New Techniques for Advanced Detection and Integration In High Throughput Fast Chromatography

Pat Fowler, Michael E. Swartz, Ph.D.

WA20697

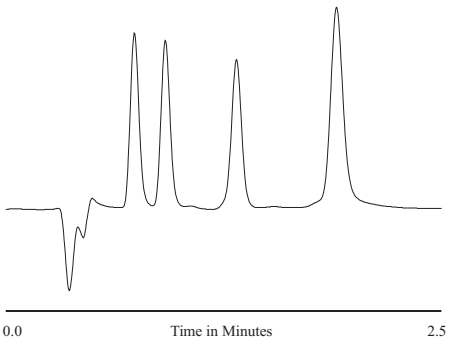
Overview

- New Challenges From Fast Chromatography
  - Photodiode Array (PDA) Detection
    - Why use a PDA?
    - Good PDA practices
    - More Reproducible Data
      - With decreasing runtimes
      - With narrow peaks
  - New Peak Integration Algorithm
    - Automation, reproducibility
    - Integration of Peak Apex
      - Shoulders easily detected and resolved

Good PDA Data Depends on Proper Settings

- Photodiode Array Detection is Enhanced by Proper Selection of Parameters
  - Digital Filter
  - Sampling Rate
  - Bandwidth
    - Optical Resolution
    - Digital Resolution
  - Lamp Energy Optimization

Fast Chromatography



Fast chromatography presents challenges in detection and integration. The peaks are narrower with very short retention times. Accurate and reproducible results depend on optimized detector and integration algorithm capabilities.

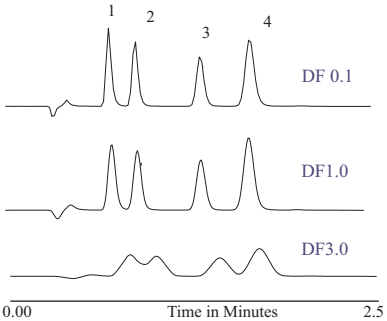
Digital Filter Setting

- Digital Filter
  - An enhanced rolling average filter applied to PDA absorbance data that reduces high-frequency noise across the entire wavelength range specified for the acquisition.
  - Longer time-constant settings
    - Increase signal/noise ratio by reducing baseline noise
    - Most effective for single peak chromatograms
    - May cause:
      - Asymmetrical broad peaks
      - Shifting in peak maxima and missing peaks
      - Reducing peak height

Why Use Photodiode Array Detection?

- Quantitatively Demonstrate Peak Homogeneity
- Confirm Peak Identity
  - Library match spectra
- Determine Trace Impurity Levels
  - Set flags for detection of impurities during routine use
- MaxPlot Capability for Method Development
  - Maximum absorbance for each peak
  - Use as Universal UV/Vis Detector
    - Document wavelength monitoring choices
    - Eight 2D channels simultaneously
- High Selectivity
  - Ability to distinguish between main peak and impurities

Effects of Digital Filter Setting



Conditions:
Xterra®MS C18 4.6X3 cm
Mobile Phase:
A) 10mM NH_4OAc, pH 6.9
B) ACN
A:B 86:14
Flow rate: 1.2 mL/min
Injection volume: 10 µL

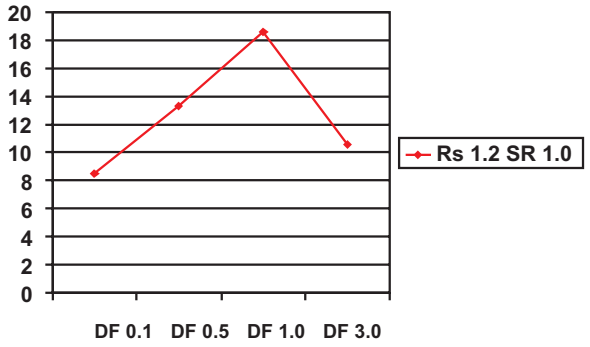
Peak ID:
1. Aminochloro-benzenesulphonamide
2. Hydrochlorothiazide
3. Methylbenzenesulphonamide
4. Triamterene

The effect of optimizing the digital filter is to reduce baseline noise and increase the S/N ratio. Too low a digital filter setting allows greater baseline noise with decreased S/N ratio and too high a setting decreases baseline noise but also decreases peak height. For this application, the best setting was determined to be 1.0.

New Techniques for Advanced Detection and Integration In High Throughput Fast Chromatography

Pat Fowler, Michael E. Swartz, Ph.D.

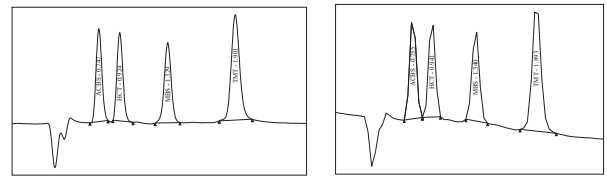
Signal/Noise Comparison



The S/N ratio for each digital filter setting is shown. The best setting for this application with the highest ratio is at the digital filter setting at 1.0 seconds.

Comparison of Sample Rates

Sample Rate 5.0 | Sample Rate 0.5



Peak Results

RT	Height	Purity1 Angle	Purity1 Threshold	Purity1 Flag	PDA Match1 Angle	PDA Match1 Threshold	PDA Match1 Spect. Name
0.747	8802	0.357	0.639	No	3.335	24.053	ACBS
0.924	8350	0.504	0.744	No	2.831	21.578	HCT
1.329	7567	4.321	1.204	Yes	2.331	21.849	MBS
1.901	9963	0.820	1.045	No	2.152	9.698	TMT

Better Results

Peak Results

RT	Height	Purity1 Angle	Purity1 Threshold	Purity1 Flag	PDA Match1 Angle	PDA Match1 Threshold	PDA Match1 Spect. Name
0.755	8723			No			
0.961	8347			No			
1.360	5426			No			
1.895	7002			No			

Missing Data

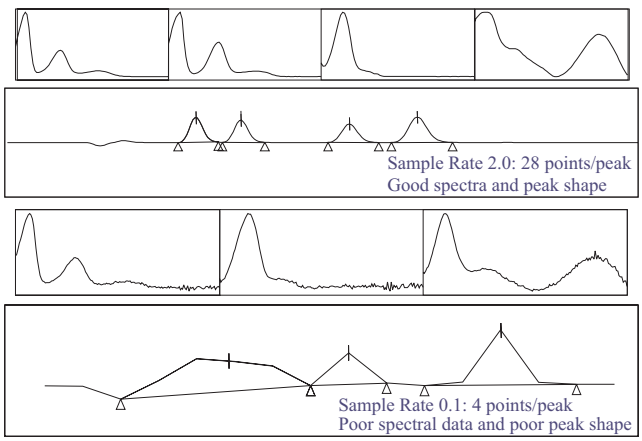
Optimization of Sampling Rates

The most accurate and reproducible peak detection depends on an adequate sampling rate during data acquisition. The optimum setting is determined by selecting the narrowest peak in the chromatogram and calculating the sampling rate by using the following formula: SR=15/W. The minimum number of points across the peak for the most accurate sampling for optimal peak integration, calibration, and quantitation is 15 points across the peak. The width of the peak is determined at the baseline of the peak in seconds. With the sampling rate set too low the spectra collected is very noisy and the chromatogram will show poor peak shape. An optimized sampling rate setting will show good spectral information and good chromatographic peak shape.

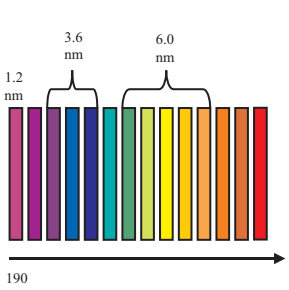
Digital Resolution (Bandwidth Setting)

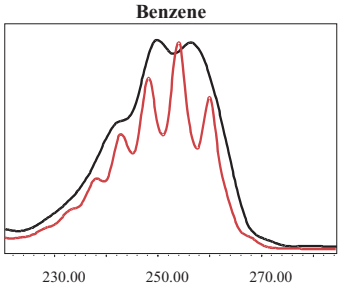
- Specifies the number of diodes that are averaged together as a single spectral data point.
  - Use smaller resolution settings ie. 1.2 nm for differentiating closely related spectra
    - Differentiating impurities from main peak
    - Determining peak homogeneity
    - Better quantitation (accuracy, precision, linearity, and specificity)
  - Small resolution values generate more data points requiring more hard disk drive space

Comparison of Sampling Rates



Digital Resolution (Bandwidth)





Less resolution at **3.6 nm** vs **1.2 nm**

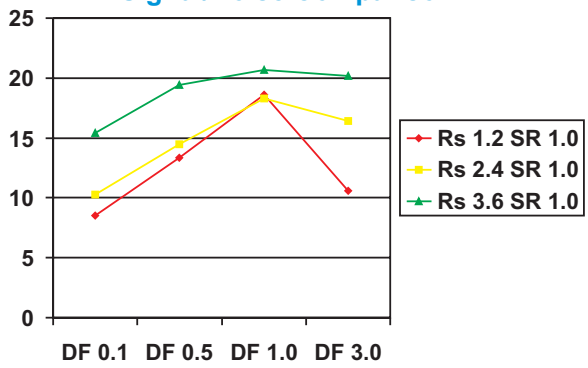
UV maxima shifted

Good digital resolution gives good quality spectral information

New Techniques for Advanced Detection and Integration In High Throughput Fast Chromatography

Pat Fowler, Michael E. Swartz, Ph.D.

Signal/Noise Comparison



The optimized settings for the digital filter, digital resolution, and sampling rate all work together to provide the best S/N ratio as well as the most accurate and reproducible chromatographic results. The best setting for this application is using the digital filter setting of 1.0, digital resolution setting of 3.6, and the sampling rate of 1.0. The best digital resolution setting for more defined spectral information is 1.2.

New Integration Algorithm Advantages

Easier:

- Automatically determines appropriate integration parameter settings
  - Auto Peak Width
  - Auto Threshold
- Should integrate well at first pass using default and automatic parameters

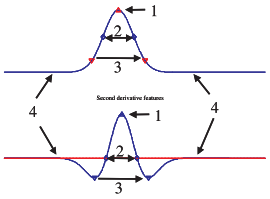
Better:

- Integrates small peaks in noisy or drifting baseline effectively
- Peak shoulders are easily detected
- Integrates negative peaks

Different Methods Have Different Requirements

- Optimized PDA Parameters Ultimately Depend on What the Method is Being Used For
  - Trace level impurities, degradation products
  - Assay (Content uniformity, potency, etc.)
- Digital filter optimized for best S/N
- Resolution, sample rate optimized for best spectral information

Features of the 2nd Derivative



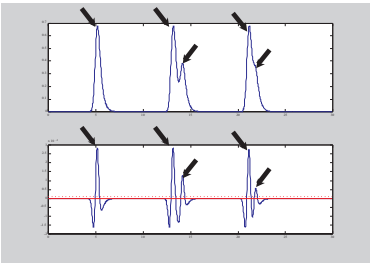
- Peak apex, maximum of curvature
- Inflection points, zero curvature
- Upslope points, minimum of curvature
- Baseline region, zero curvature

(Y scale of 2nd derivative plot = curvature)

Optimum Peak Integration For Fast Chromatography

- Peak Apex Track Integration
  - New algorithm is easier to use and works better than traditional integration
  - Based on measuring the curvature (the rate of change of slope) of the chromatogram (2nd derivative).
- Traditional Integration vs. Apex Track Integration
  - Traditional detects peaks by initially looking at peak start
  - Apex Track detects peaks by initially looking for the peak apex

Example of Peak Detection

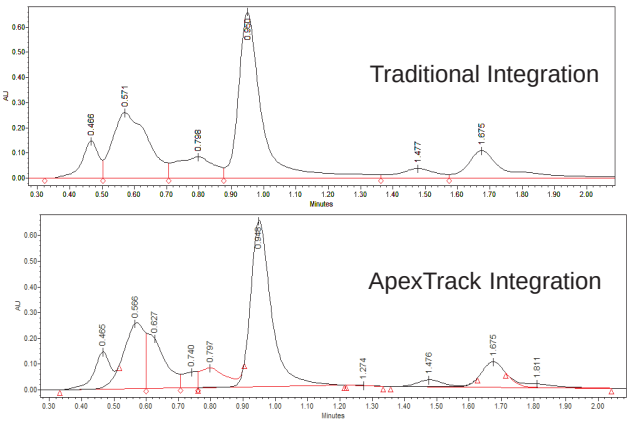


- Top plot: simulated chromatogram
- Bottom plot: 2nd derivative
- Arrows indicate peak apices
- Note that shoulders are cleanly resolved and easily detected

New Techniques for Advanced Detection and Integration In High Throughput Fast Chromatography

Pat Fowler, Michael E. Swartz, Ph.D.

Integration Algorithm Comparison



Integration Algorithm Comparison

Traditional integration compares the peak slope against a fixed threshold to identify the point of liftoff and touchdown. The algorithms in this method work down the chromatogram to determine peak touchdown when a suitable threshold is met for finding the apices, valleys, and other features. ApexTrack integration uses the peak apex for detecting peaks. The algorithm for this method searches for the peak apex initially then works downward and outward using the second derivative to determine the baseline. Peak detection is independent of baseline location. Peak shoulders can be easily identified. Identification of peak clusters are found when the expanding baselines meet and fuse together.

Conclusion

- Addressed Challenges in Fast Chromatography
  - PDA
    - Optimize Sample Rate Using the Narrowest Peak of Interest in the Chromatogram
    - Optimize the Digital Filter Setting Using Signal/Noise
    - Optimize the Spectral Resolution Setting Using the Spectra of the Compound(s)
  - Peak Integration
    - ApexTrack Integration
      - Based on measuring the curvature of the chromatogram (2nd derivative)
      - Faster more reproducible integration of difficult peaks, shoulders, and valleys