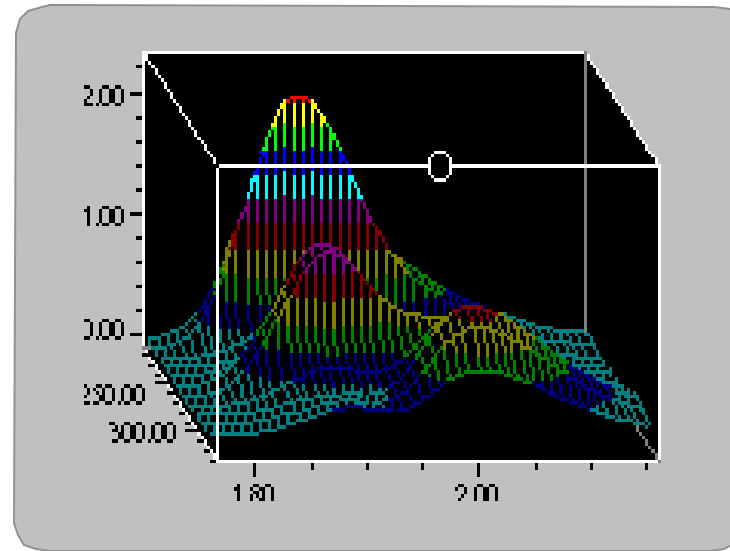
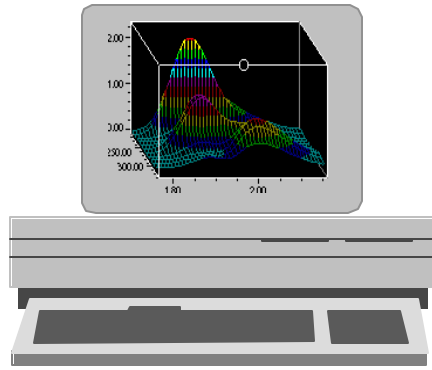


Photodiode Array



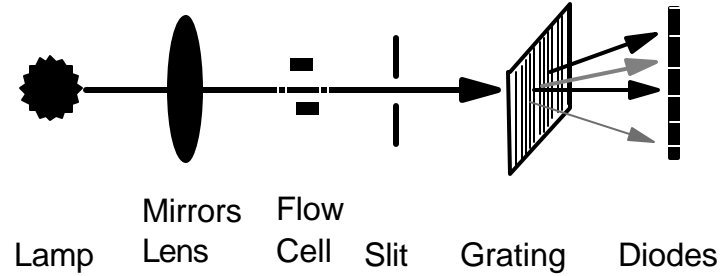
Advanced Detection Technologies
for Compound Identification

Photodiode Array

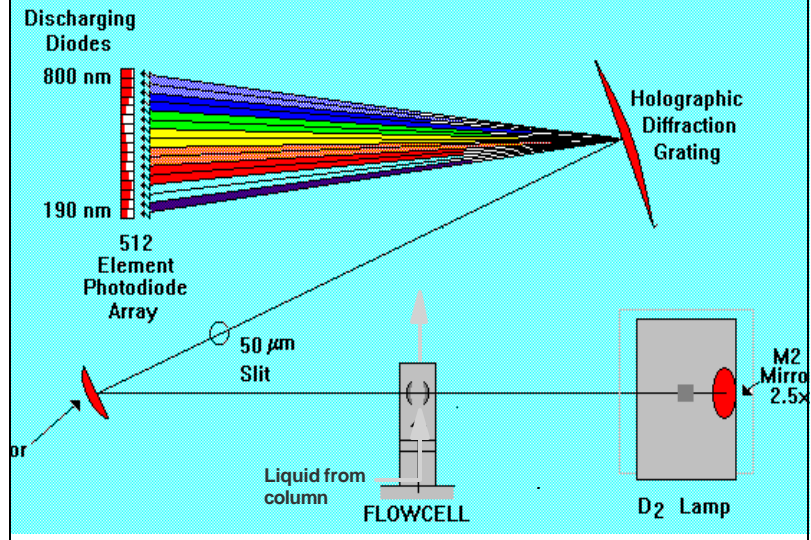


Advanced Detection Technologies
for Compound Identification

PDA Optics Diagram

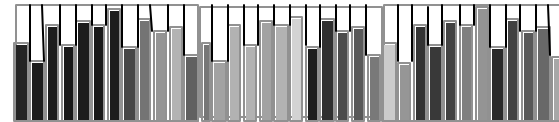


Principle of Measurement

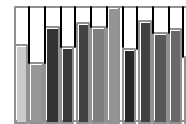


How the Diode Array Works

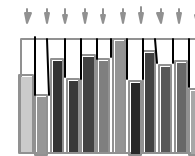
The λ of light striking the diode is determined by its position relative to the stationary grating



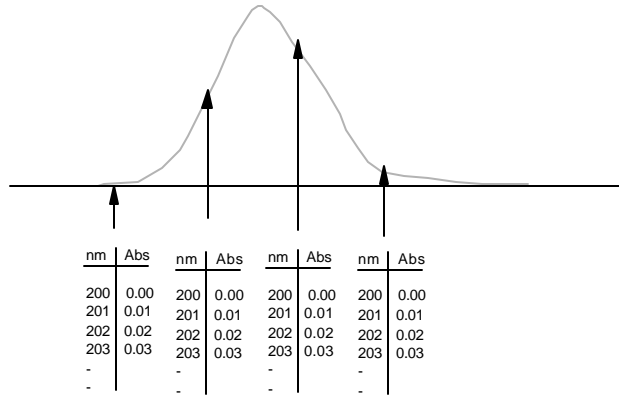
Diodes discharged



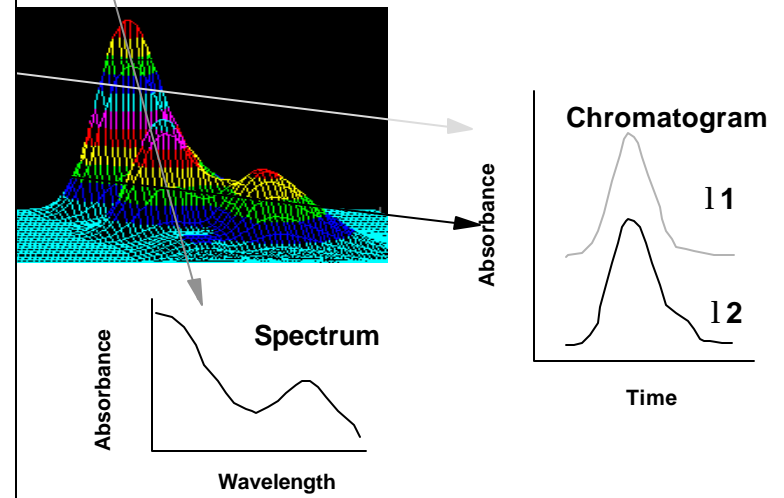
Diodes recharging



The Data is 3D

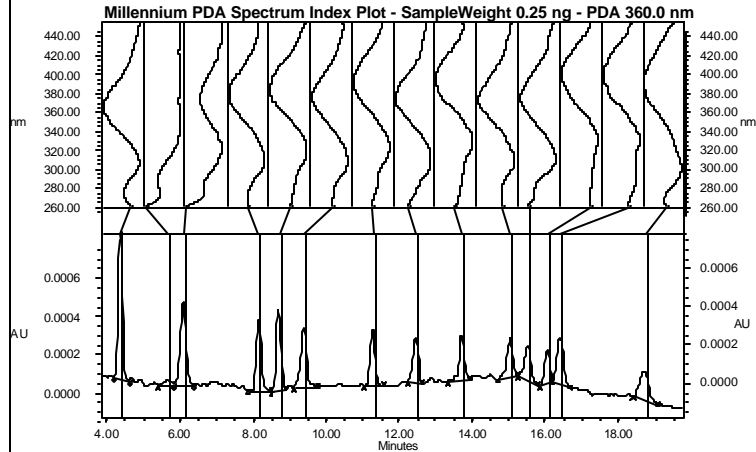


Extraction of 3D Data

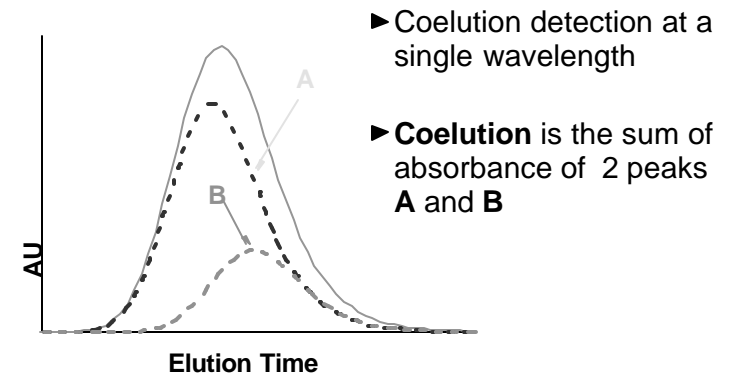


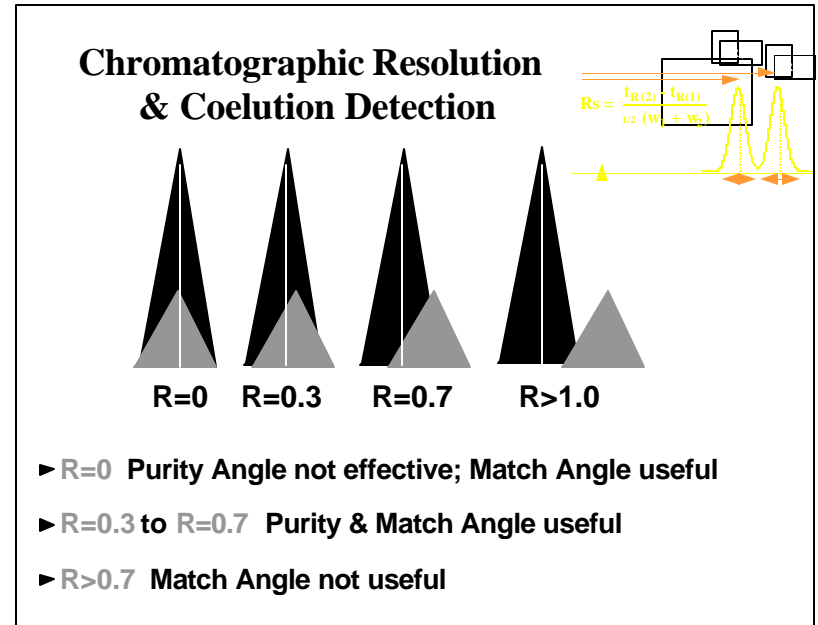
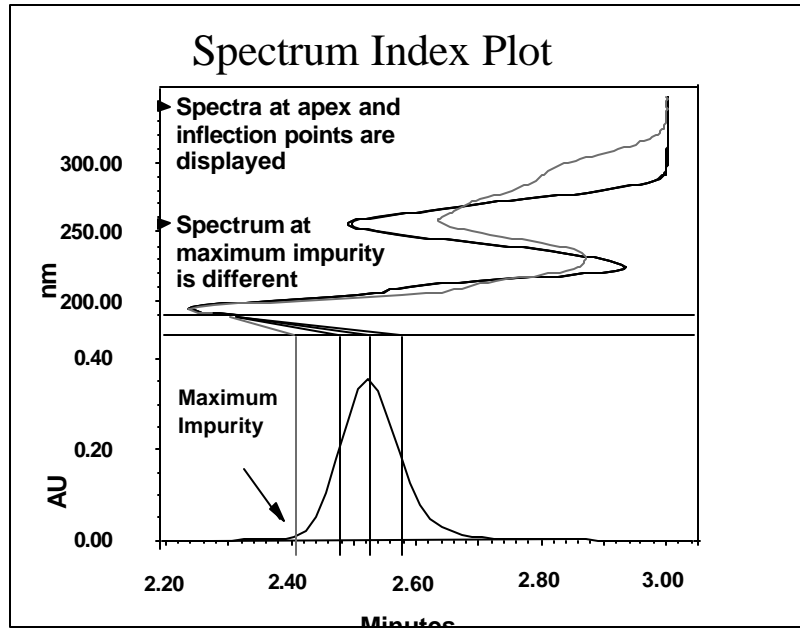
996 PDA Spectrum Index Plot

DNPH Derivatives 0.25 ng Each Peak



Coelution of 2 Peaks





Photodiode Array Technology

Spectral Analyses

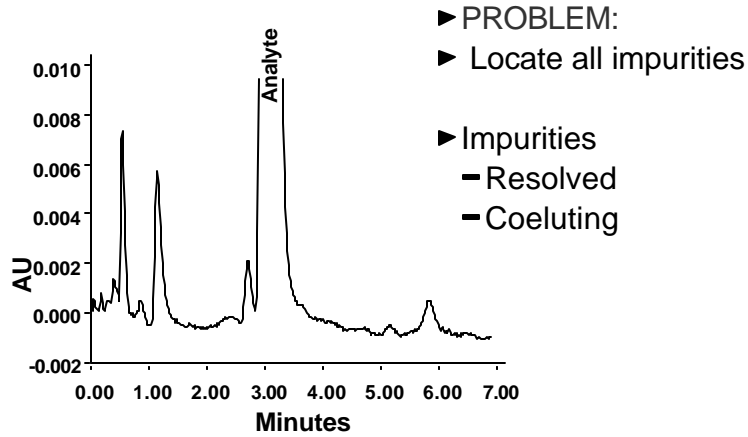
The diagram shows a computer monitor displaying a spectral plot with multiple overlapping peaks. The plot has a y-axis from 0.00 to 2.00 and an x-axis from 2.00 to 3.00. Below the monitor is a keyboard.

- ▶ 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

Major Peak and Minor Peaks

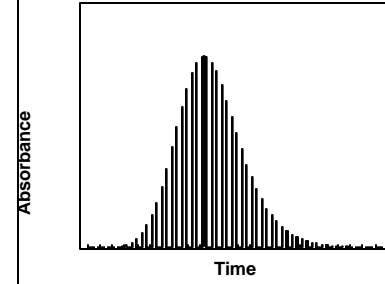


Good quality spectral

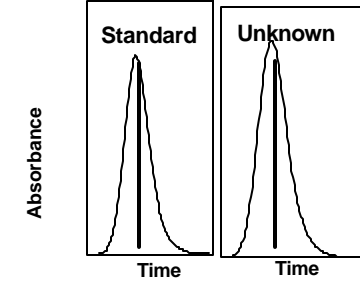
Information is important for:

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

Purity verification

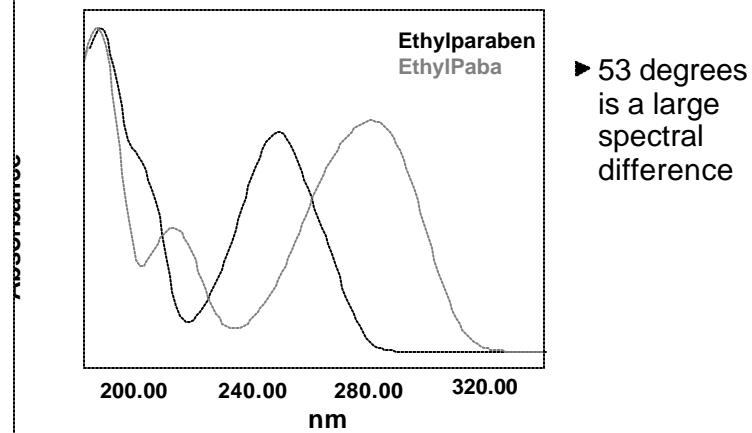


Library identification

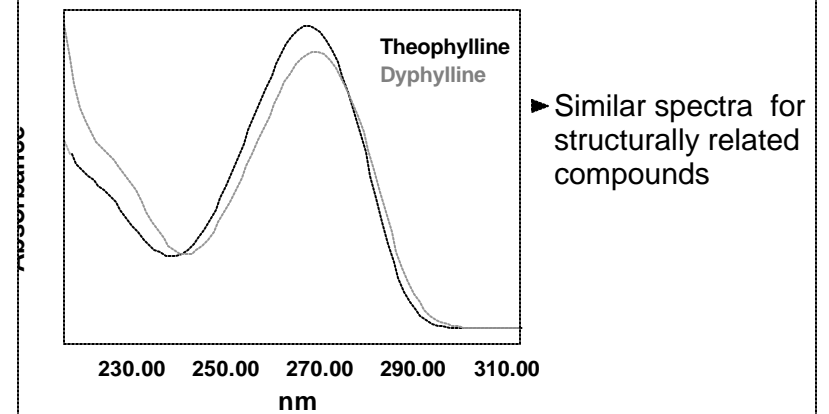


- Peak Purity analyzes all spectra (minimum 15) within a peak
- Apex spectrum is the reference spectrum
- Matching compares the unknown apex spectrum of the peak with a reference spectrum in a library

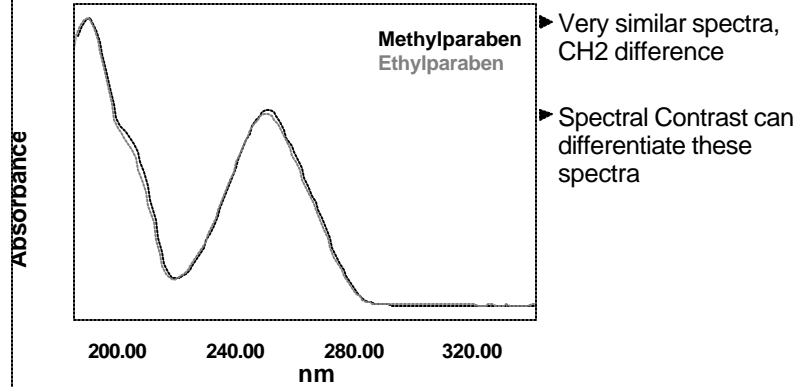
Spectral Contrast 53 Degrees



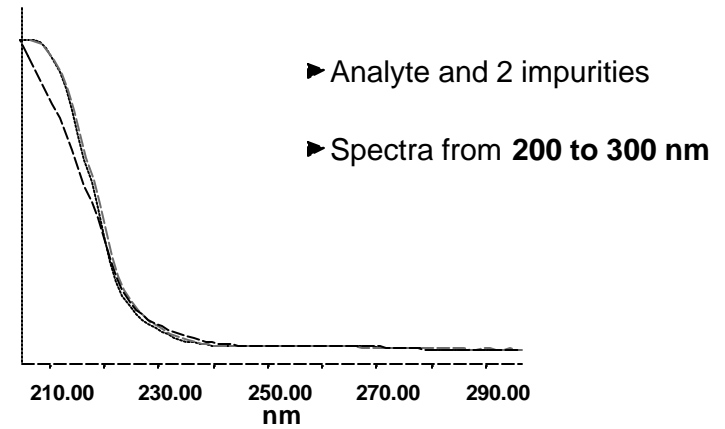
Spectral Contrast 10 Degrees



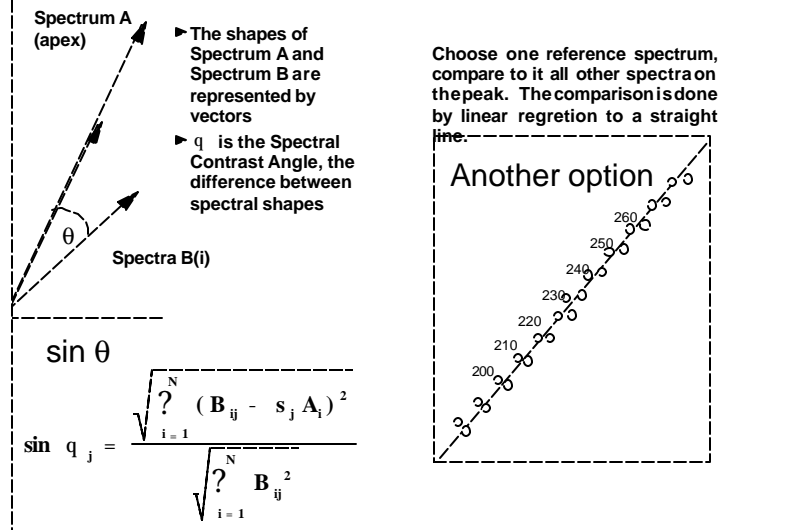
Spectral Contrast 0.5 Degrees



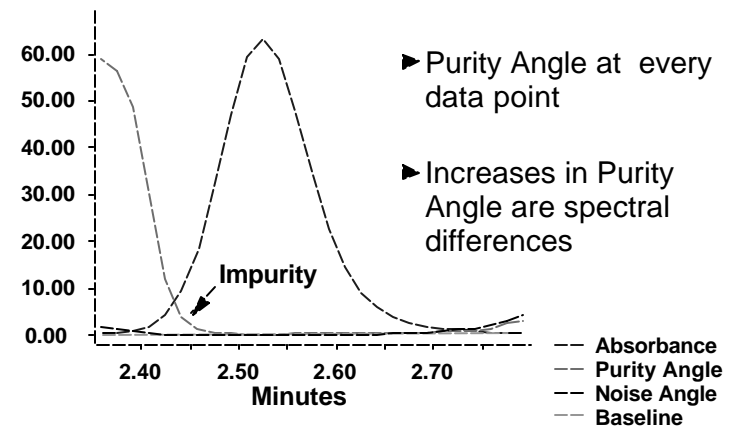
Very Similar Spectra



Spectral Comparison



Peak Purity Plot

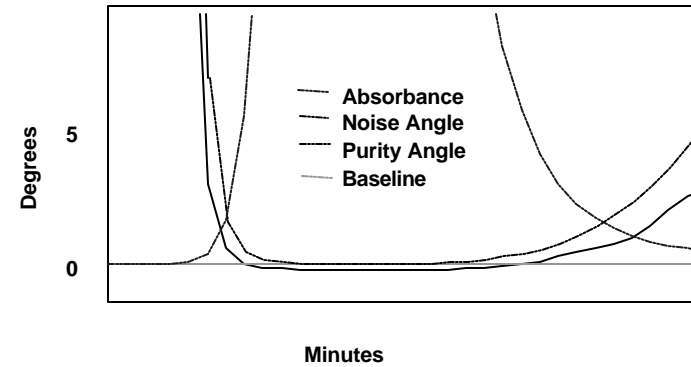


Interpretation of Peak Purity Plots

Peak Purity Plots can indicate

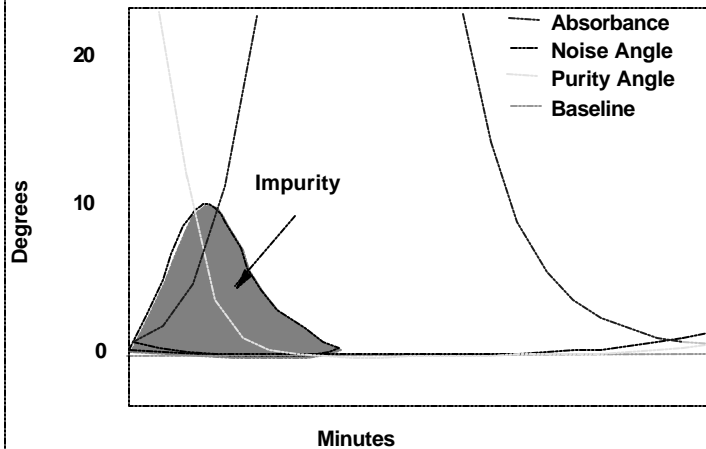
- ▶ Peak homogeneity
- ▶ Spectral homogeneity
- ▶ Coeluting impurities
- ▶ Spectral differences due to artifacts

Purity Plot Chemically Pure Compound



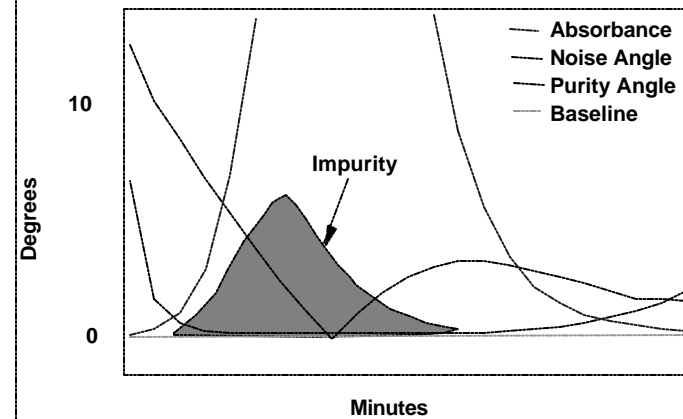
- ▶ Purity Angle less than Noise Angle, ideal situation

Purity Plot: Mixture of 2 Compounds

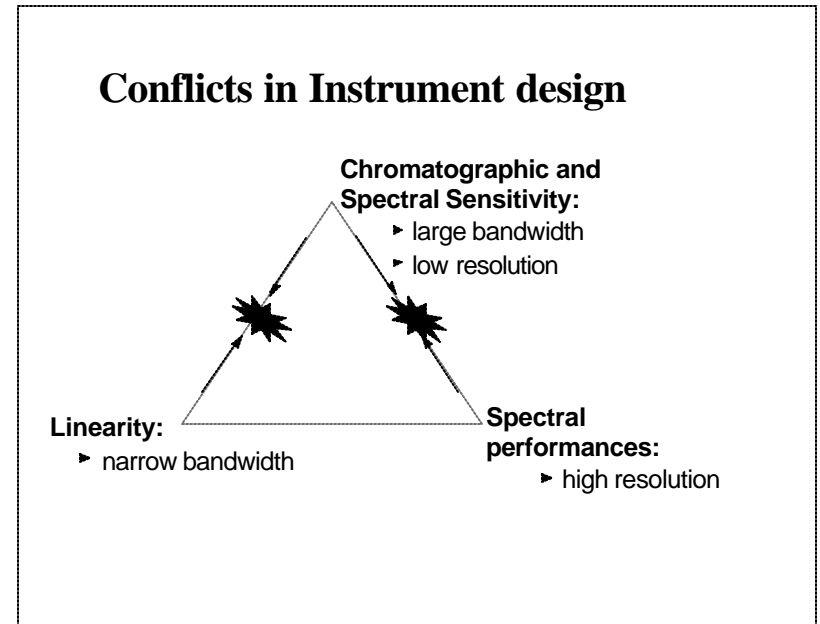
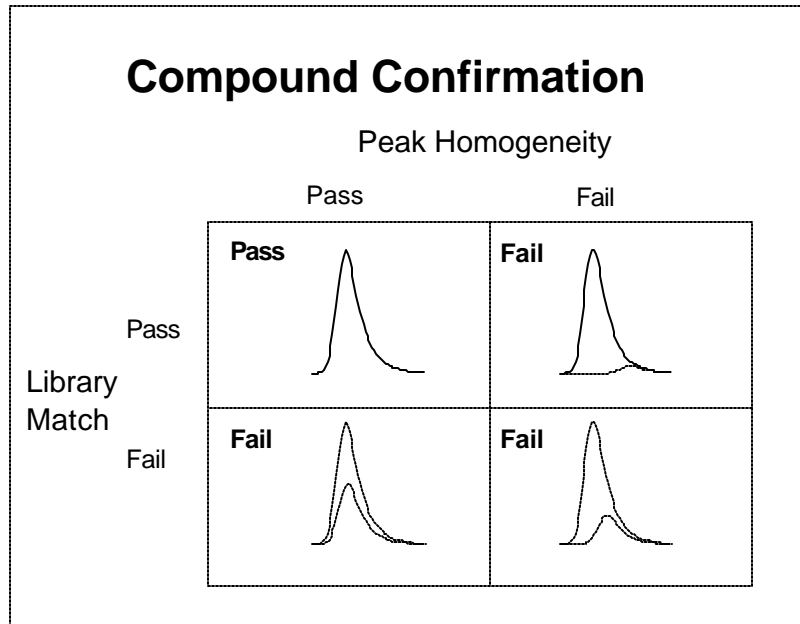
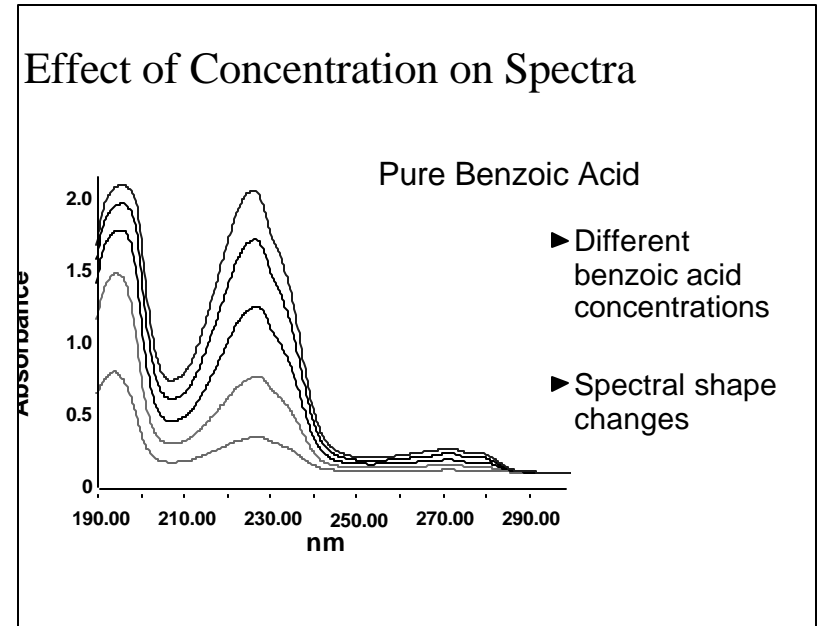
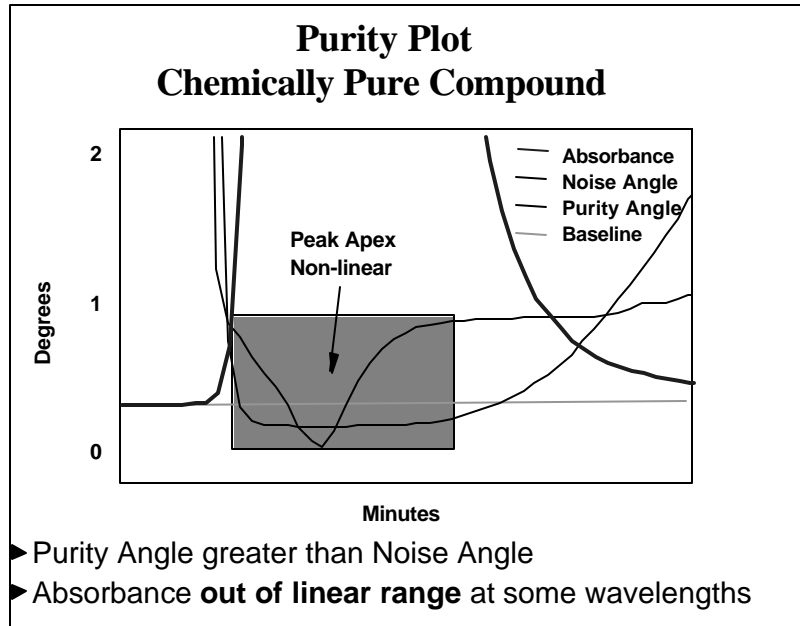


- ▶ Purity Angle is greater than Noise Angle - coelution on the front of the peak

Purity Plot: Mixture of 2 Compounds

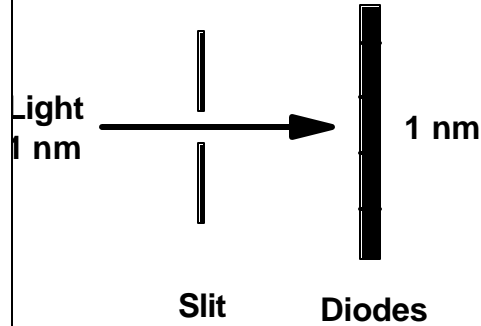


- ▶ Purity Angle is greater than Noise Angle - coelution near the peak apex



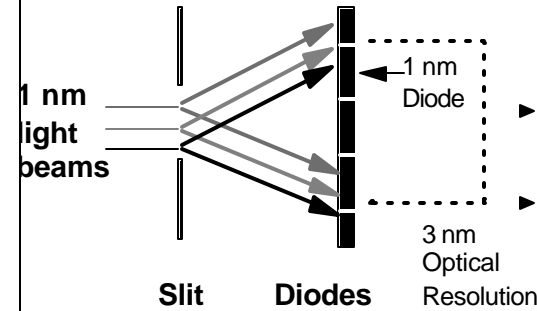
Optical vs. Diode Resolution

IDEAL



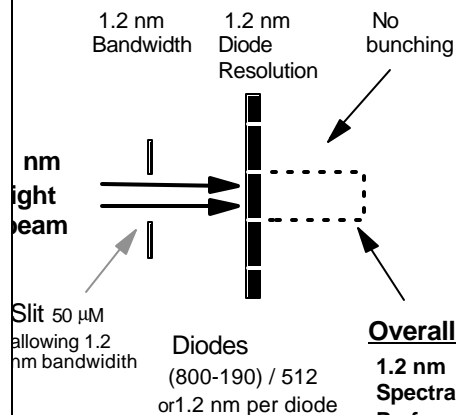
- ▶ High optical resolution is 1 nm
- ▶ Optical resolution affects linearity

Optical vs. Diode (Numeric) Resolution



- ▶ **Diode resolution** (numeric resolution) is equal to wavelength coverage divided by diode number
- ▶ Hardware determines **optical resolution**, 3 nm
- ▶ Optical resolution, determines the quality of spectra

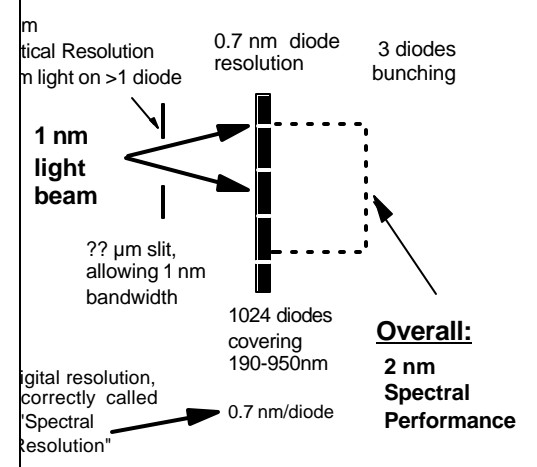
996 Spectral Performance



- ▶ Slit width, # diodes, and WL range (hardware) determine optical resolution: 1.2 nm
- ▶ nm/diode (hardware) determine diode resolution, 1.2 nm
- ▶ Diode 'bunching' (software) determines the **overall spectral performance, 1.2 nm**

Overall:
1.2 nm Spectral Performance

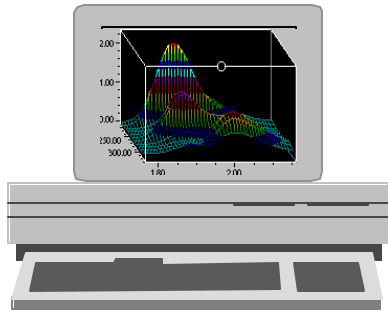
Brand X Spectral Performance 1nm slit, 2 nm 'bunch'



- ▶ Slit width, # diodes, and WL range (hardware) determine optical resolution: 1 nm
- ▶ nm/diode (hardware) determine diode resolution, 0.7 nm
- ▶ Diode 'bunching' (software) determines the **spectral performance, 2 nm**

Overall:
2 nm Spectral Performance

Photodiode Array Technology Optical Performance

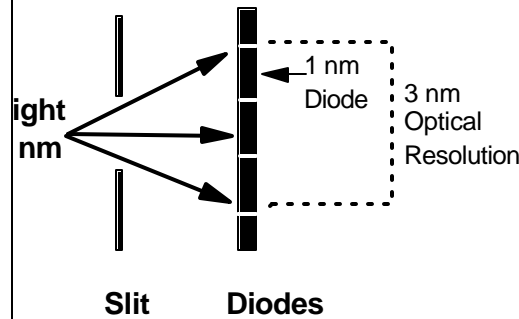


Linearity
Optical Resolution
Sensitivity

Importance of Detector Linearity

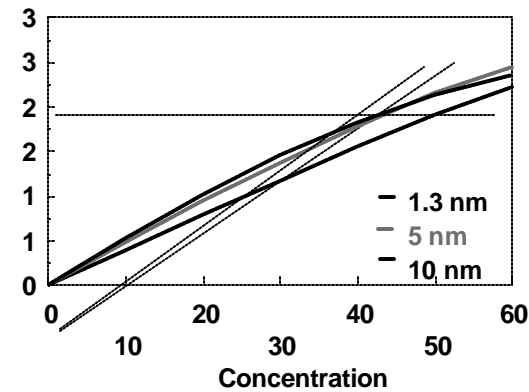
- ▶ Quantitation
 - Major peaks
 - Minor peaks
- ▶ Spectral Analyses
 - Library Matching
 - Peak Purity/Peak Homogeneity

Optical vs. Diode Resolution



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

Effects of Optical Resolution on Linearity



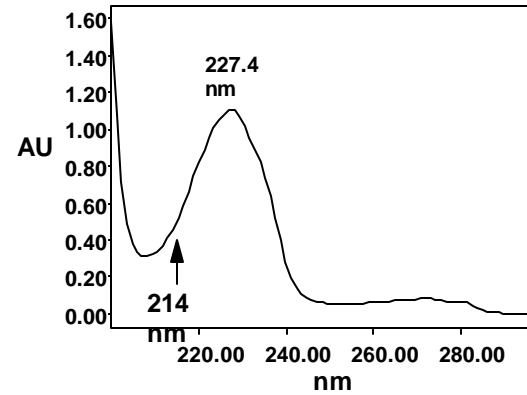
- ▶ 1.3 nm resolution is more linear than 5 or 10 nm
- ▶ Wide bandwidth is non-linear

Technical approaches to gain in linearity

Increase optical resolution

One forbidden technical approach: using a prism in place of a grating, since prisms are non linear

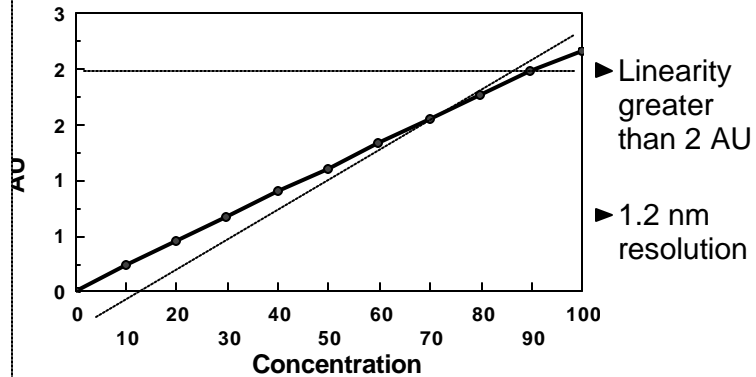
Benzoic Acid Spectrum



► 214 nm is on a spectral slope

► Linearity requires good optical resolution

Linearity 214 nm Benzoic acid



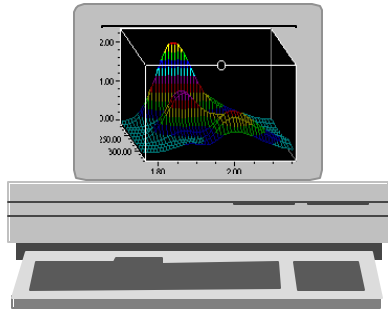
► Linearity greater than 2 AU

► 1.2 nm resolution

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

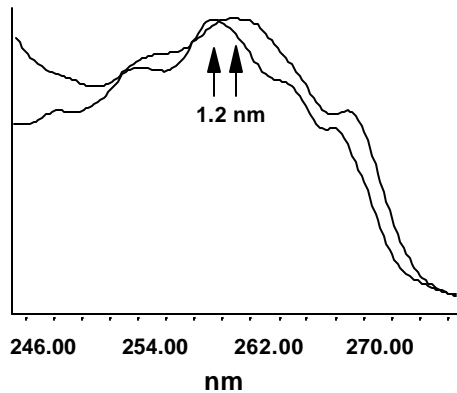
Common Perceptions

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

Factors Affecting Spectral Resolution

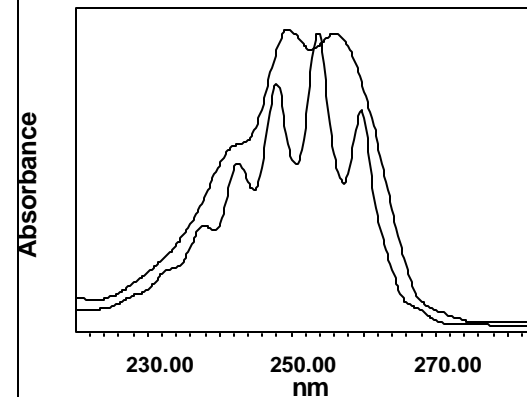
- ▶ Optical resolution
- ▶ Diode or digital resolution
- ▶ Slit width and bandwidth

Spectral Fine Structure

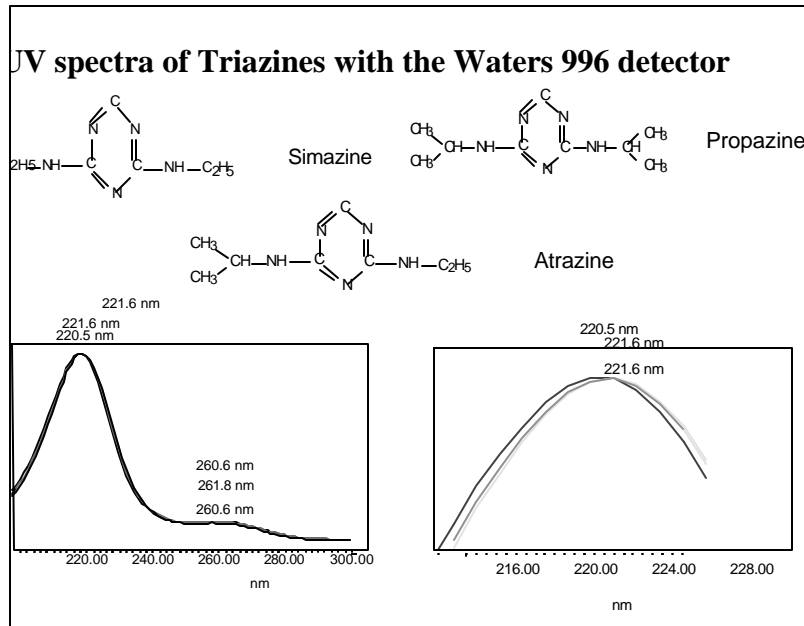


- ▶ Analyte and one impurity spectra from 245 to 275 nm
- ▶ 1.2 nm resolution

Spectral Resolution - 1.2 nm vs. 3.6 nm



- ▶ Benzene spectra
- ▶ Less resolution at 3.6 nm vs. 1.2 nm
- ▶ UV maxima shifted



Features and Advantages Optical Resolution

FEATURES

Less than 2 nm optical and diode resolution

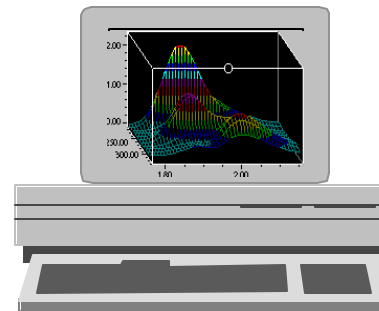
ADVANTAGES

- ▶ Differentiation of similar spectra
- ▶ Visualizing spectral fine structure
- ▶ Linearity 190 to 800 nm to 2 AU

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

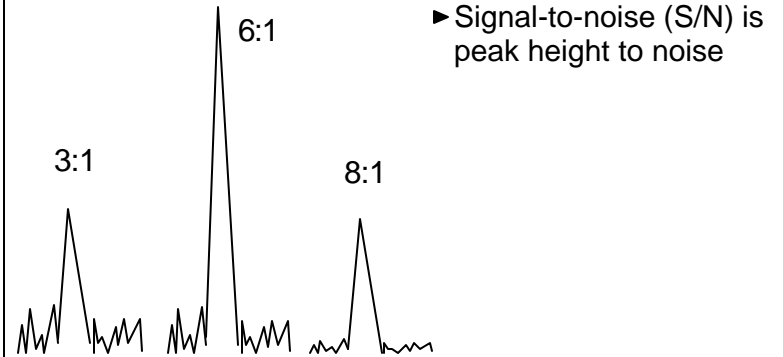


Linearity
Optical Resolution
Sensitivity

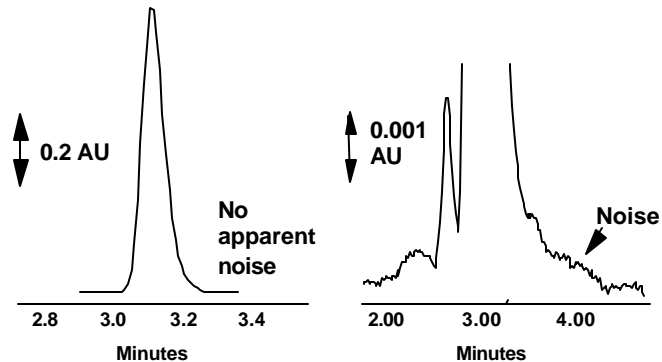
Importance of Sensitivity

- ▶ 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



Chromatographic Sensitivity Signal-to-Noise Ratio



Perceptions

Photodiode array detectors (PDA) are much less sensitive than variable wavelength detectors

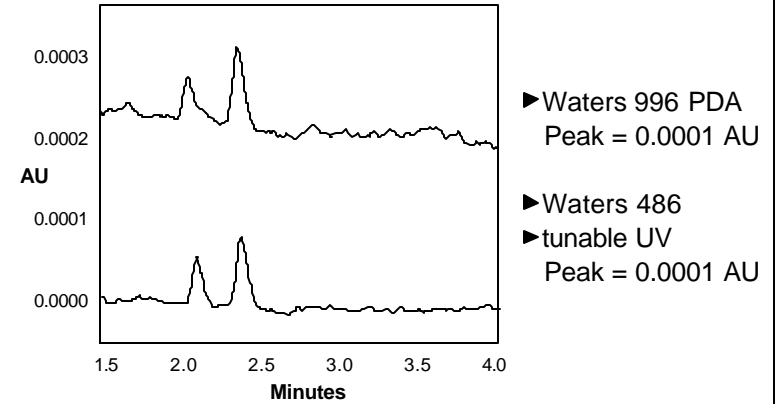
PDA detectors are noisy

PDA detectors can not be used for quantitation of minor peaks

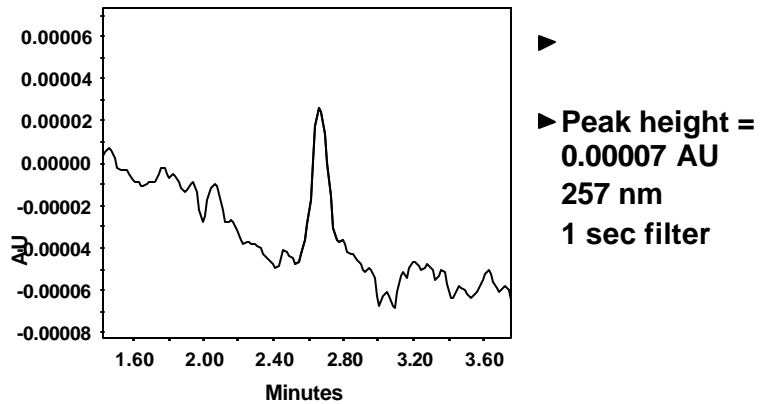
Technological Advances in PDA Detectors

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

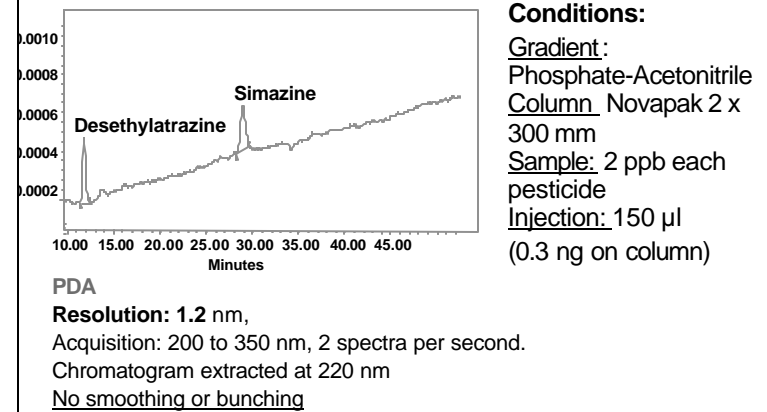
Waters 996 Chromatographic Sensitivity

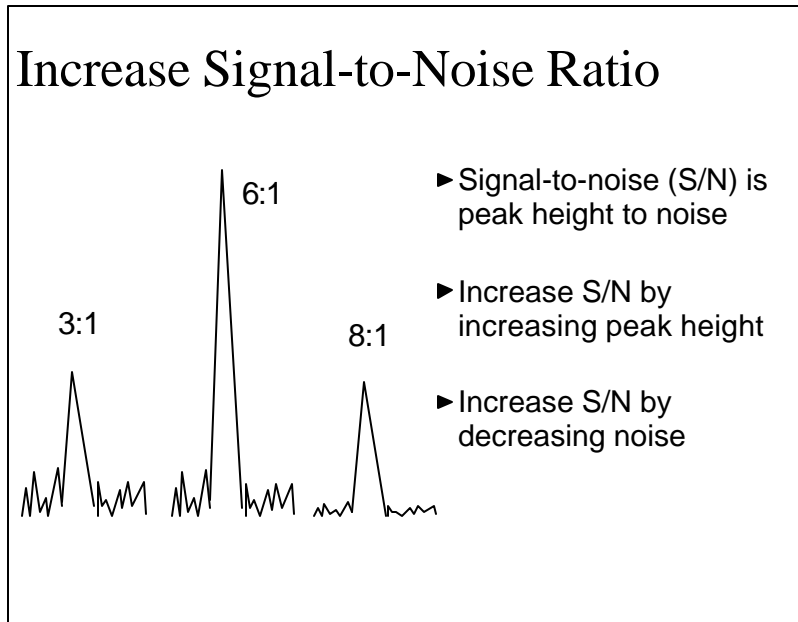
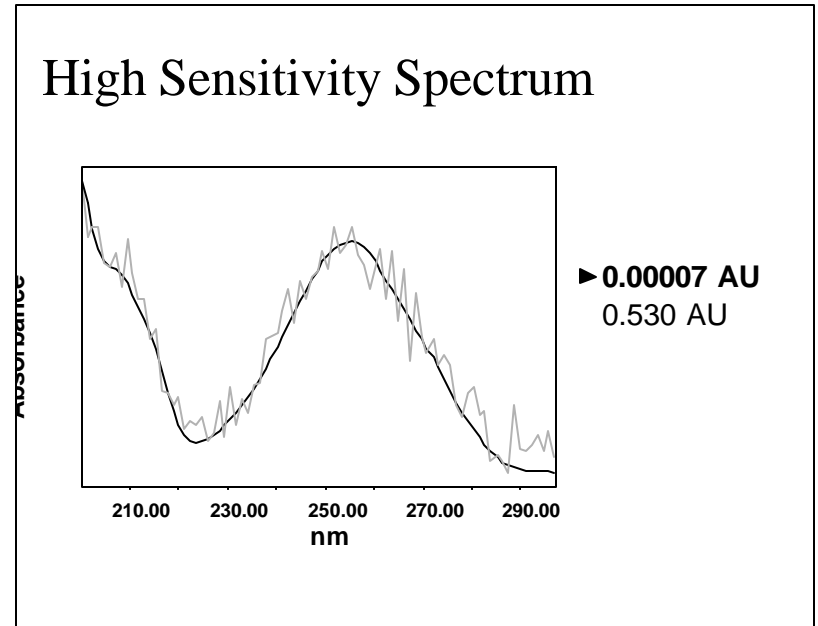
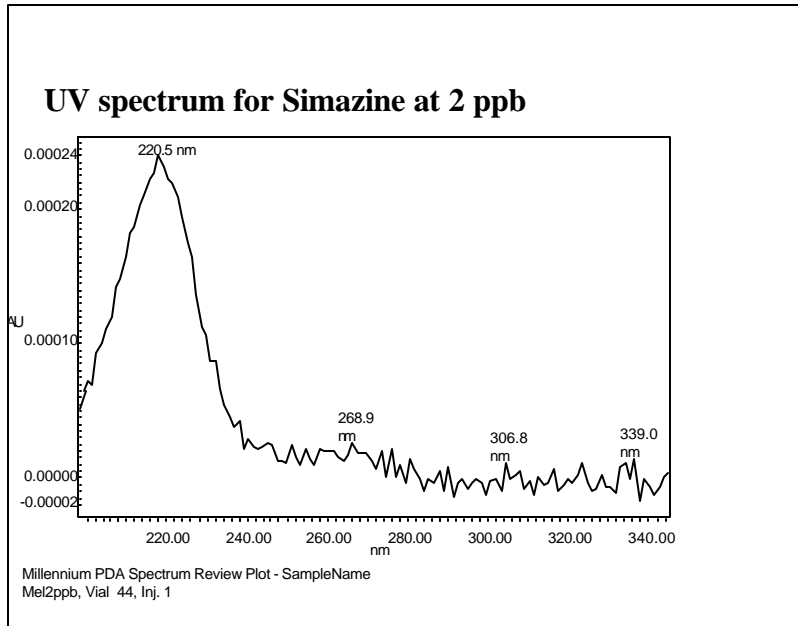


High Sensitivity Chromatogram



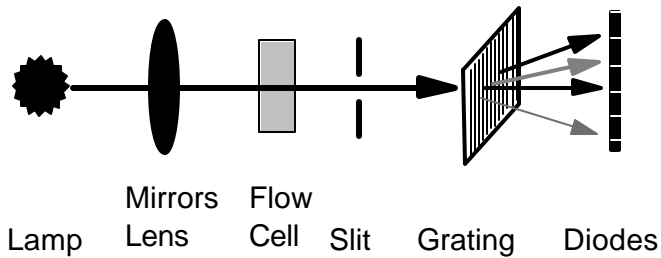
Chromatographic Sensitivity Triazine herbicides at detection limit





- ### 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

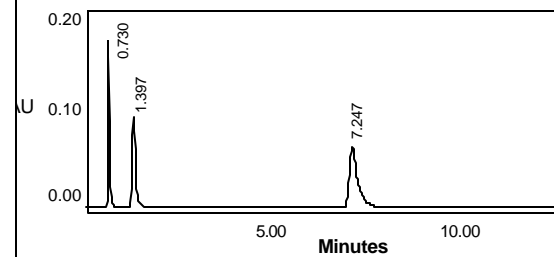
Traditional approaches:

- ▶ 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)

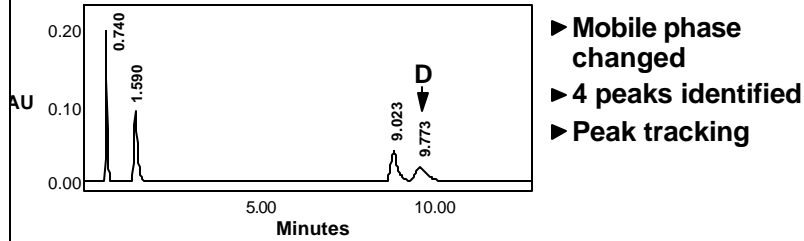
Method Development #1



PROBLEM
4 Compounds
3 Peaks

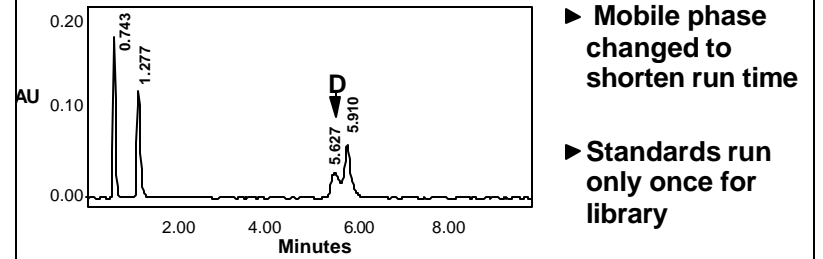
#	Ret Time (min)	Area (uV*sec)	Match Spectrum Name	Match Angle	Match Thresh.
1	0.730	651471	Peak A	0.096	1.163
2	1.397	655846	Peak B	0.071	1.284
3	7.247	1019807	Peak C	0.883	1.640

Method Development #2



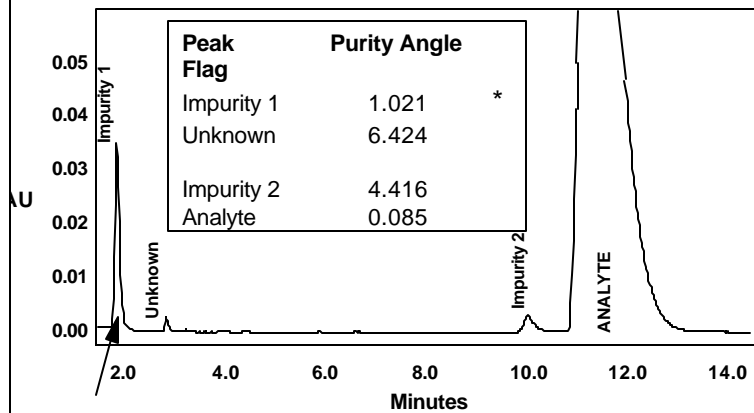
#	Ret Time (min)	Area (uV*sec)	Match Spectrum Name	Match Angle	Match Thresh.
1	0.740	660273	Peak A	0.042	1.203
2	1.590	666849	Peak B	0.026	1.347
3	9.023	560464	Peak C	0.079	1.839
4	9.773	434562	Peak D	0.516	2.747

Method Development #3

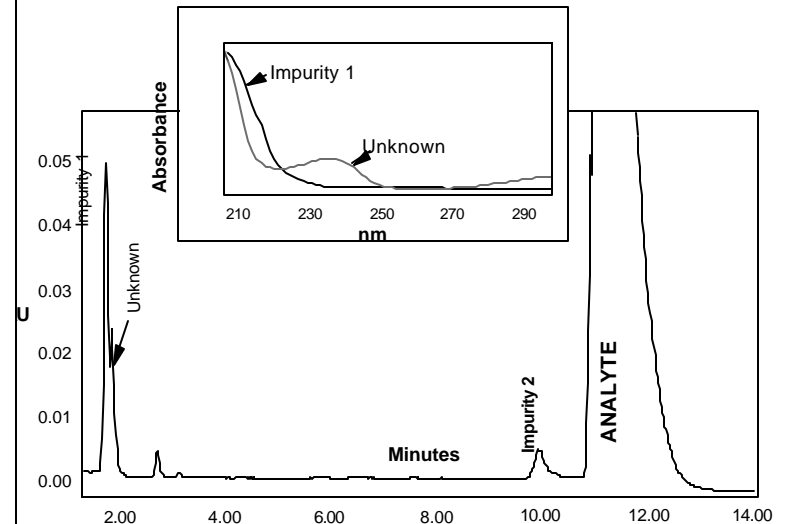


#	Ret Time (min)	Area (uV*sec)	Match Spectrum Name	Match Angle	Match Thresh.
1	0.743	652303	Peak A	0.139	1.161
2	1.277	654077	Peak B	0.125	1.274
3	5.627	366935	Peak D	1.455	2.366
4	5.910	682223	Peak C	0.369	1.649

Stability Test at 8 Weeks



Stability Test at 12 Weeks



Stability Data at 12 Weeks

#	Ret		Match		Purity		
	Time (Min)	Area %	Spectrum Name	Match Angle	Flag	Angle	Flag
1	1.777	4.95	Impurity 1	0.792	*	33.261	*
2	1.960	0.27					
3	2.743	0.28				6.461	
4	3.143	0.06	Impurity 2	6.542		12.879	
5	9.927	0.93				25.868	*
6	11.193	93.50	Analyte	0.154		0.092	

Peak Purity Using Photodiode Array Detection

- ▶ Spectral homogeneity or peak homogeneity
- ▶ **NEVER** Chemical Purity
 - Impurity has absorbance
 - Impurity is present in high enough concentration
 - Impurity is spectrally different from the analyte

Positive Compound Identification and Monitoring

- ▶ Integrated PDA with Mass Detector enables:
 - PDA peak purity to investigate peak homogeneity
 - UV and mass spectral information to be used from the same run for positive compound identification
 - UV monitoring of separation for diagnostic purposes and quantitation