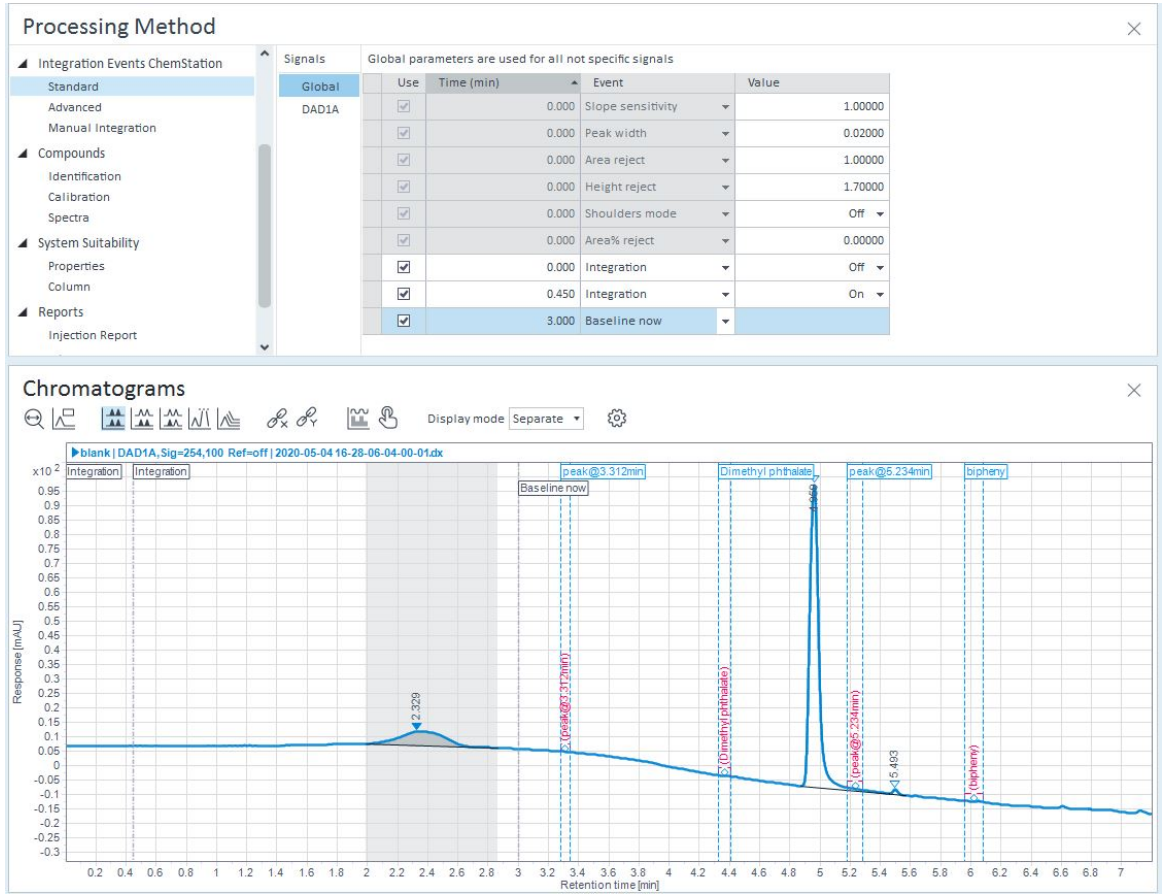
Integration Important Events

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

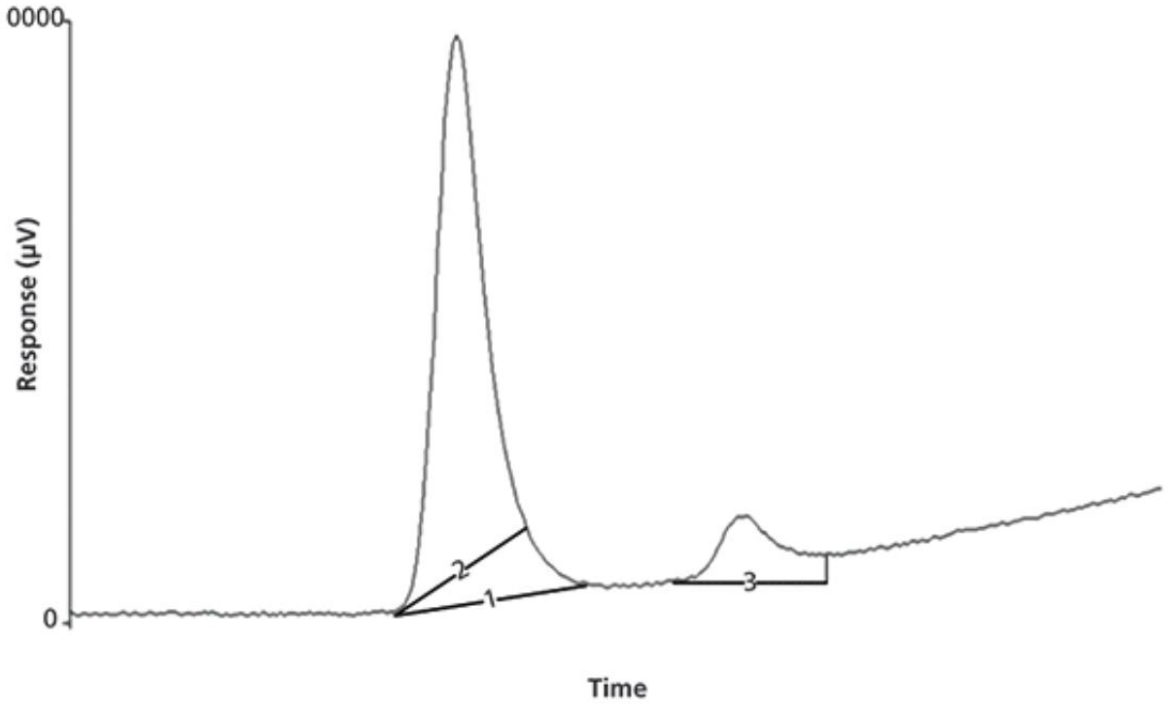
Spikes in the baseline

"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 Enviroment –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

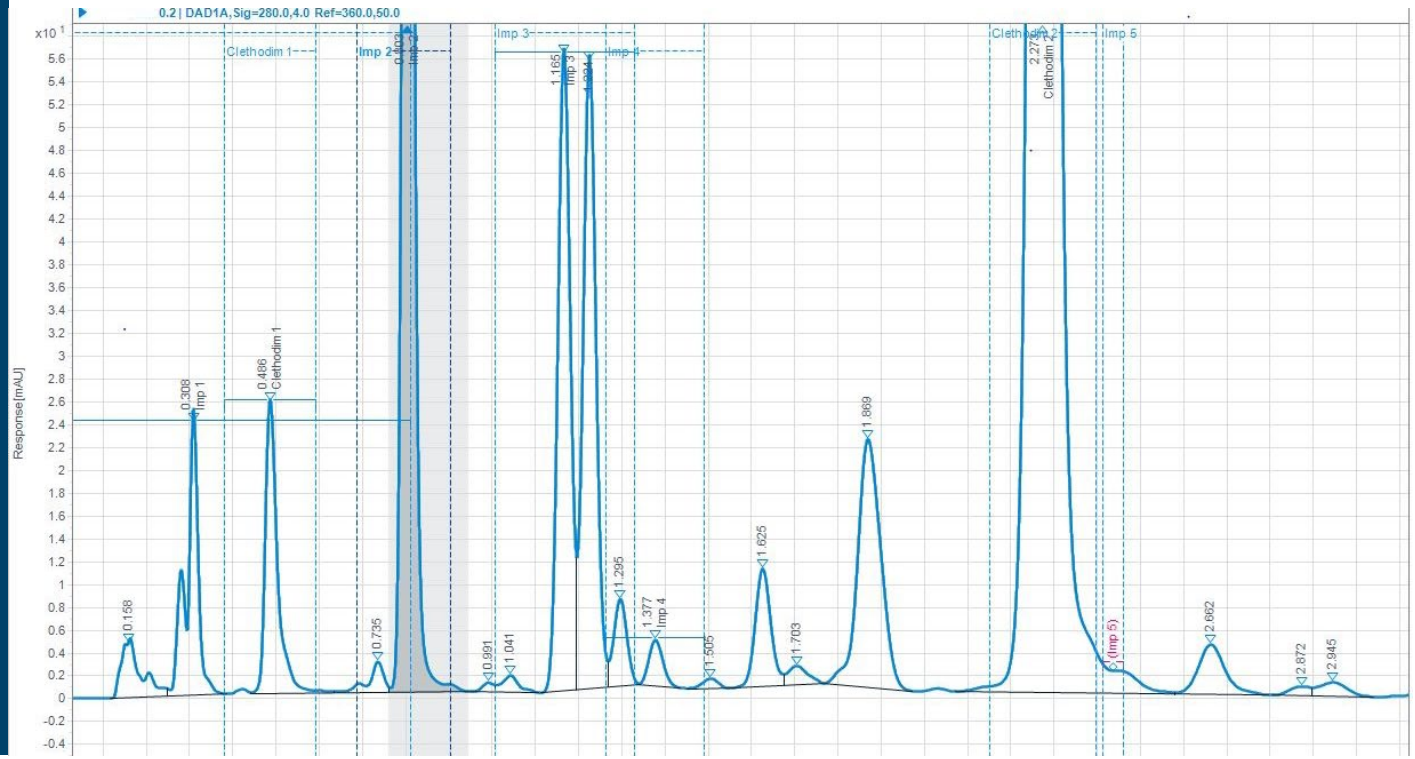
Scientific defensible

Consistent and appropriate integrations

Should not be performed to meet an acceptance criteria

Good idea to documentation justification for the manual integration.

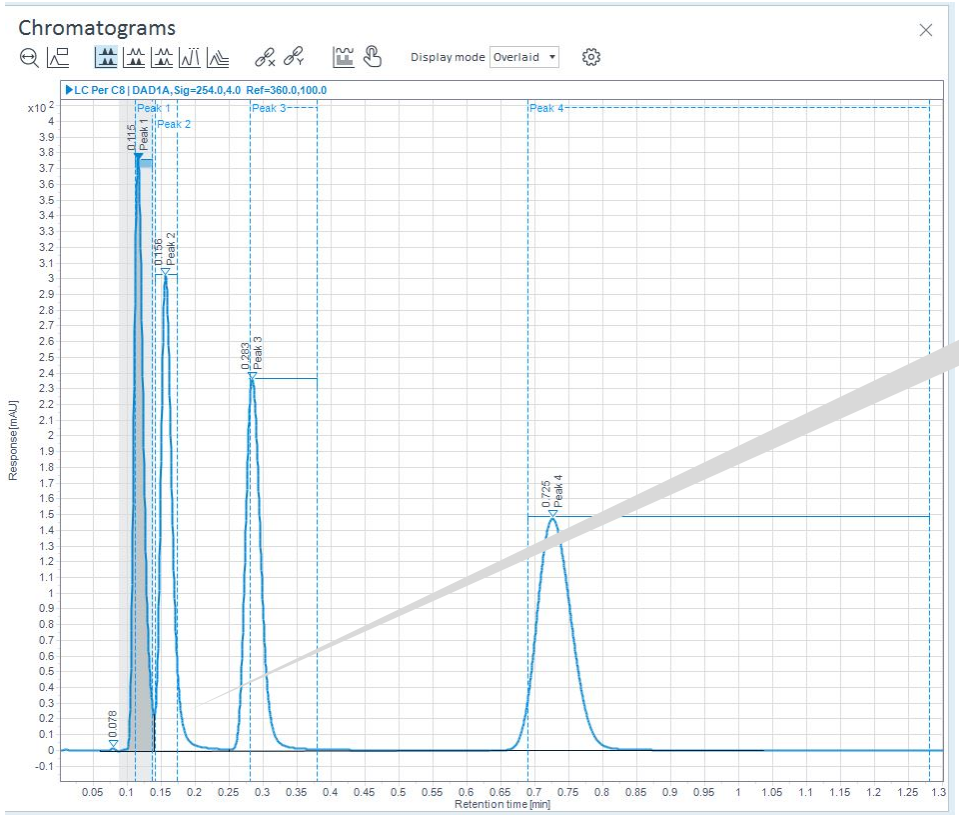
Integration Challenges

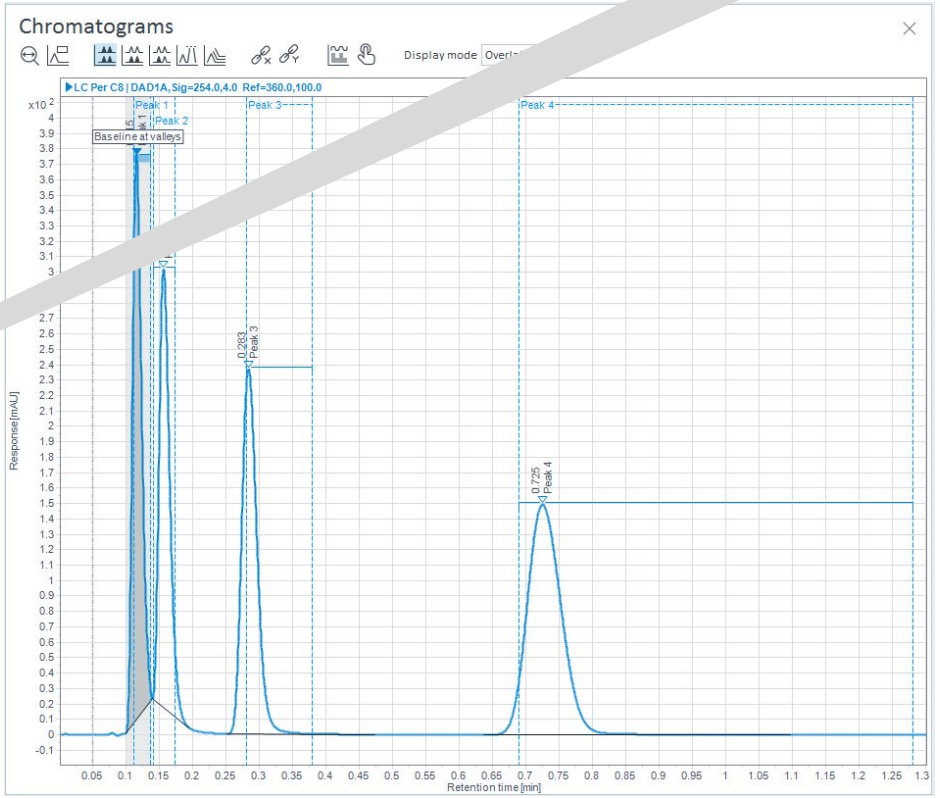


Integration Challenges

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

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

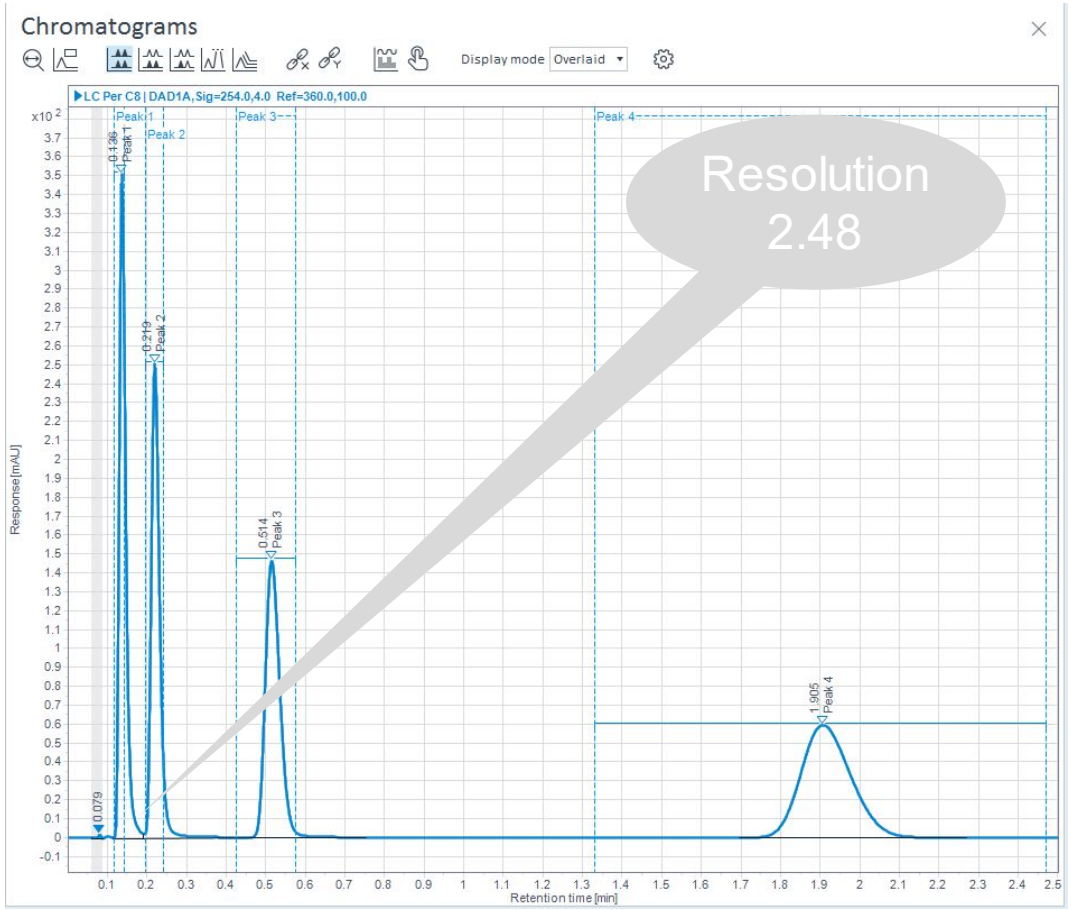




Resolution 1.48

Integration Challenges

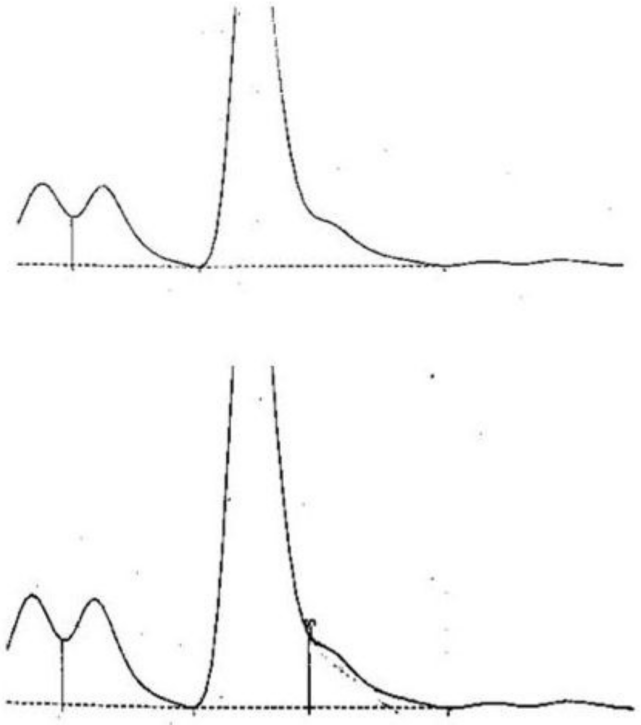
Drop Vertical Versus Valley to Base

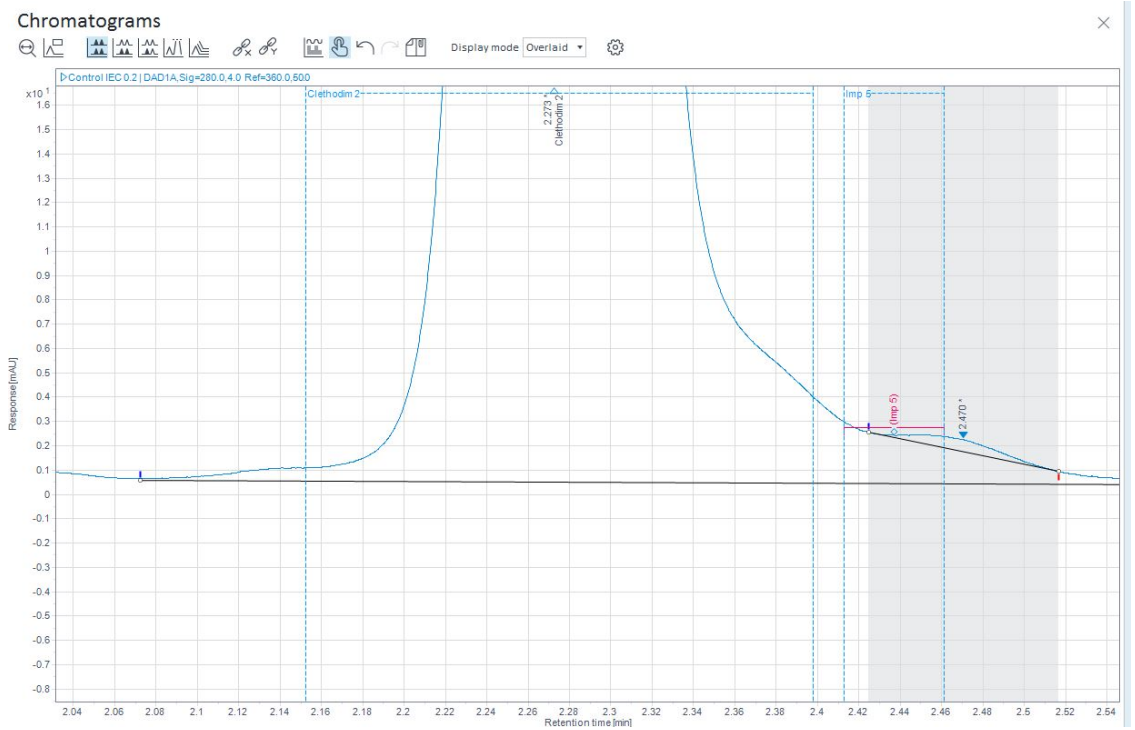


Integ. Code	Peak 1		Peak 2		% Error	
	RT	Area	RT	Area	Peak 1	Peak 2
BV	0.115	381.1122	0.156	364.428	94.3	97.4
VB	0.115	351.3416	0.156	314.4827	86.9	84.0
BB	0.136	404.1422	0.218	374.1953		

Integration Challenges

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





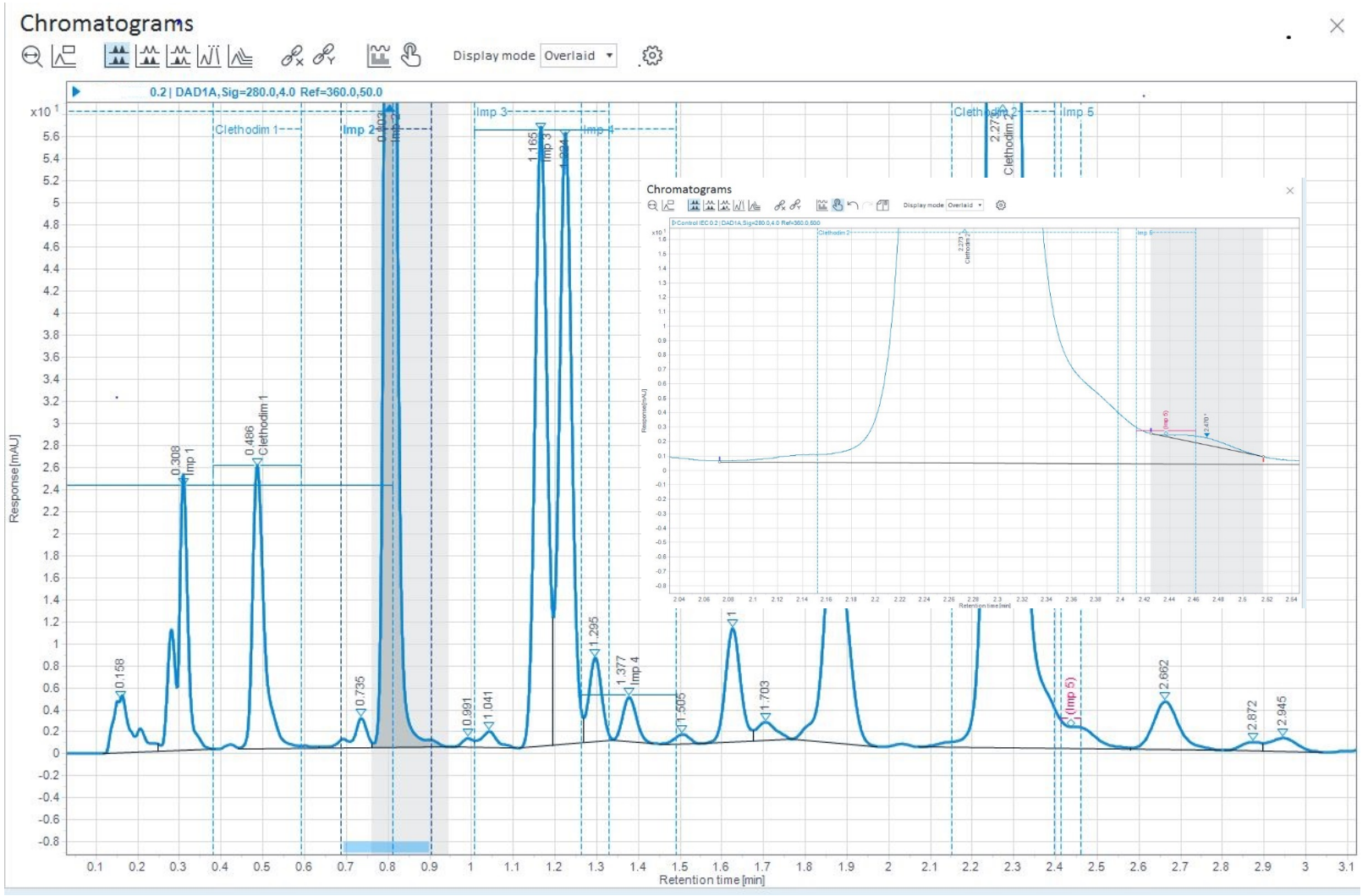
Integration Challenges

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

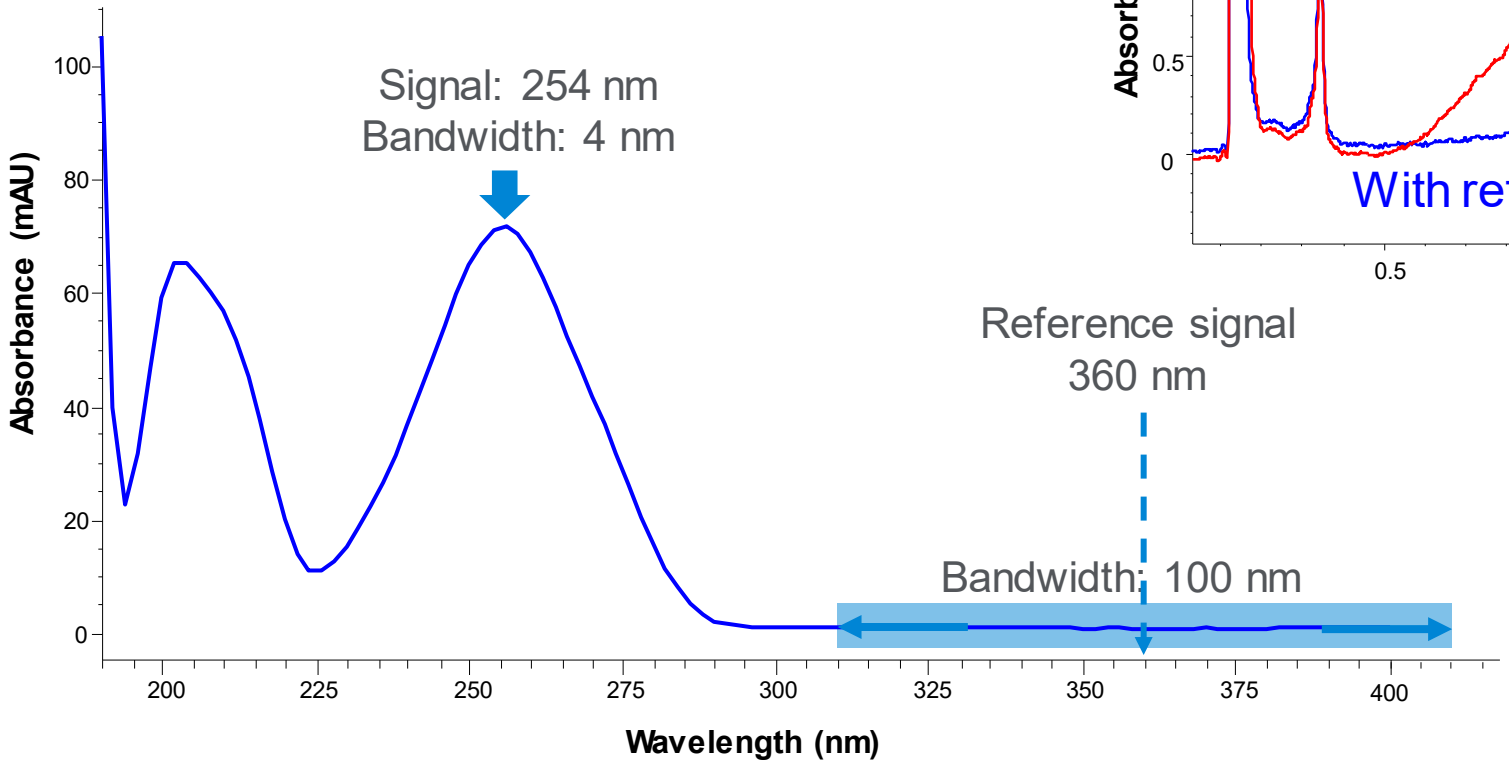


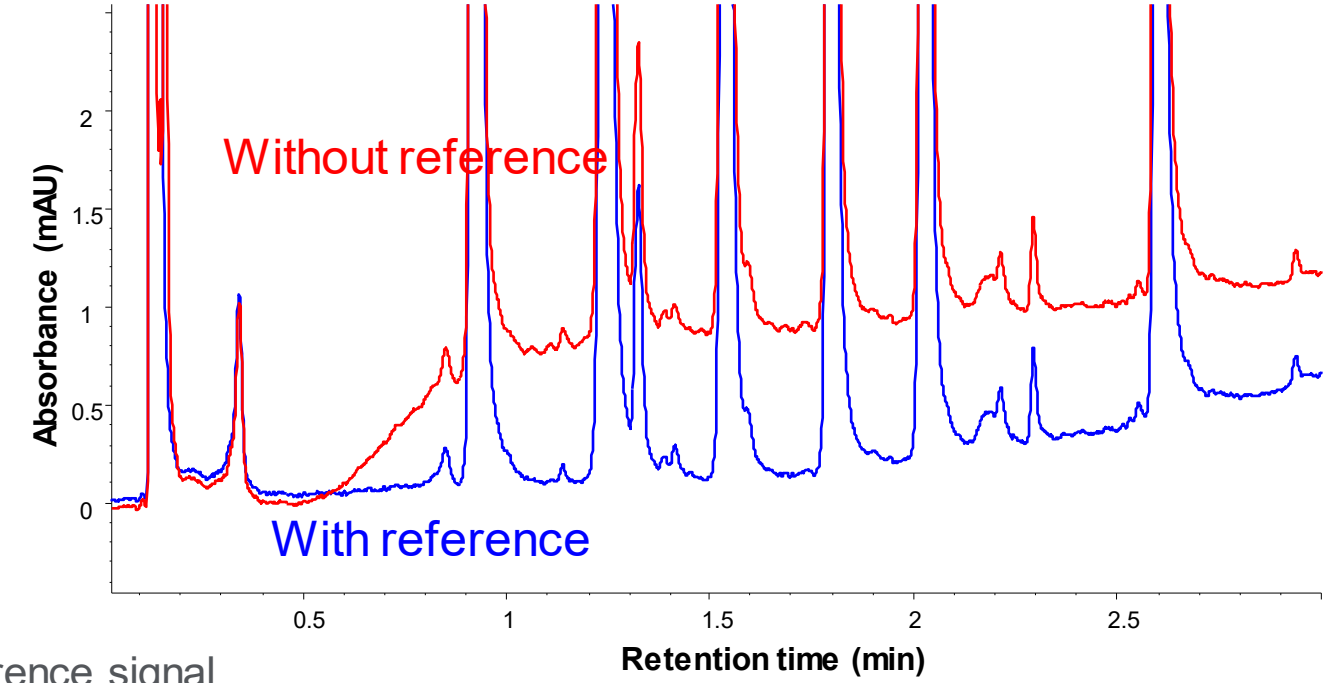
- **UV setting affecting integration**

DAD Setting — Choose the right signal and reference

Signals

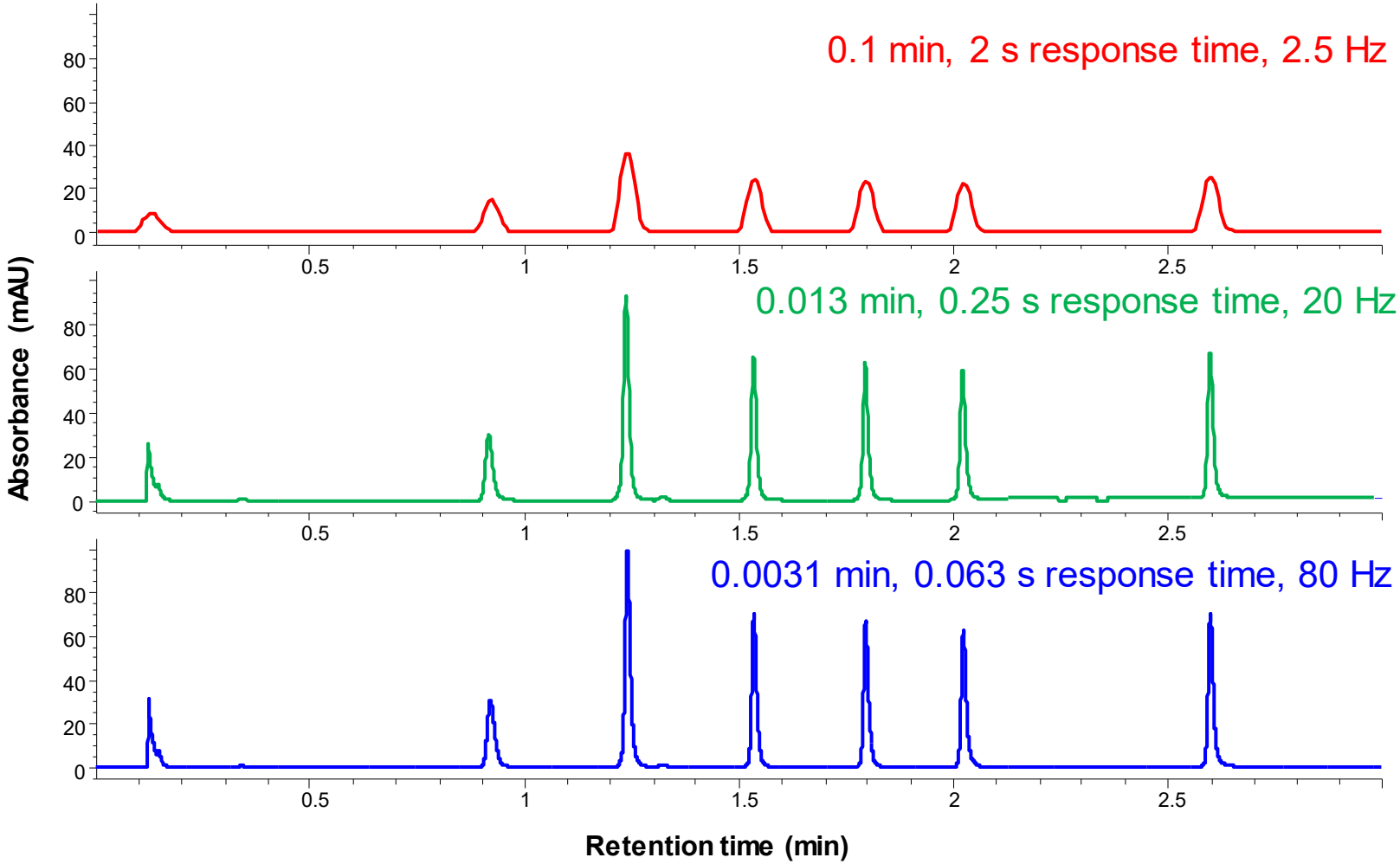
	Acquire	Wavelength	Bandwidth	Reference Wavelength	Reference Bandwidth	
Signal A	☑	254.0	4.0	☑ 360.0	100.0	nm
Signal B	☑	254.0	4.0	☐ 360.0	100.0	nm

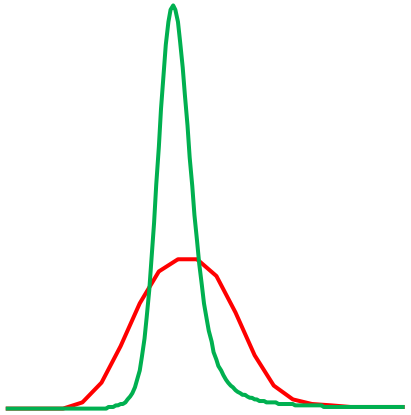




Gradient: 10-100% ACN in 3 min

DAD Setting — Choose the right sampling rate

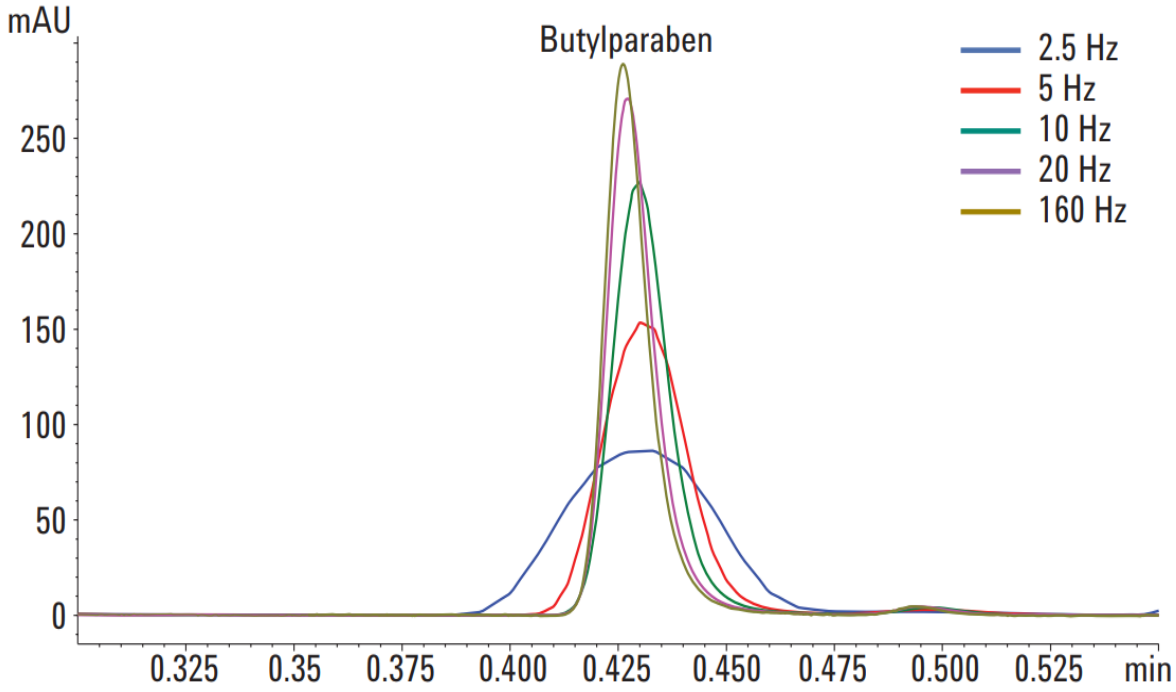


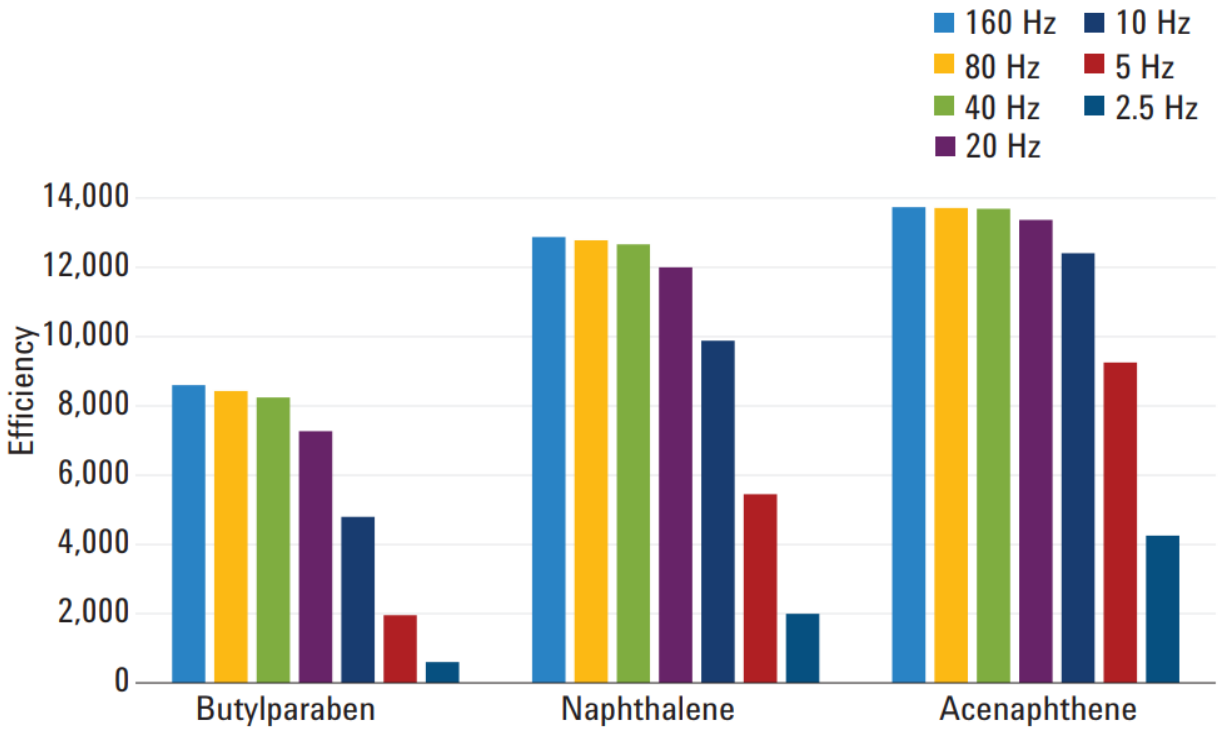


Changes in **Peak Width** and **Resolution**

Column: ZORBOX Eclipse Plus C18, 2.1x50 mm, 1.8 µm
Column temperature: 35 °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

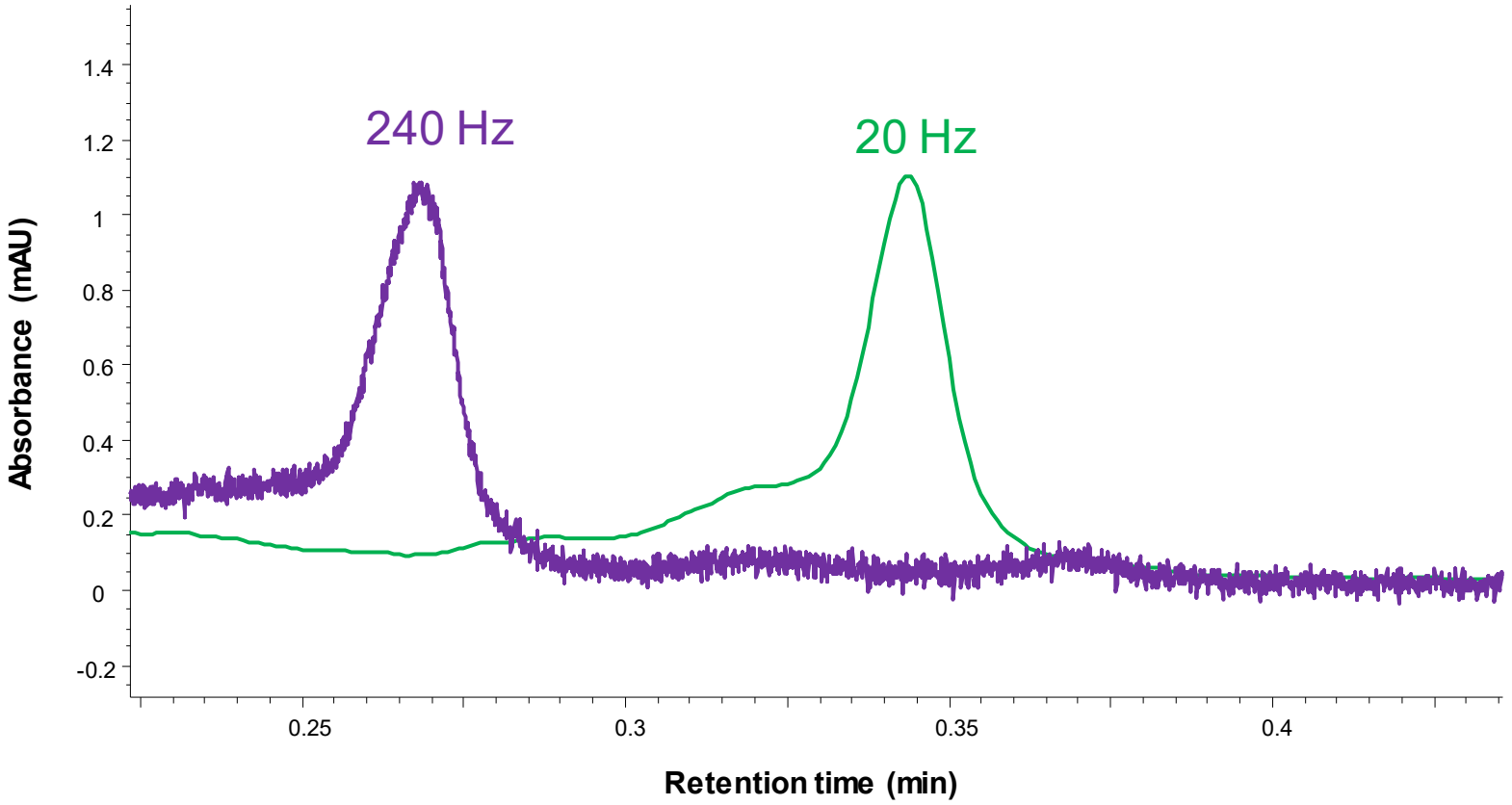
Data Rates May Impact Observed Resolution



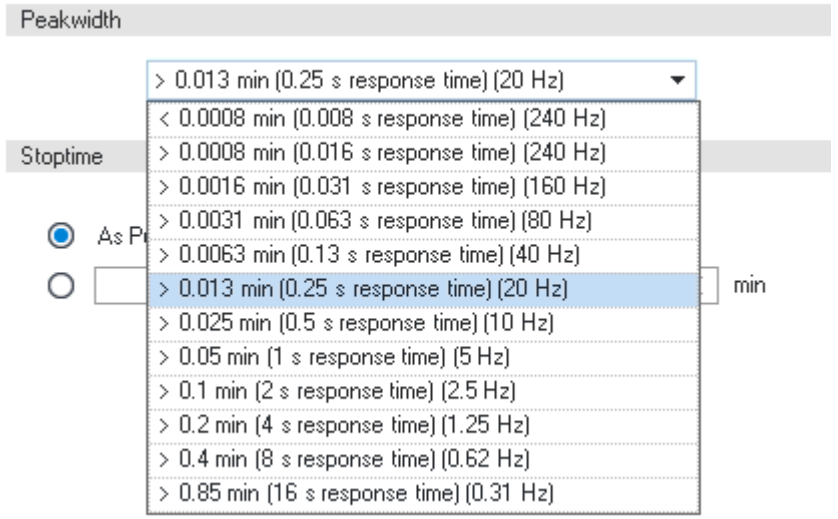


Fast data collection rates must be used with Agilent InfinityLab Poroshell 1.9 µm columns to accurately measure the efficiency of the column, especially for early eluting compounds such as butylparaben (k′ = 1.3).

DAD Setting — Choose the right sampling rate



Do Not Use Peakwidth Smaller Than Necessary



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Column temperature: 35 °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 widthsetting
- 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

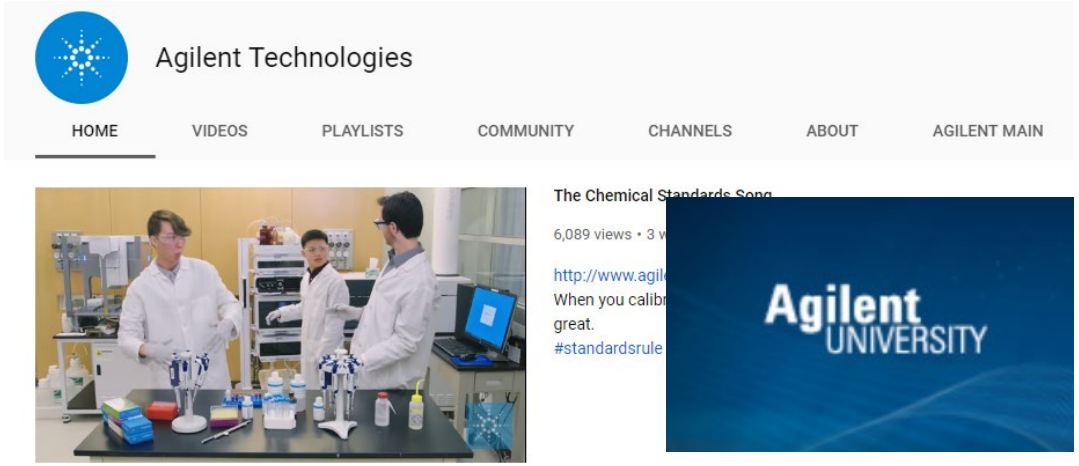
5989-6639EN
Principles in Preparative HPLC

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):

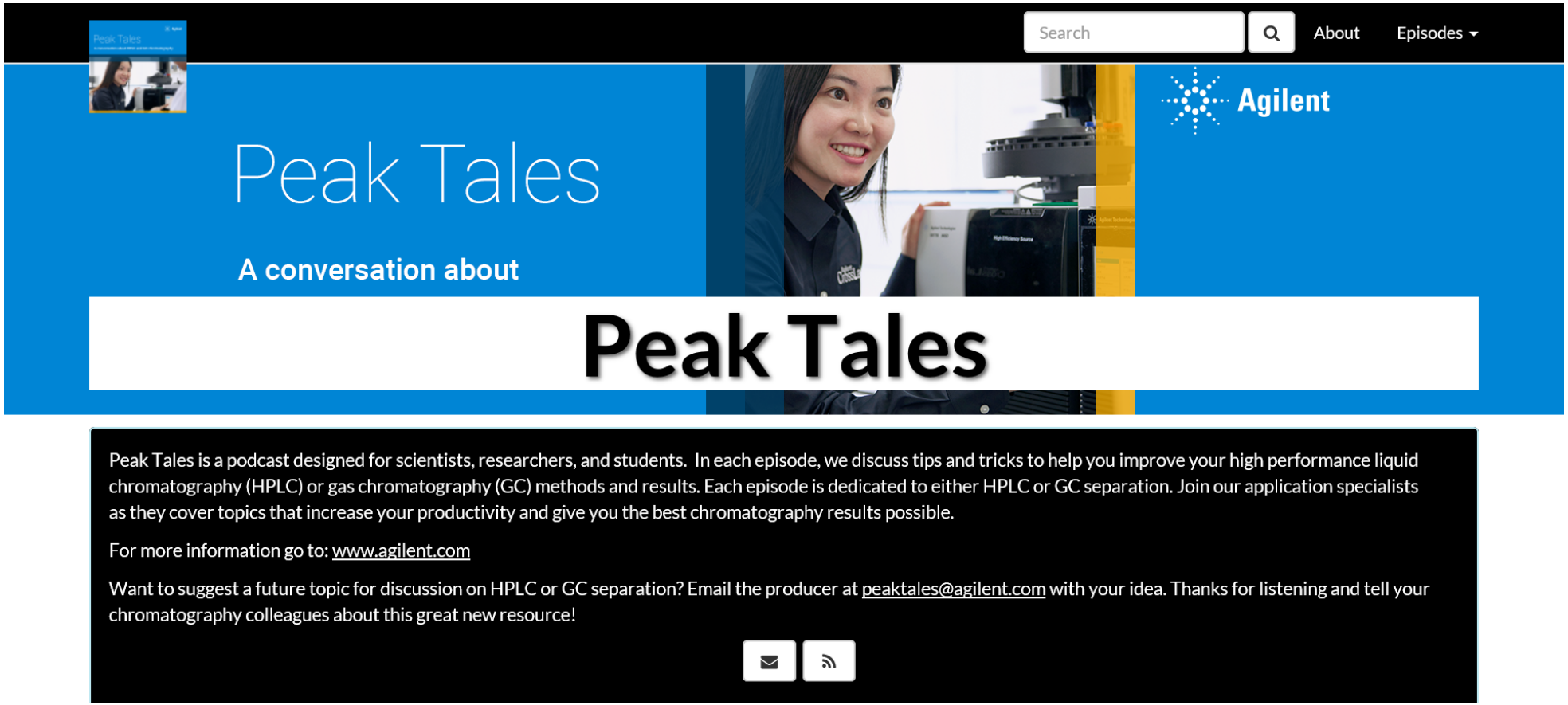
1-800-227-9770 Phone Tree Navigation Assistance

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



Thanks for your attention!

Thanks for attending today,
Greg & Sue