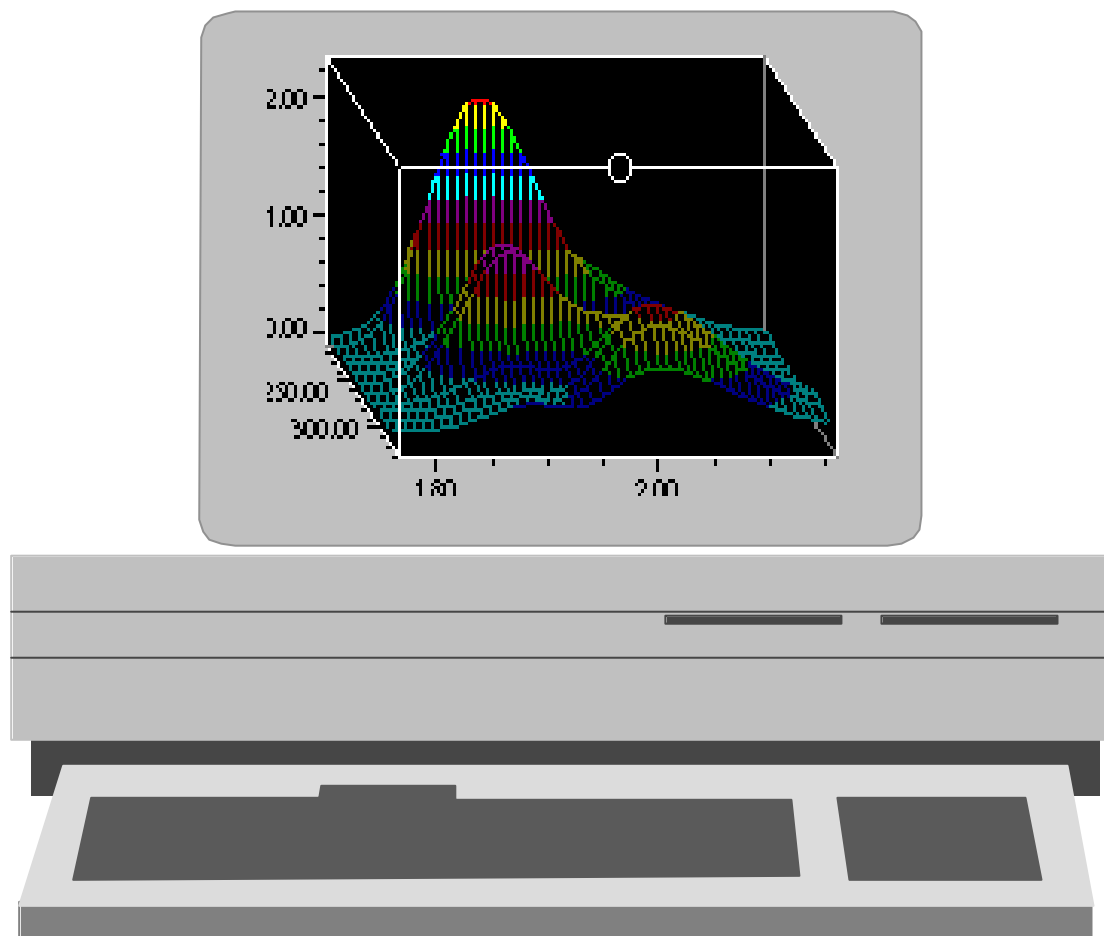
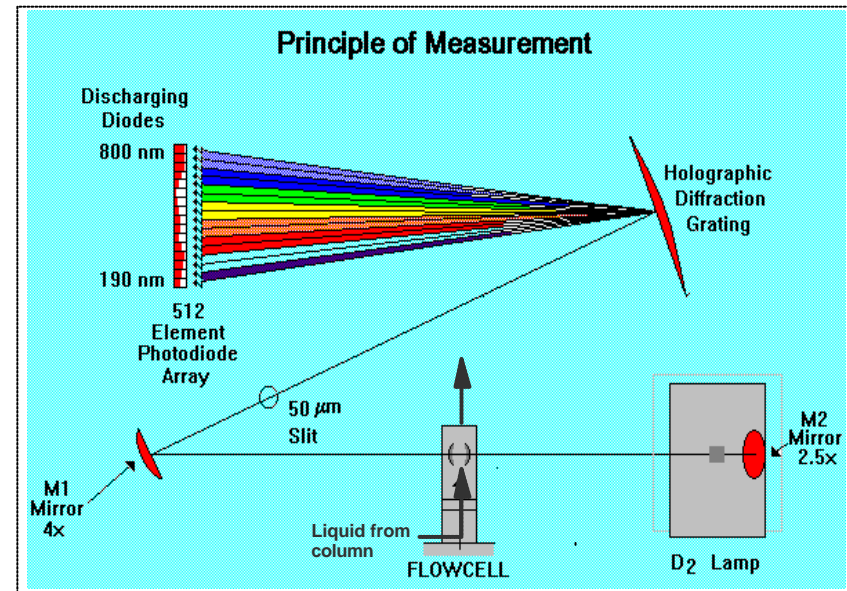
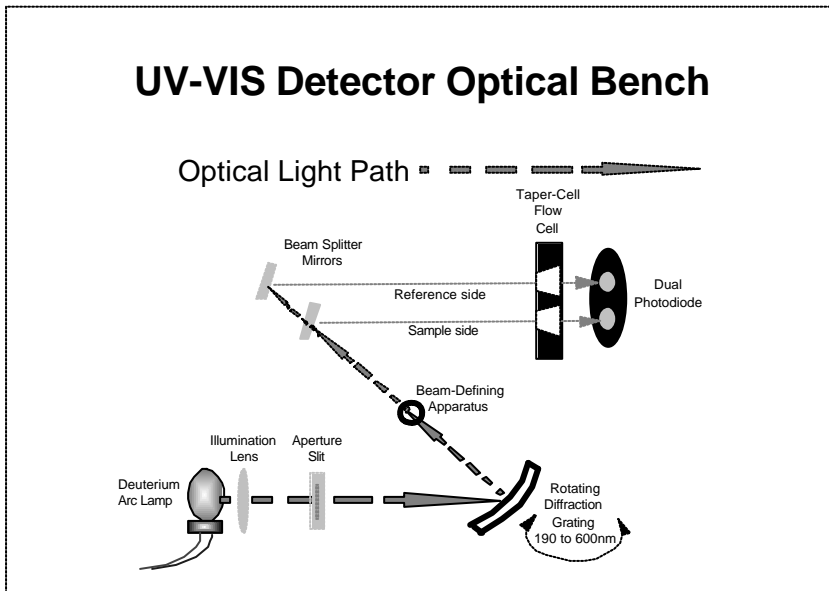
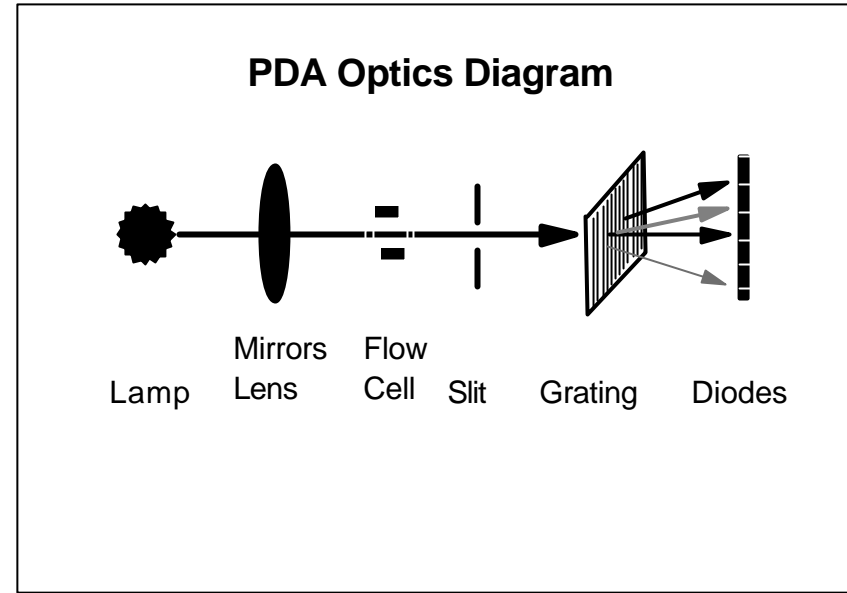
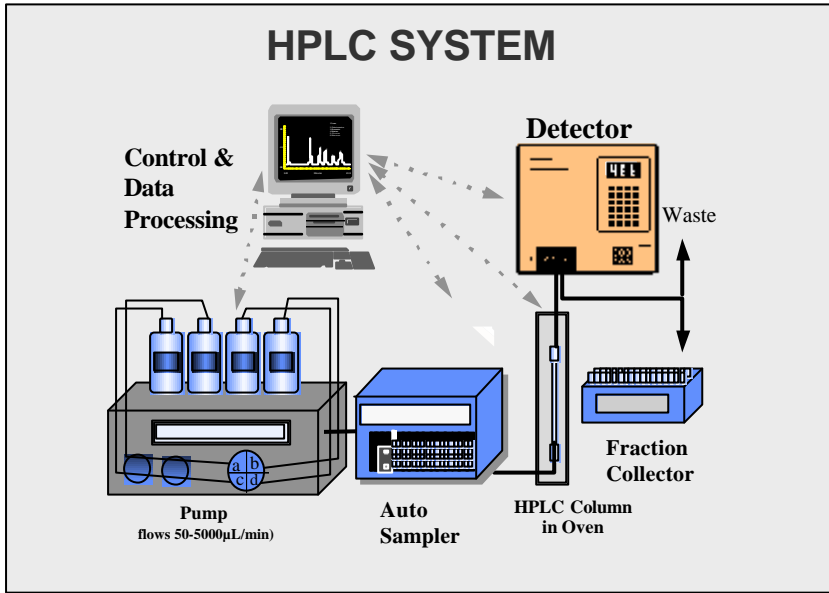


Photodiode Array

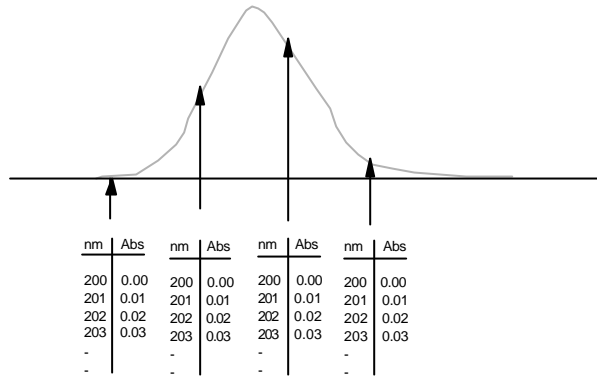


Advanced Detection Technologies
for Compound Identification

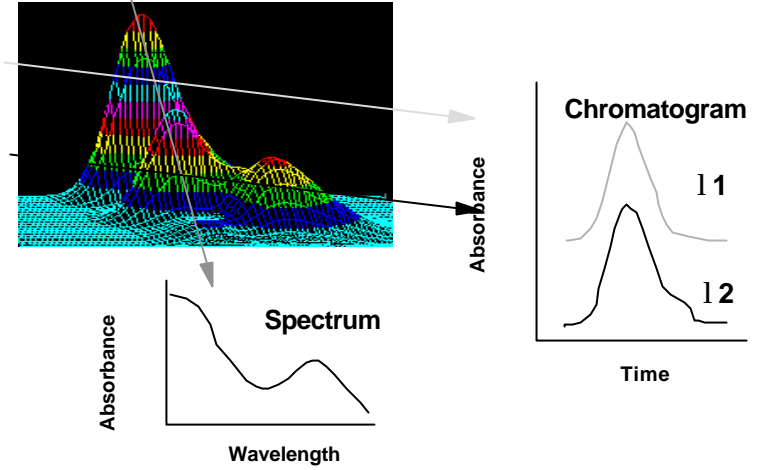
Diode Array Detectors



The Data is 3D

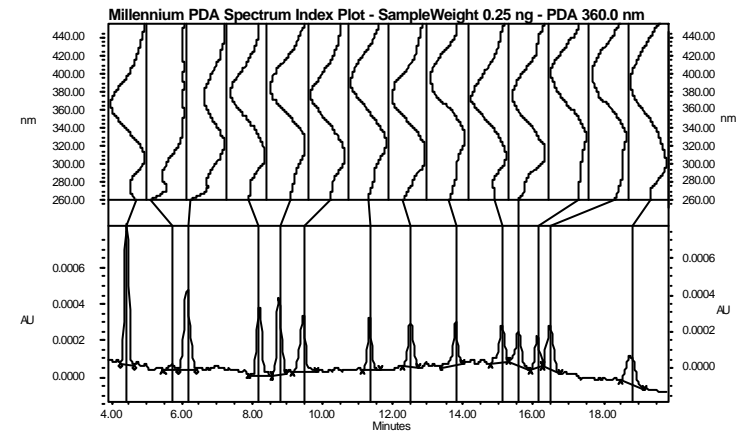


Extraction of 3D Data

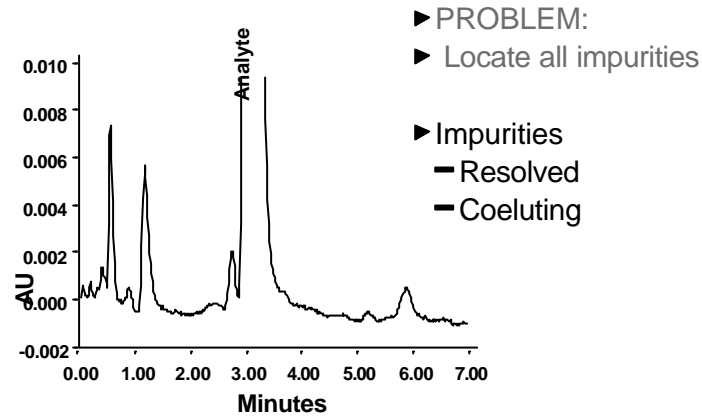


Software Demonstration of 3D data

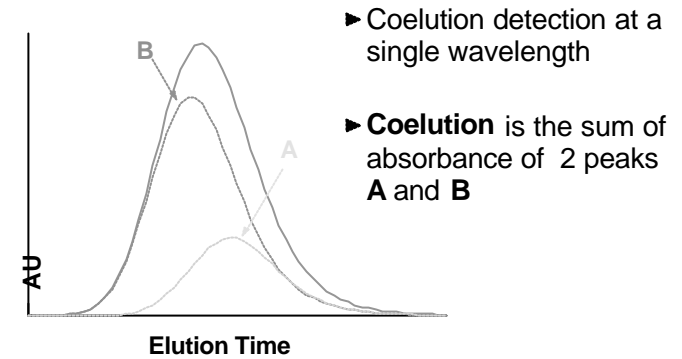
PDA Spectrum Index Plot DNPH Derivatives 0.25 ng Each Peak



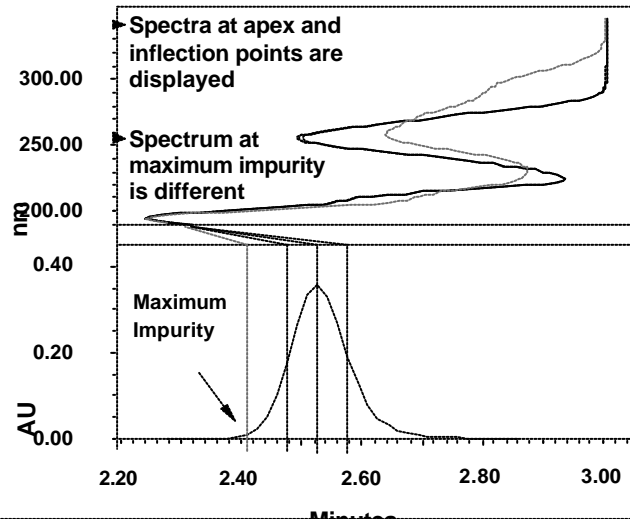
Major Peak and Minor Peaks



Coelution of 2 Peaks

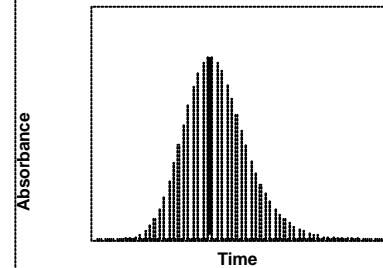


Spectrum Index Plot



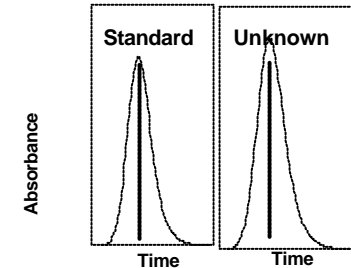
Good quality spectral information is important for:

Purity verification



- Peak Purity analyzes all spectra (minimum 15) within a peak
- Apex spectrum is the reference spectrum

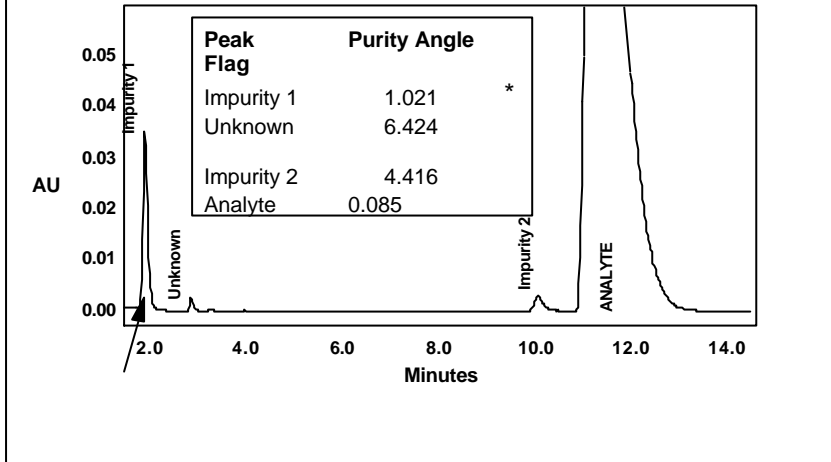
Library identification



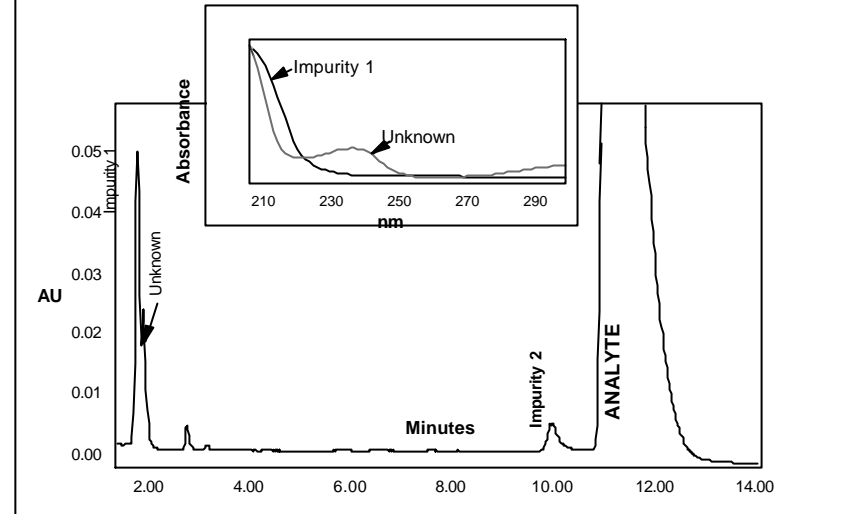
- Matching compares the unknown apex spectrum of the peak with a reference spectrum in a library

$$\sin q_j = \frac{\sqrt{\sum_{i=1}^n (B_{ij} - s_j A)^2}}{\sqrt{\sum_{i=1}^n B_{ij}^2}}$$

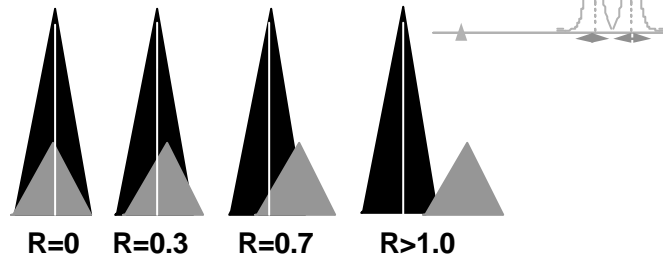
Stability Test at 8 Weeks



Stability Test at 12 Weeks



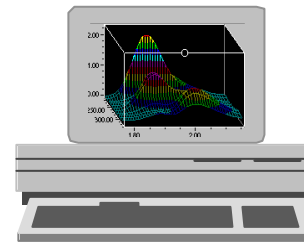
Chromatographic Resolution & Coelution Detection



- ▶ **R=0** Purity Angle not effective; Match Angle useful
- ▶ **R=0.3 to R=0.7** Purity & Match Angle useful
- ▶ **R>0.7** Match Angle not useful

Photodiode Array Technology

Spectral Analyses



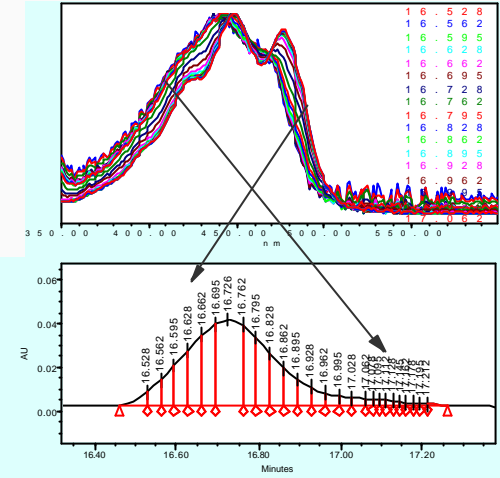
- ▶ **Library Matching**
 - Compound identification
 - Coelution detection
- ▶ **Peak Purity Analysis**
 - Peak purity/peak homogeneity
 - Coelution detection

Importance of Spectral Analyses

- ▶ Library Matching
 - Compound identification
 - Coelution detection

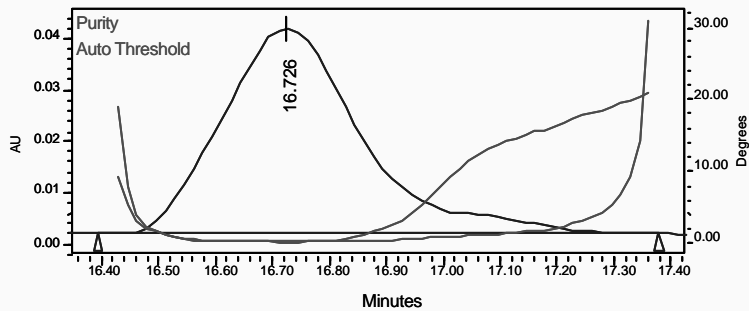
- ▶ Peak Purity Analysis
 - Peak purity/peak homogeneity
 - Coelution detection

Spectra Collected from a Peak with Impurity



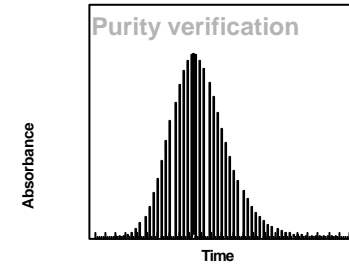
CAROTENOIDS Purity Plot of Peak 4 - Not Pure

Purity Flag	Maximum Impurity	Purity Threshold	Purity Angle
Yes	17.078	0.404	1.885

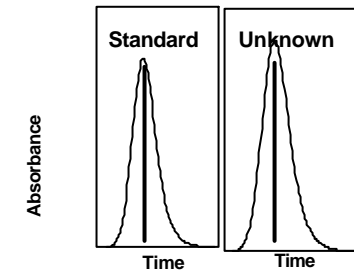


Comparison between Spectra

$$\sin q_j = \frac{\sqrt{\sum_{i=1}^N (B_{ij} - s_j A_i)^2}}{\sqrt{\sum_{i=1}^N B_{ij}^2}}$$



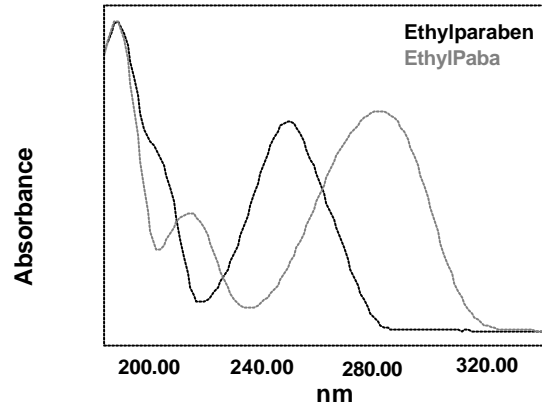
Library identification



- ▶ Peak Purity analyzes all spectra (minimum 15) within a peak
- ▶ Apex spectrum is the reference spectrum

- ▶ Matching compares the unknown apex spectrum of the peak with a reference spectrum in a library

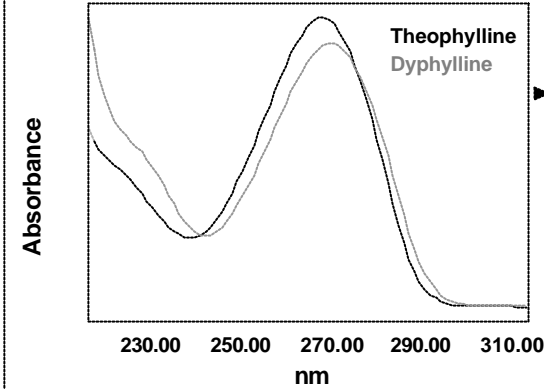
53 Degrees in a scale of 0-90



► 53 degrees is a large spectral difference

$$\sin \theta_j = \frac{\sqrt{\sum_{i=1}^n (B_{ij} - s_j A)^2}}{\sqrt{\sum_{i=1}^n B_{ij}^2}}$$

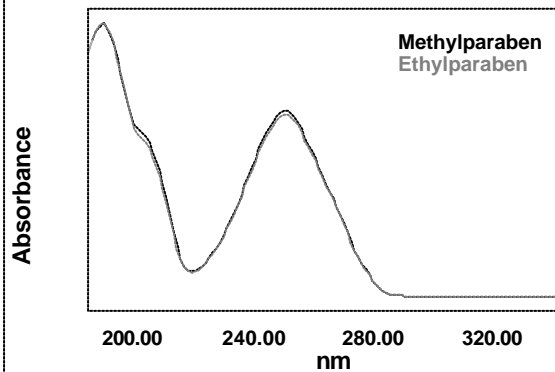
10 Degrees in a scale of 0-90



► Similar spectra for structurally related compounds

$$\sin \theta_j = \frac{\sqrt{\sum_{i=1}^n (B_{ij} - s_j A)^2}}{\sqrt{\sum_{i=1}^n B_{ij}^2}}$$

0.5 Degrees in a scale of 0-90

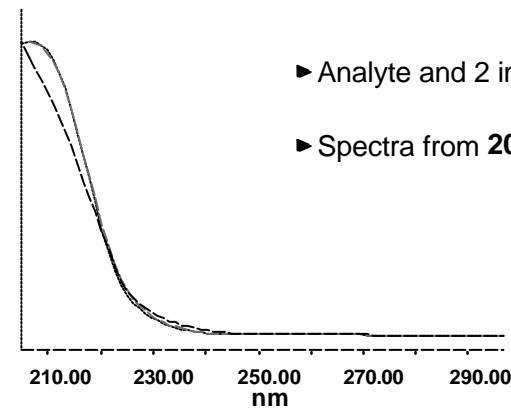


► Very similar spectra, CH2 difference

► Spectral Contrast can differentiate these spectra

$$\sin \theta_j = \frac{\sqrt{\sum_{i=1}^n (B_{ij} - s_j A)^2}}{\sqrt{\sum_{i=1}^n B_{ij}^2}}$$

Very Similar Spectra



► Analyte and 2 impurities

► Spectra from 200 to 300 nm

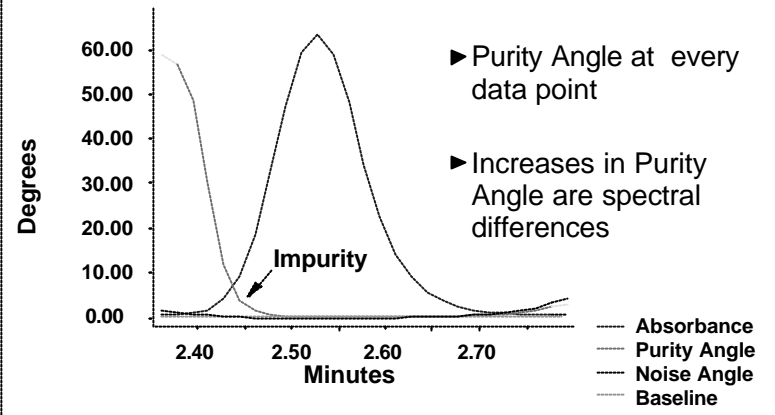
$$\sin \theta_j = \frac{\sqrt{\sum_{i=1}^n (B_{ij} - s_j A)^2}}{\sqrt{\sum_{i=1}^n B_{ij}^2}}$$

Interpretation of Peak Purity Plots

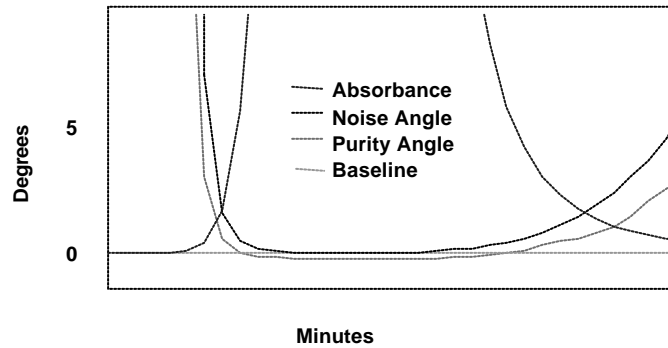
Peak Purity Plots can indicate

- ▶ Peak homogeneity
- ▶ Spectral homogeneity
- ▶ Coeluting impurities
- ▶ Spectral differences due to artifacts

Peak Purity Plot

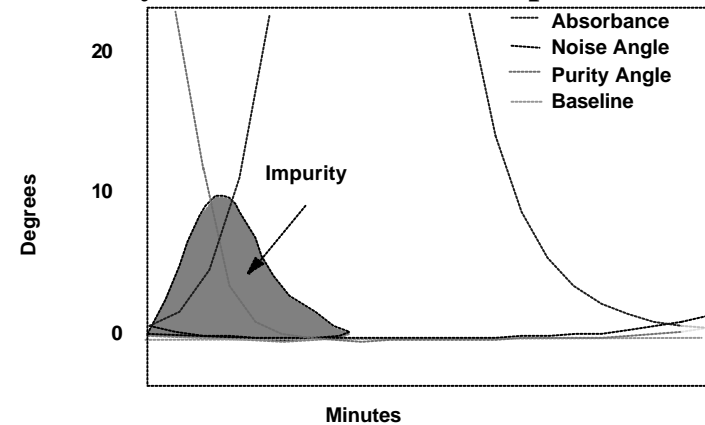


Purity Plot Chemically Pure Compound



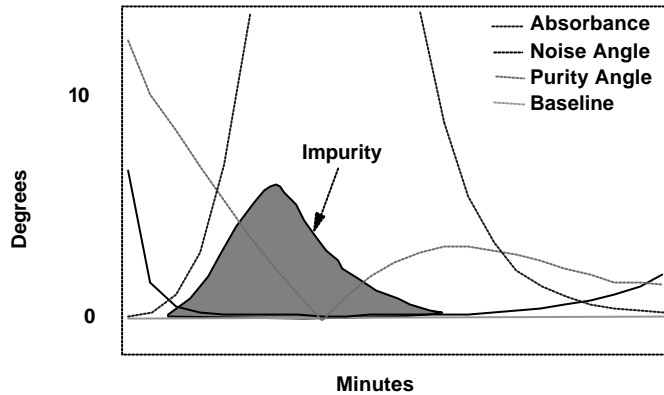
▶ Purity Angle less than Noise Angle, ideal situation

Purity Plot: Mixture of 2 Compounds



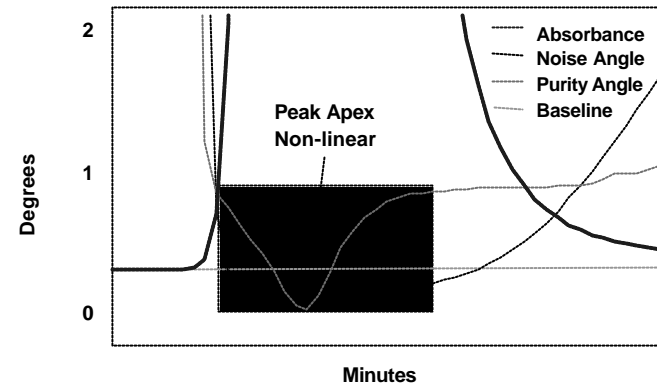
▶ Purity Angle is greater than Noise Angle - coelution on the front of the peak

Purity Plot: Mixture of 2 Compounds



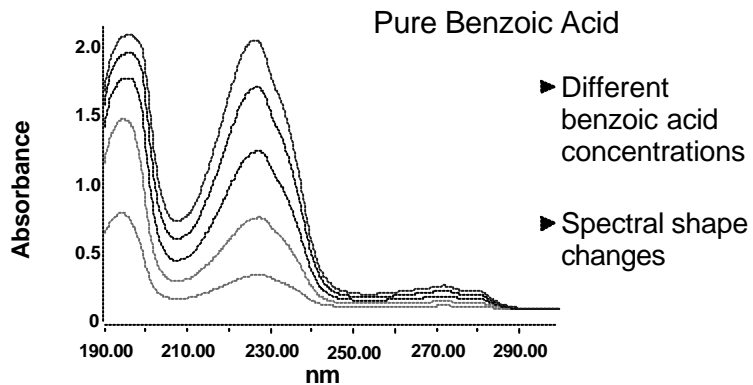
► Purity Angle is greater than Noise Angle - coelution near the peak apex

Purity Plot Chemically Pure Compound



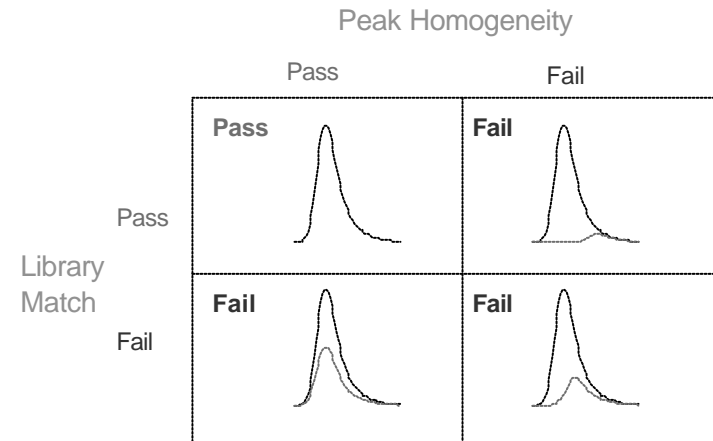
► Purity Angle greater than Noise Angle
► Absorbance **out of linear range** at some wavelengths

Effect of Concentration on Spectra

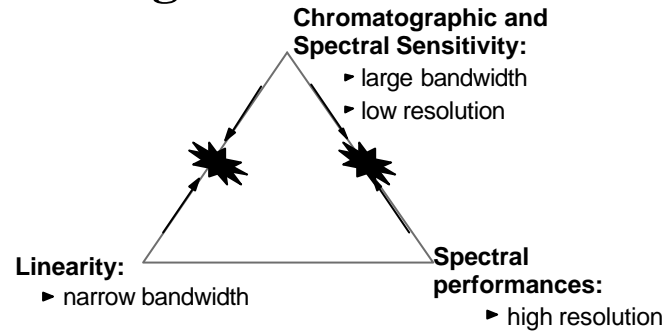


► Different benzoic acid concentrations
► Spectral shape changes

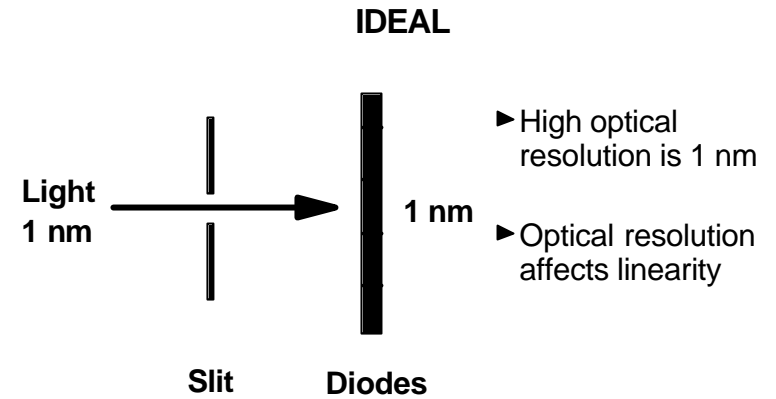
Compound Confirmation



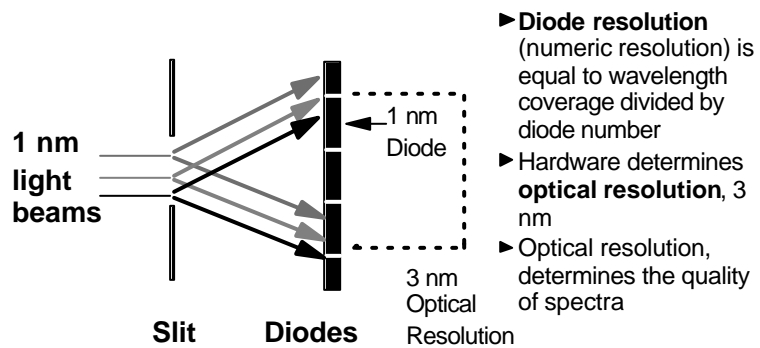
Conflicts in Instrument design



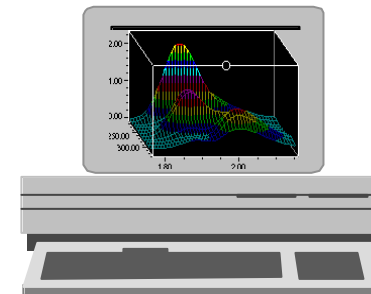
Optical vs. Diode Resolution



Optical vs. Diode (Numeric) Resolution



Photodiode Array Technology Optical Performance

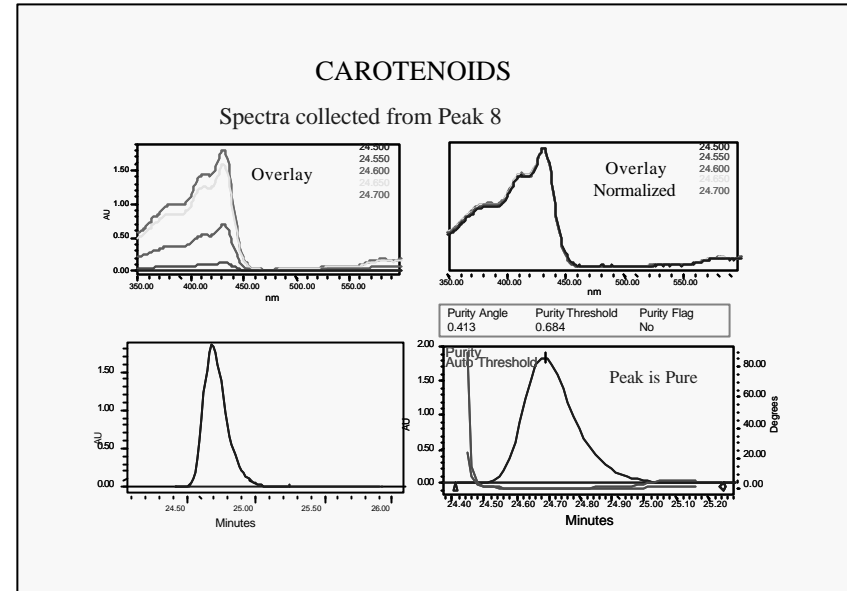


Linearity
Optical Resolution
Sensitivity

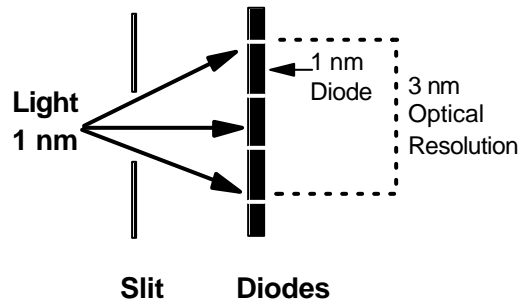
Importance of Detector Linearity

- ▶ Quantitation
 - Major peaks
 - Minor peaks

- ▶ Spectral Analyses
 - Library Matching
 - Peak Purity/Peak Homogeneity

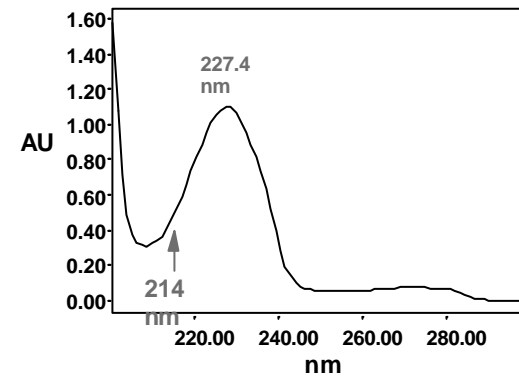


Optical vs. Diode Resolution



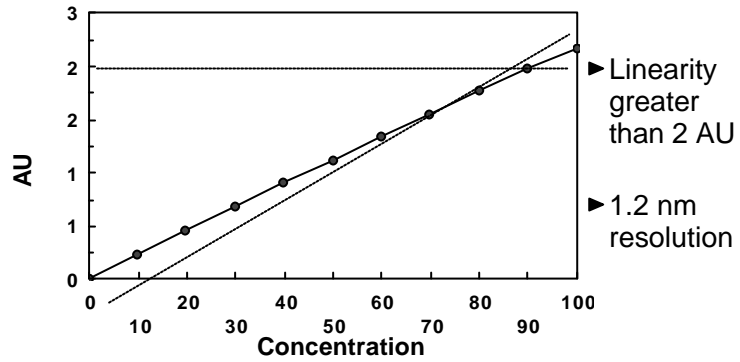
- ▶ Slit width determines optical resolution, 3 nm
- ▶ 1 nm per diode is 1 nm diode resolution

Benzoic Acid Spectrum

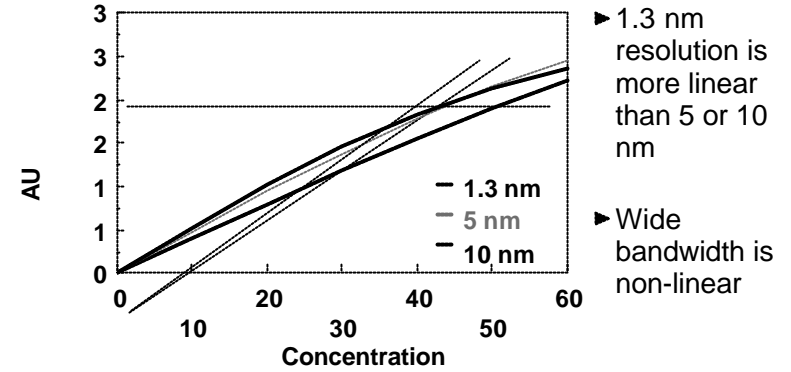


- ▶ 214 nm is on a spectral slope
- ▶ Linearity requires good optical resolution

Linearity 214 nm Benzoic acid



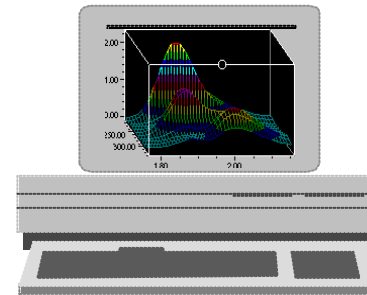
Effects of Optical Resolution on Linearity



Other Causes of Non-Linearity

- ▶ Second order effects
- ▶ Stray light
- ▶ Chemical interactions

Photodiode Array Technology Optical Performance



Linearity
Optical Resolution
Sensitivity

Resolution

Resolution can be improved by:

- ▶ 1) using a small slit
- ▶ 2) selecting a narrow bandwidth
- ▶ 3) Reducing the wavelength covering (nm/diode)
- ▶ 4) Enlarging the number of diodes

Overall quality of optics design and manufacturing is a crucial factor

Resolution

Drawbacks:

- ▶ 1) Small slit: less energy means more noise
- ▶ 1) Reduce the wavelength range: lack of information in the visible
- ▶ 2) More diodes: smaller diodes means noisier signal (less energy on each diode)

Quality of optics design and manufacturing: means important R&D plus QC efforts from the supplier

Importance of Optical Resolution

- ▶ Differentiation of Spectral Differences
 - Similar spectra
 - Spectral fine structure
- ▶ Spectral Analyses
 - Library matching
 - Peak purity / peak homogeneity

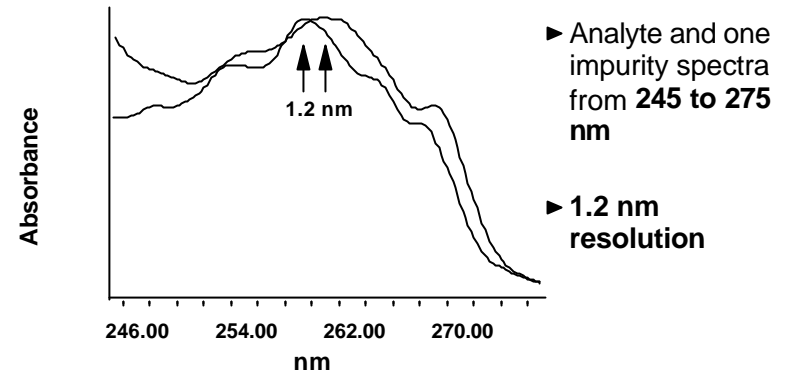
Common Perceptions

- ▶ Most UV spectra have very broad spectral peaks
- ▶ Good optical resolution is only required when there is spectral fine structure

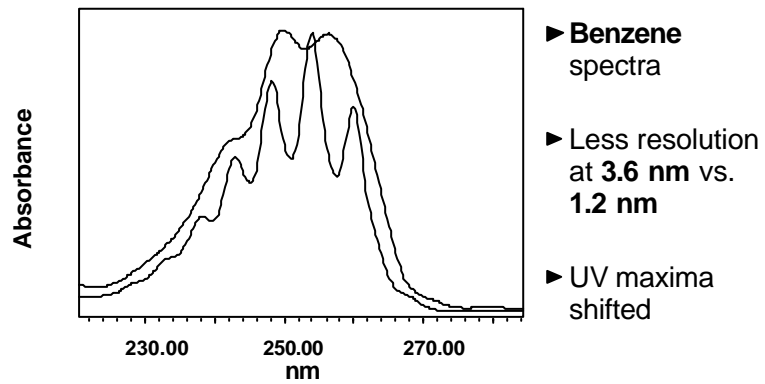
Factors Affecting Spectral Resolution

- ▶ Optical resolution
- ▶ Diode or digital resolution
- ▶ Slit width and bandwidth

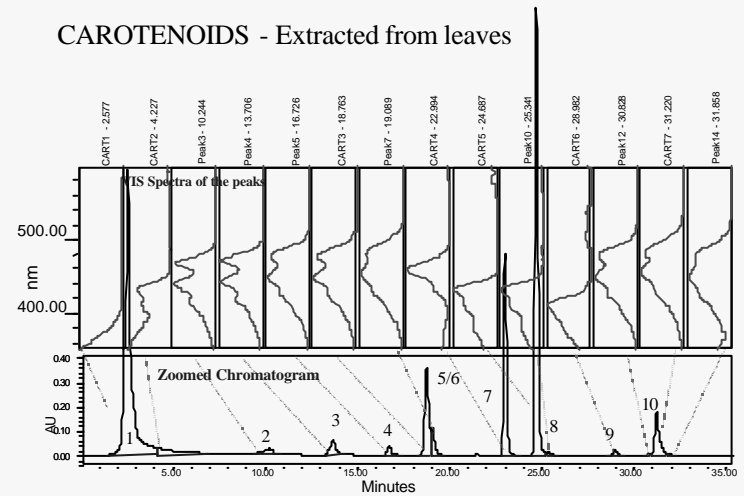
Spectral Fine Structure



Spectral Resolution - 1.2 nm vs. 3.6 nm



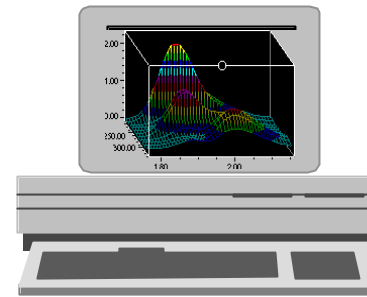
CAROTENOIDS - Extracted from leaves



Benefits of Good Optical Resolution

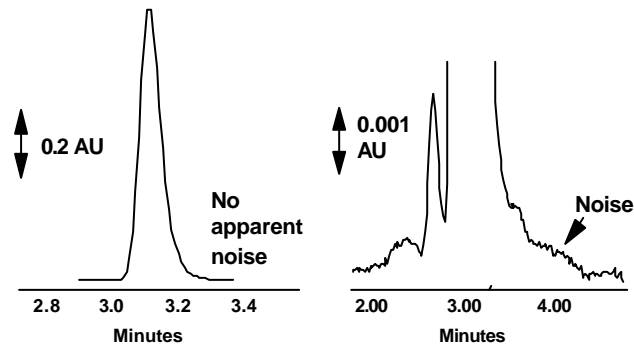
- ▶ Peak confirmation
 - Confidence in compound identification
 - Confidence in peak homogeneity with good peak purity analysis
- ▶ Good detector linearity
 - Quantitation at high and low concentrations
 - Spectral analyses
 - Identification of major and minor compounds

Photodiode Array Technology Optical Performance



Linearity
Optical Resolution
Sensitivity

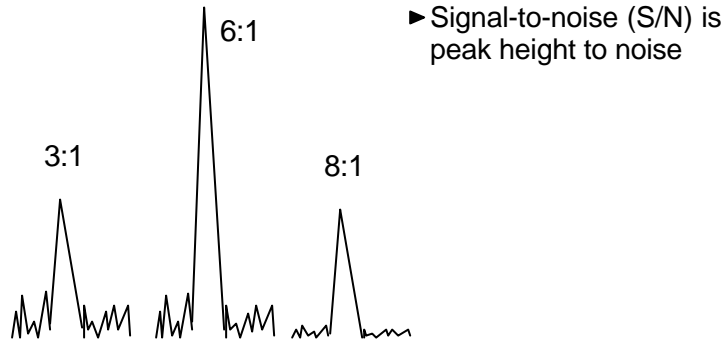
Chromatographic Sensitivity Signal-to-Noise Ratio



Importance of Sensitivity

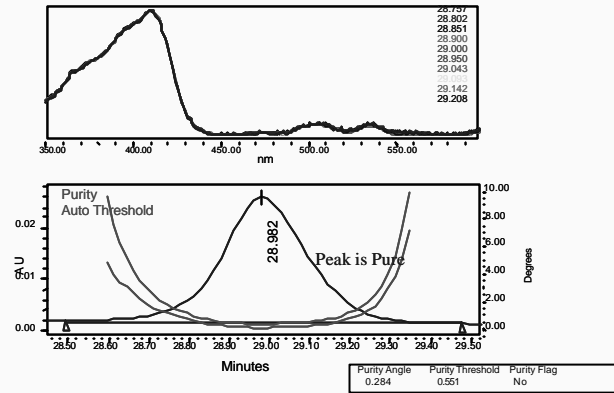
- ▶ Detection of Low Concentrations of Analytes
 - Detection of impurities, metabolites, by-products and degradation products
 - Quantitation
- ▶ Detection of Spectra at Low Concentrations
 - Peak identification
 - Peak purity / peak homogeneity

Signal-to-Noise Ratio

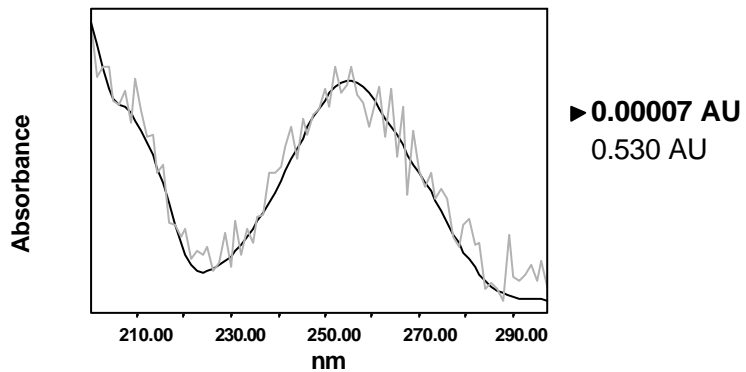


CAROTENOIDS

Spectra collected from Peak 9



High Sensitivity Spectrum



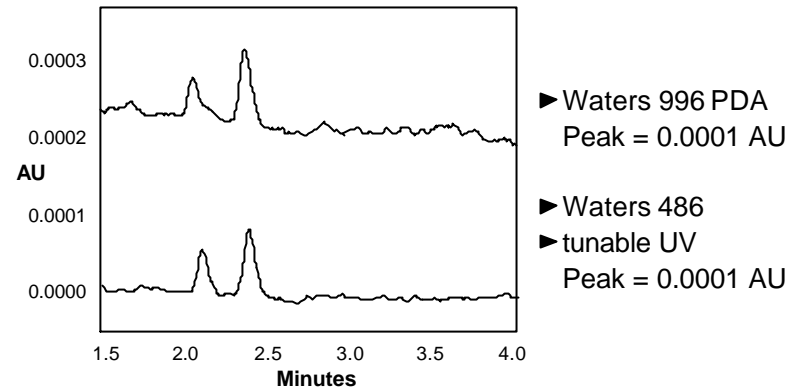
Perceptions

- Photodiode array detectors (PDA) are much less sensitive than variable wavelength detectors
- PDA detectors are noisy
- PDA detectors can not be used for quantitation of minor peaks

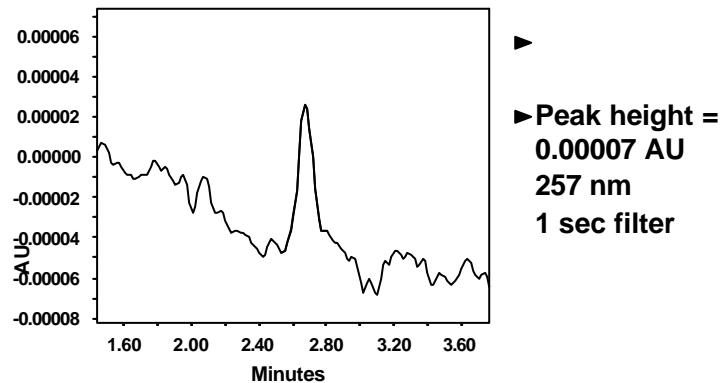
Technological Advances in PDA Detectors

- ▶ New designs to improve signal-to-noise performance
- ▶ Increased chromatographic sensitivity
- ▶ Increased spectral sensitivity
- ▶ Enhanced software for improved performance

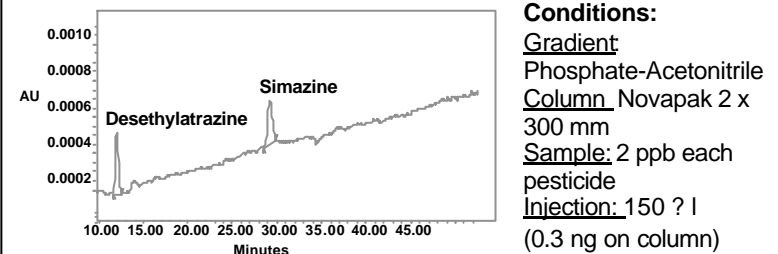
Waters 996 Chromatographic Sensitivity



High Sensitivity Chromatogram



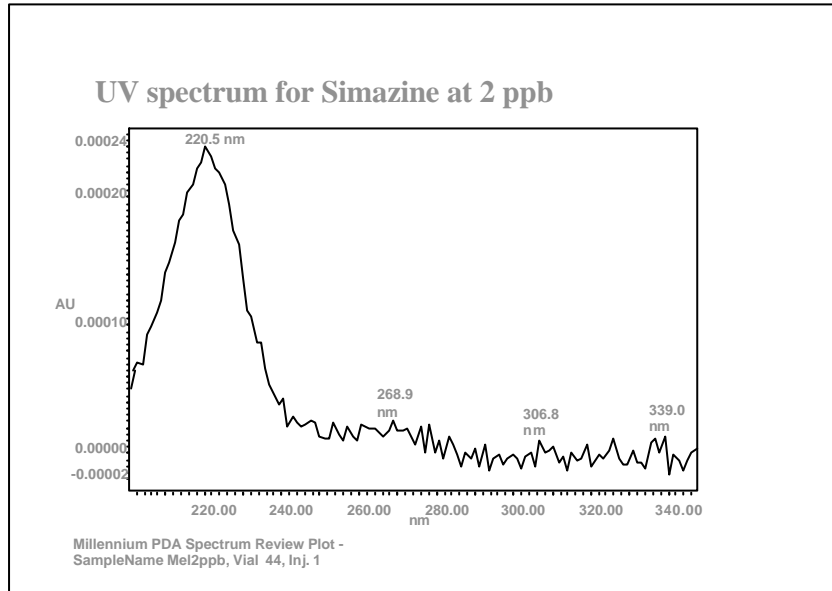
Chromatographic Sensitivity Triazine herbicides at detection limit



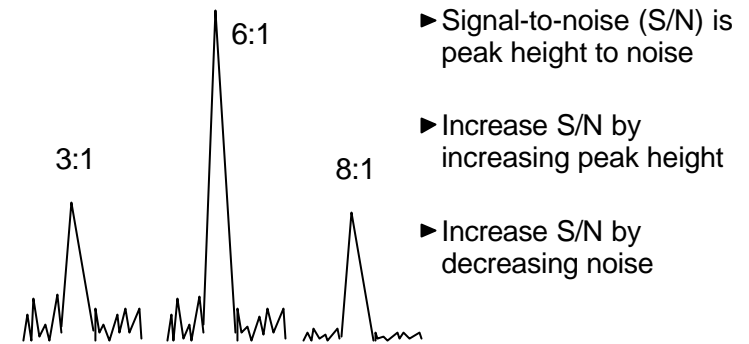
PDA

Resolution: 1.2 nm,
Acquisition: 200 to 350 nm, 2 spectra per second.
Chromatogram extracted at 220 nm
No smoothing or bunching

Diode Array Detectors



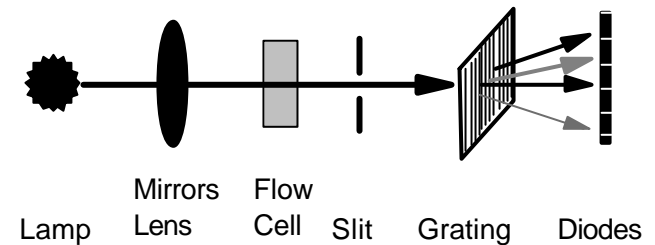
Increase Signal-to-Noise Ratio



Factors Increasing Signal

- ▶ Increase sample concentration
- ▶ Increase injection volume
- ▶ Wavelength
- ▶ Low volume flow cell

PDA Optics Diagram



- ▶ Each component in the optics path will affect noise

Factors Affecting Noise

- ▶ Optics bench design
- ▶ Lamp energy
- ▶ Wavelengths
- ▶ Mobile phase
- ▶ Resolution
- ▶ Filter

Technical approaches to gain in chromatographic sensitivity

Traditional approaches:

- ▶ enlarge slit width (decrease resolution)
- ▶ change optical resolution (affects spectrum)
- ▶ diode bunching (affects spectrum)
- ▶ noise smoothing (affects peak shape and height)
- ▶ reference wavelength subtraction (loss of information in the subtracted band)

Sophisticated approaches:

- ▶ optimize the optics design: minimum dispersion, good focus of light on the diodes
- ▶ lamp optimization software (eliminate the need for different slits)