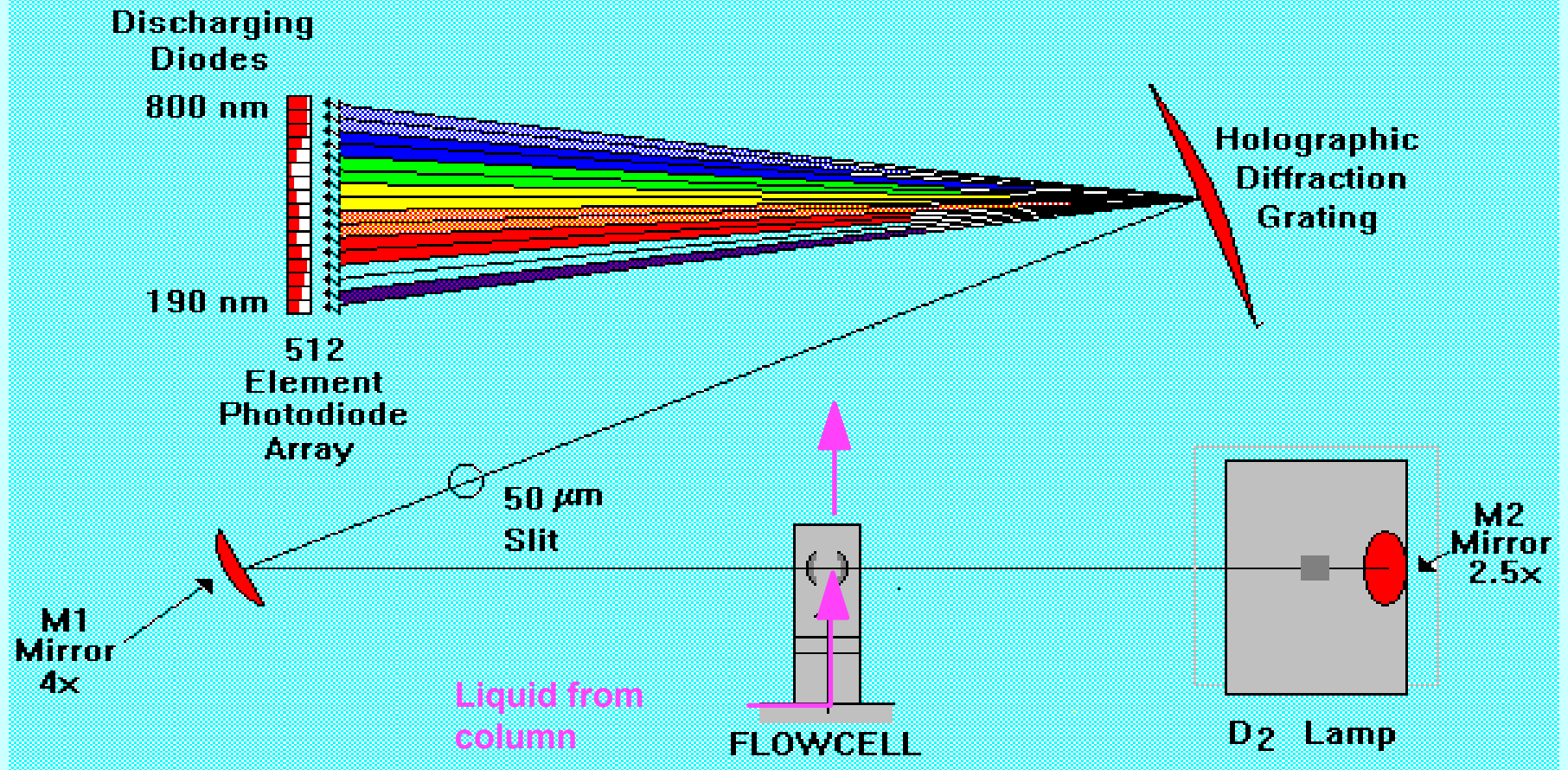


# Considerations in Peak Purity Measurements

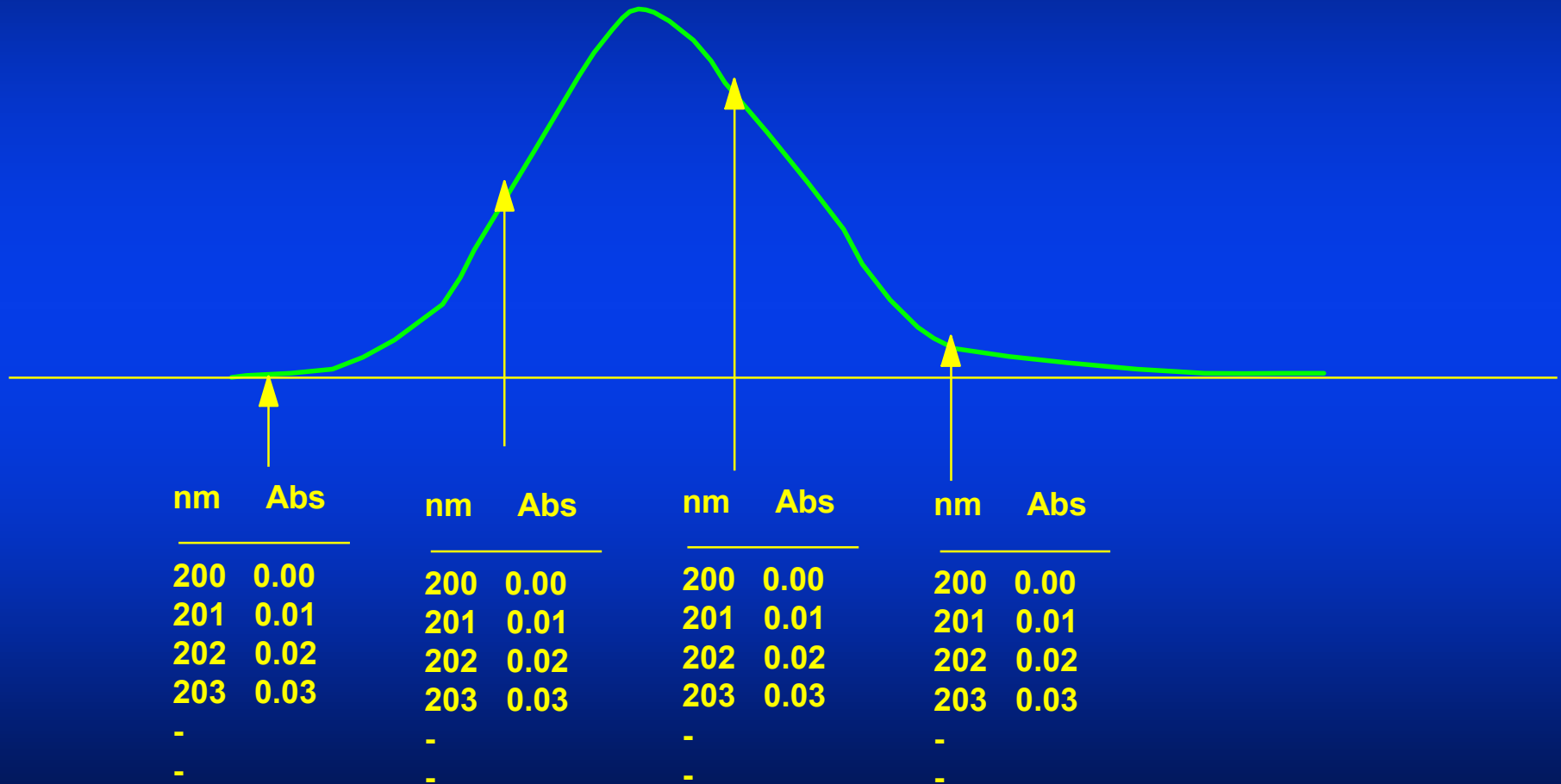
Shulamit Levin  
Analytical Chemistry Department  
Medtechnica

# Photodiode array Detector

## Principle of Measurement

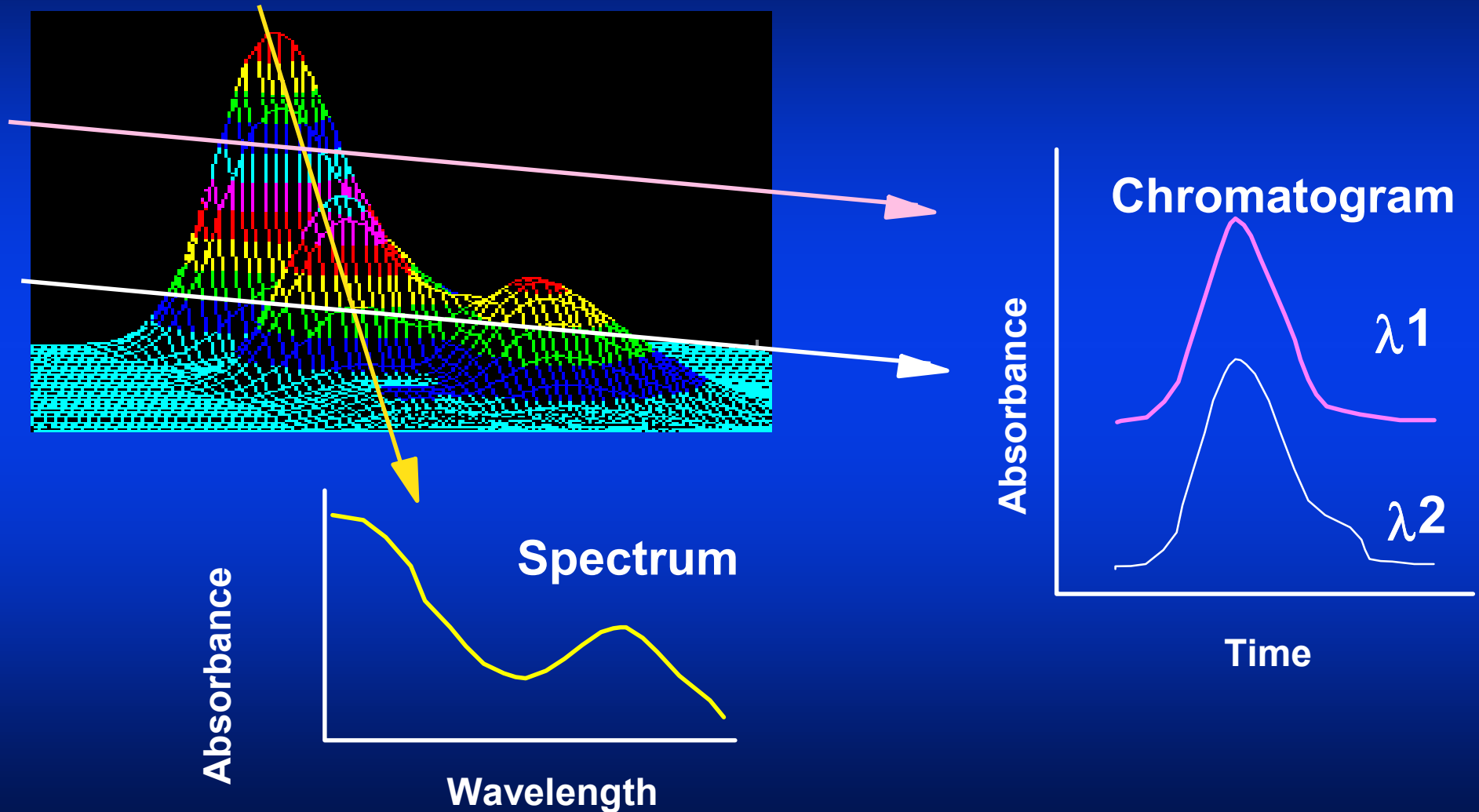


# The Data is 3D – behind every point in the chromatogram hides a spectrum!

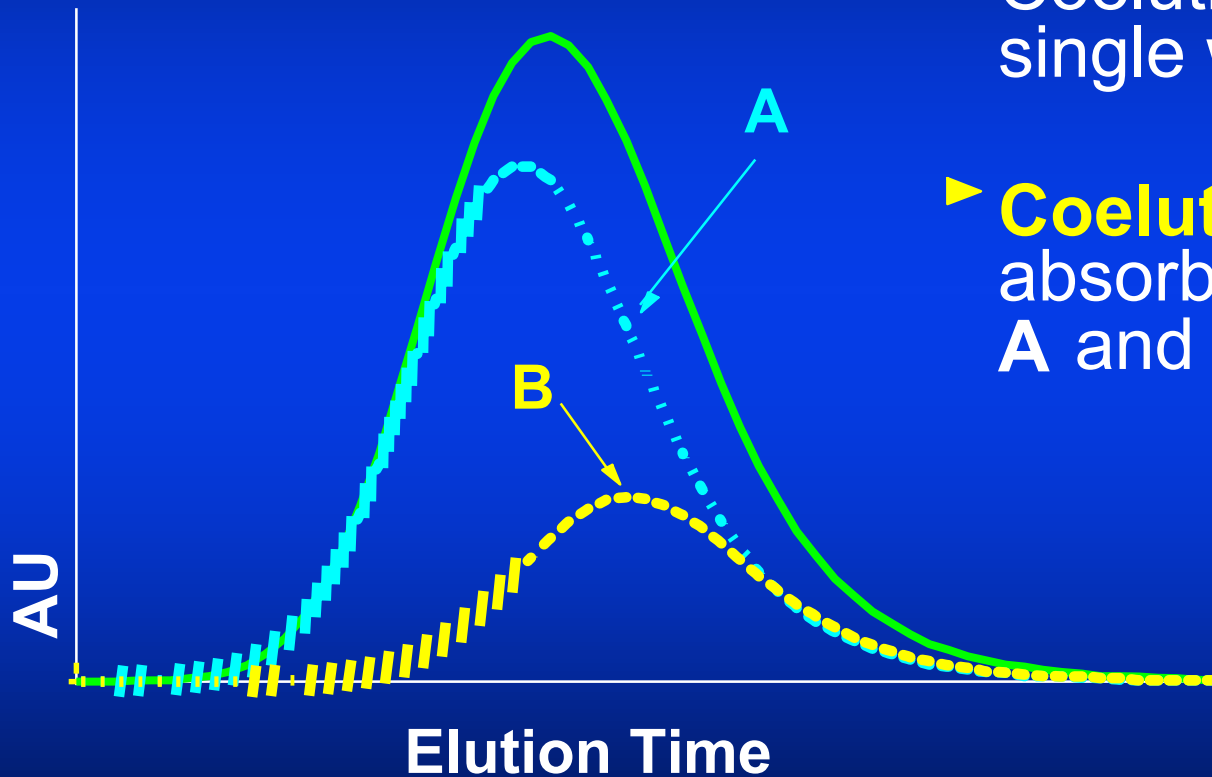


# Extraction of 3D Data

XY plane = Chromatogram ; ZY plane = spectrum

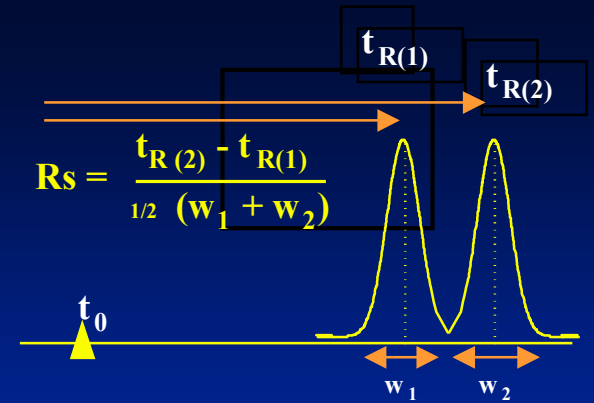
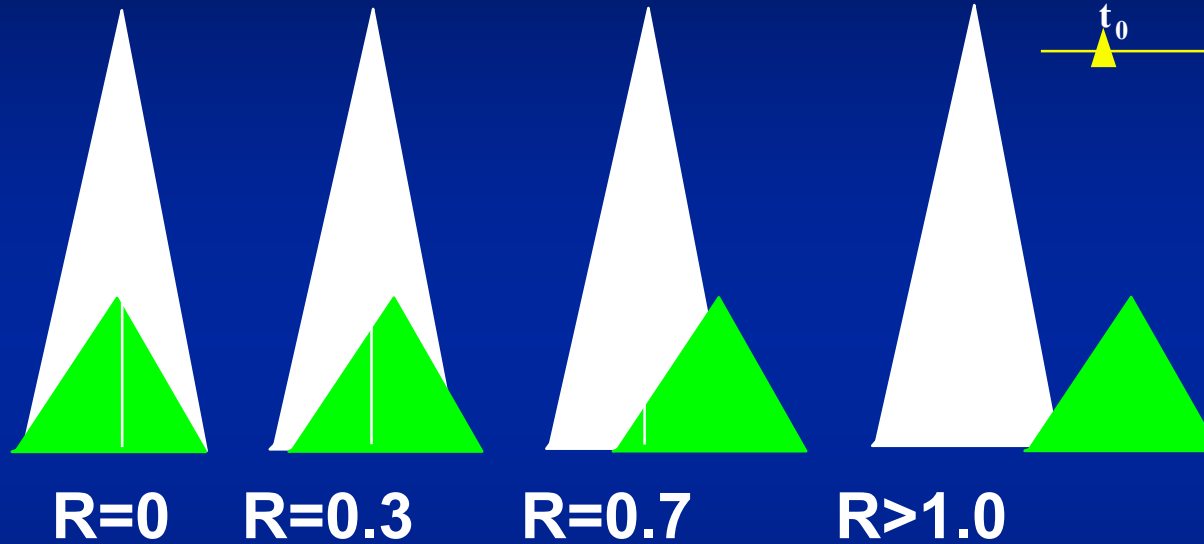


# Coelution of 2 Peaks



- ▶ Coelution detection at a single wavelength
- ▶ **Coelution** is the sum of absorbance of 2 peaks A and B

# Chromatographic Resolution & Coelution Detection

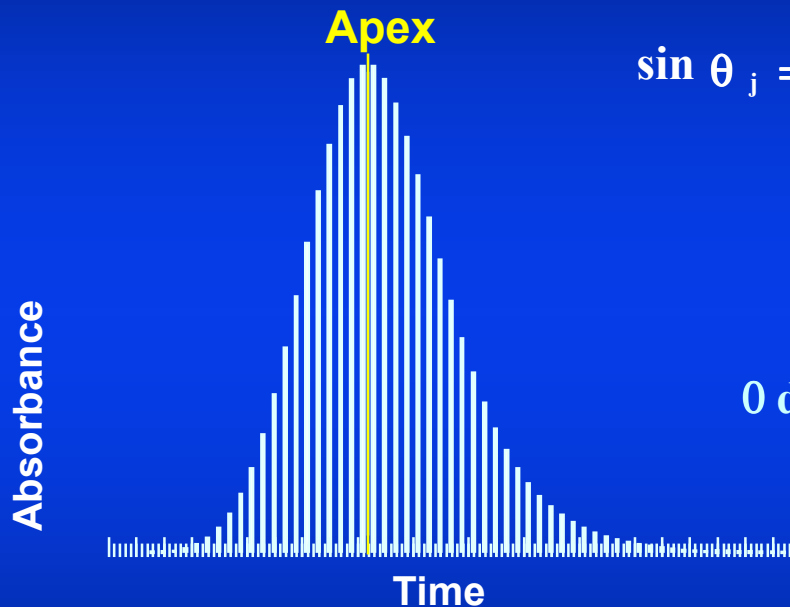


- ▶  $R=0$  Purity Angle not effective; Match Angle useful
- ▶  $R=0.3$  to  $R=0.7$  Purity & Match Angle useful
- ▶  $R>0.7$  Match Angle not useful

# Peak Purity and Spectral Matching Principles:

Spectral contrast angle:

## Purity verification



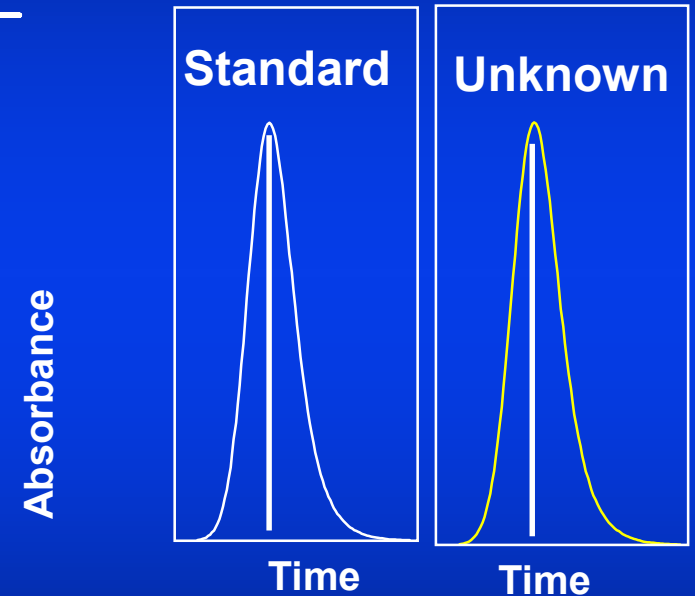
$$\sin \theta_j = \frac{\sqrt{\sum_{i=1}^N (B_{ij} - s_j A_i)^2}}{\sqrt{\sum_{i=1}^N B_{ij}^2}}$$

$$0 \leq \sin \theta \leq 1$$

$$0 \text{ deg} \leq \theta \leq 90 \text{ deg}$$

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

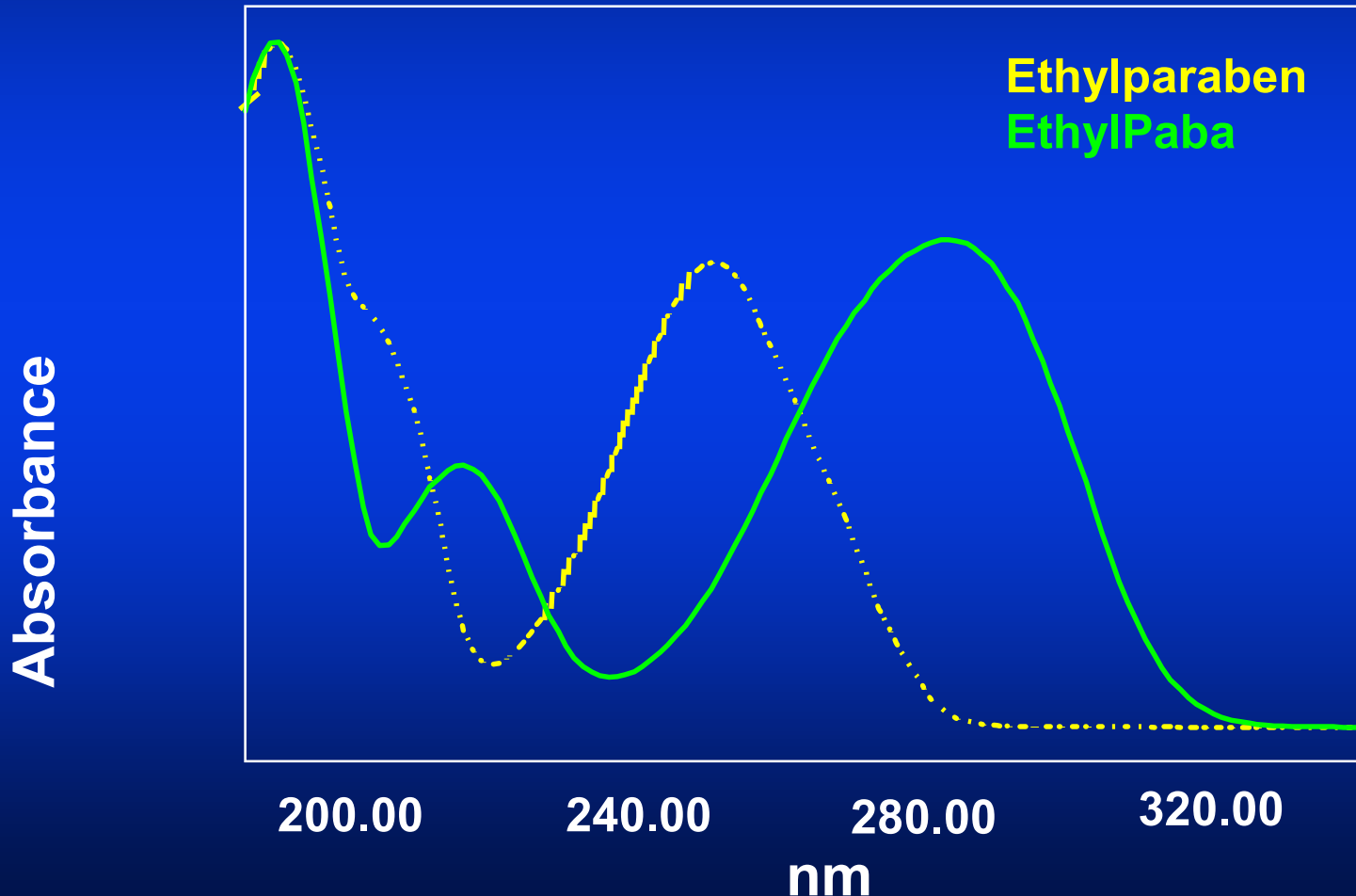
## Library identification



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

# Spectral Contrast Angle = 53 Degrees

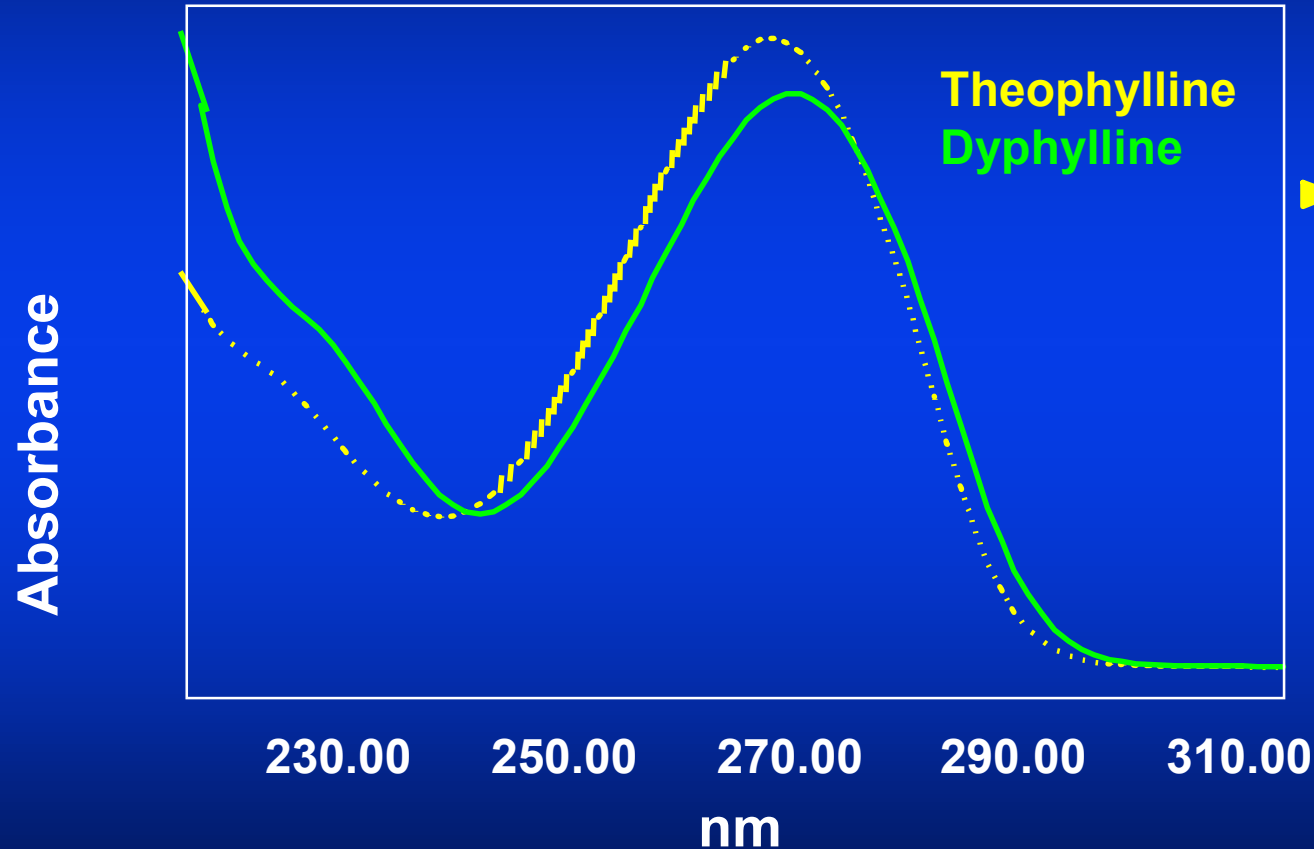
## Very large difference



► 53 degrees is a large spectral difference



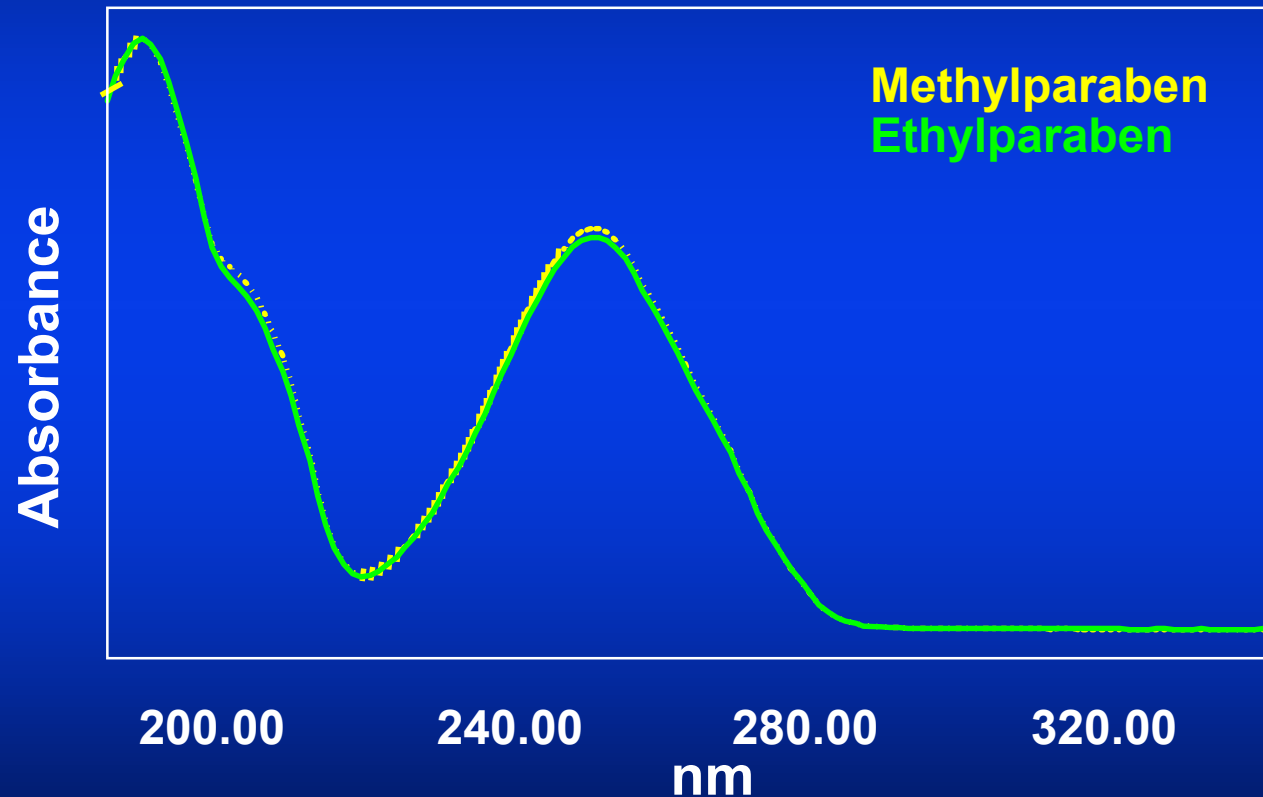
# Spectral Contrast 10 Degrees



Theophylline  
Dyphylline

▶ Similar spectra for structurally related compounds

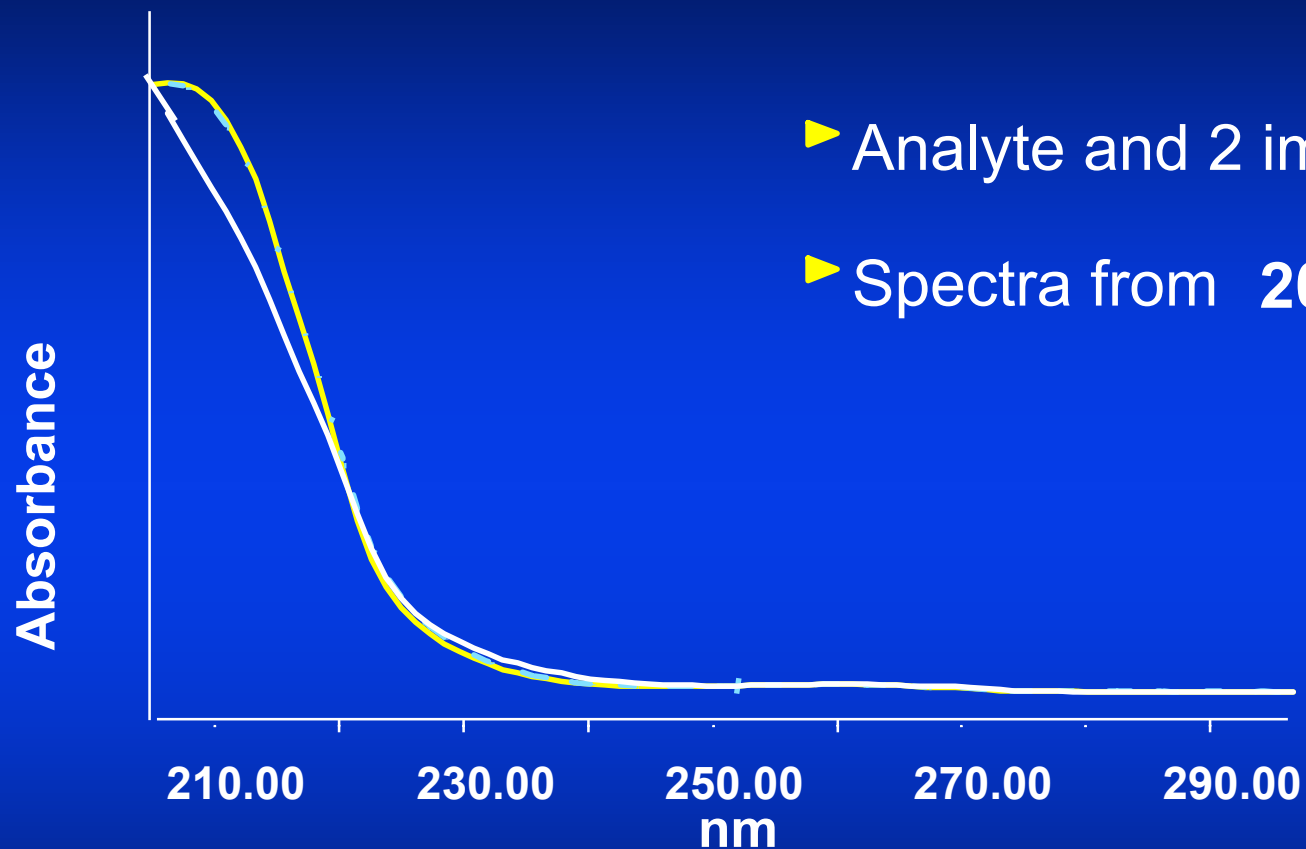
# Spectral Contrast 0.5 Degrees



▶ Very similar spectra, CH<sub>2</sub> difference

▶ Spectral Contrast can differentiate these spectra

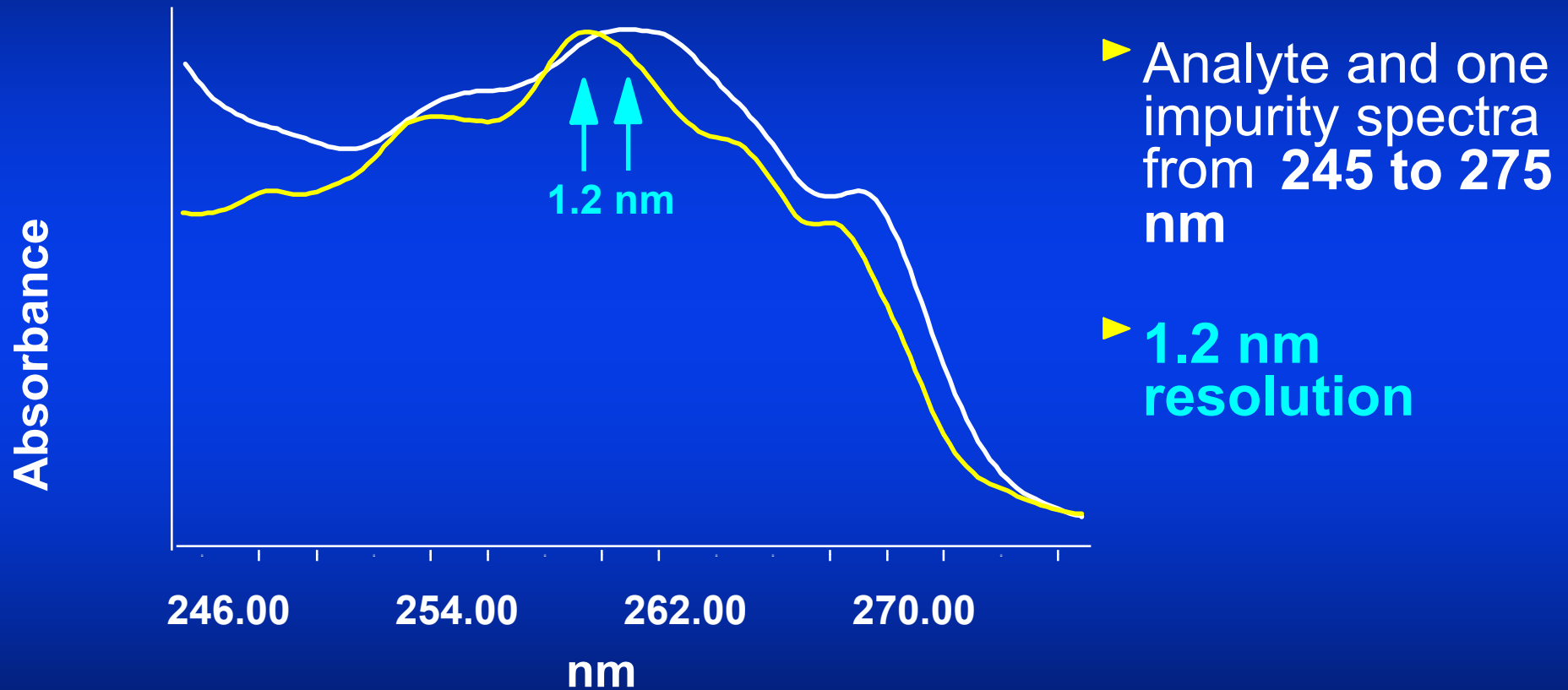
# Very Similar Spectra



▶ Analyte and 2 impurities

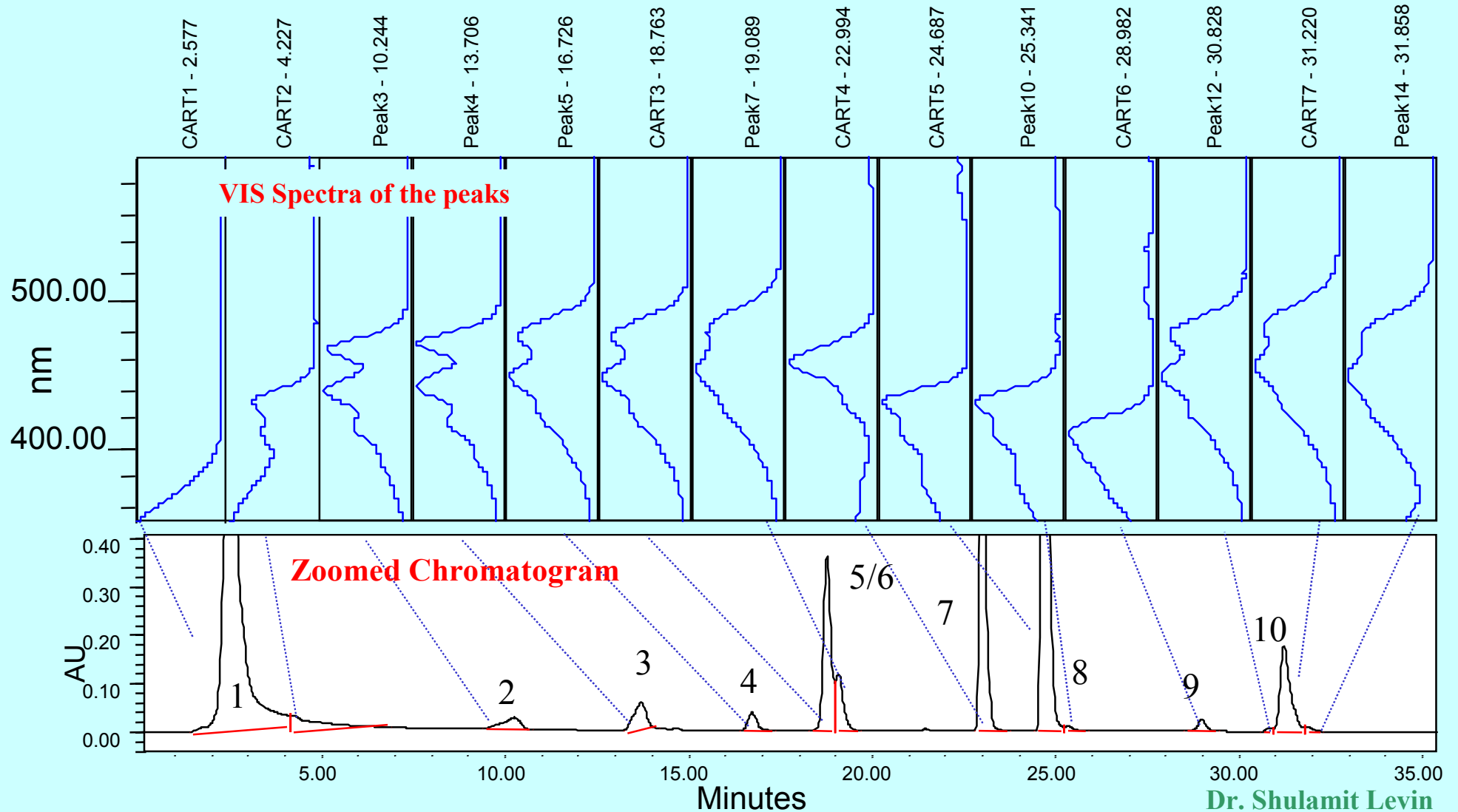
▶ Spectra from 200 to 300 nm

## Detection of Spectral Fine Structure Requires 1.2 nm Resolution



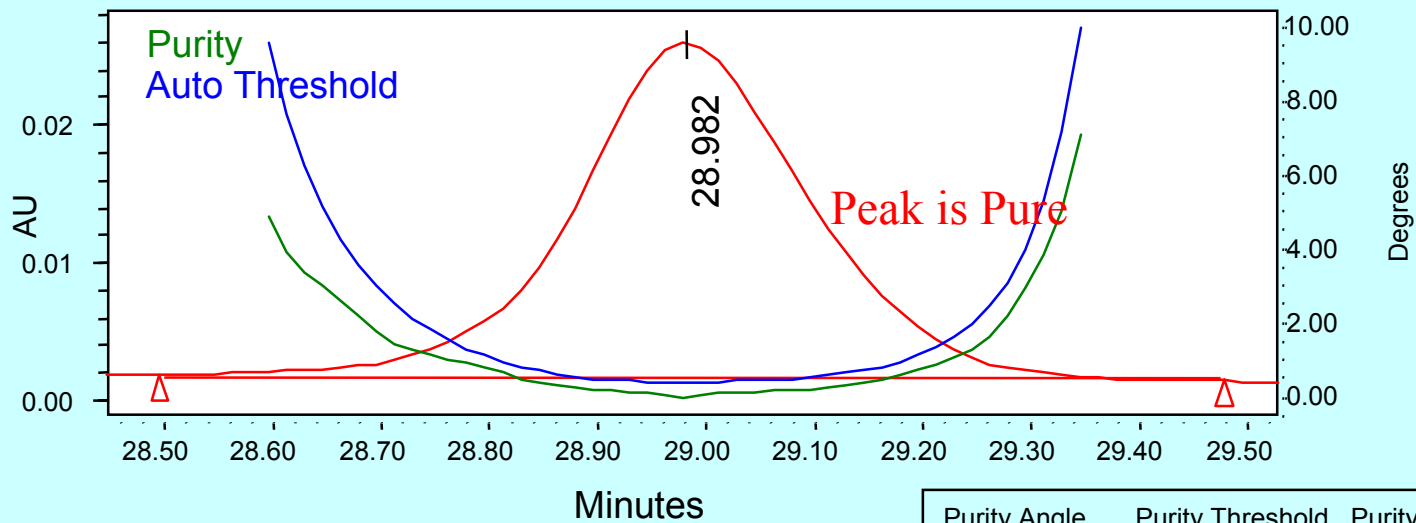
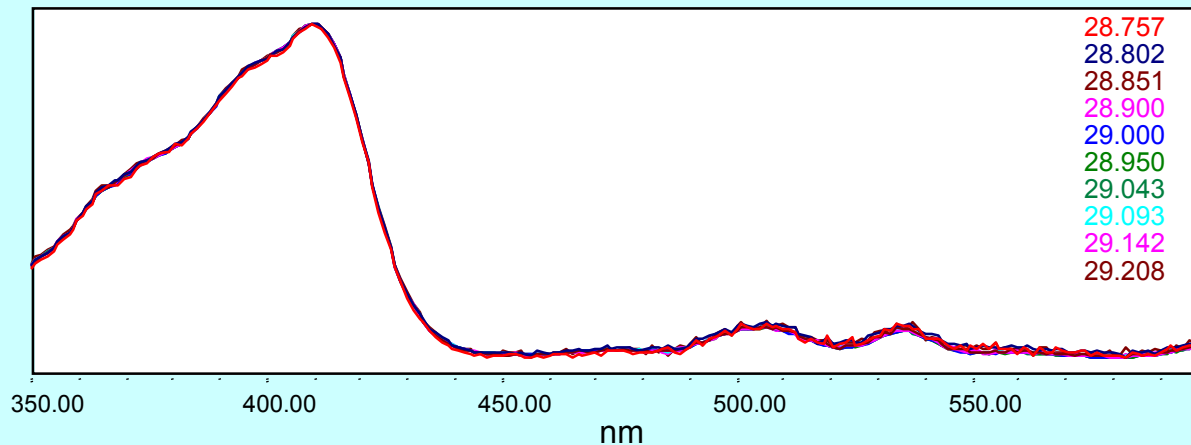
# CAROTENOIDS - Extracted from leaves

## Spectrum Index presentation



# An Example for Pure Peak

## Spectra collected from Peak 9

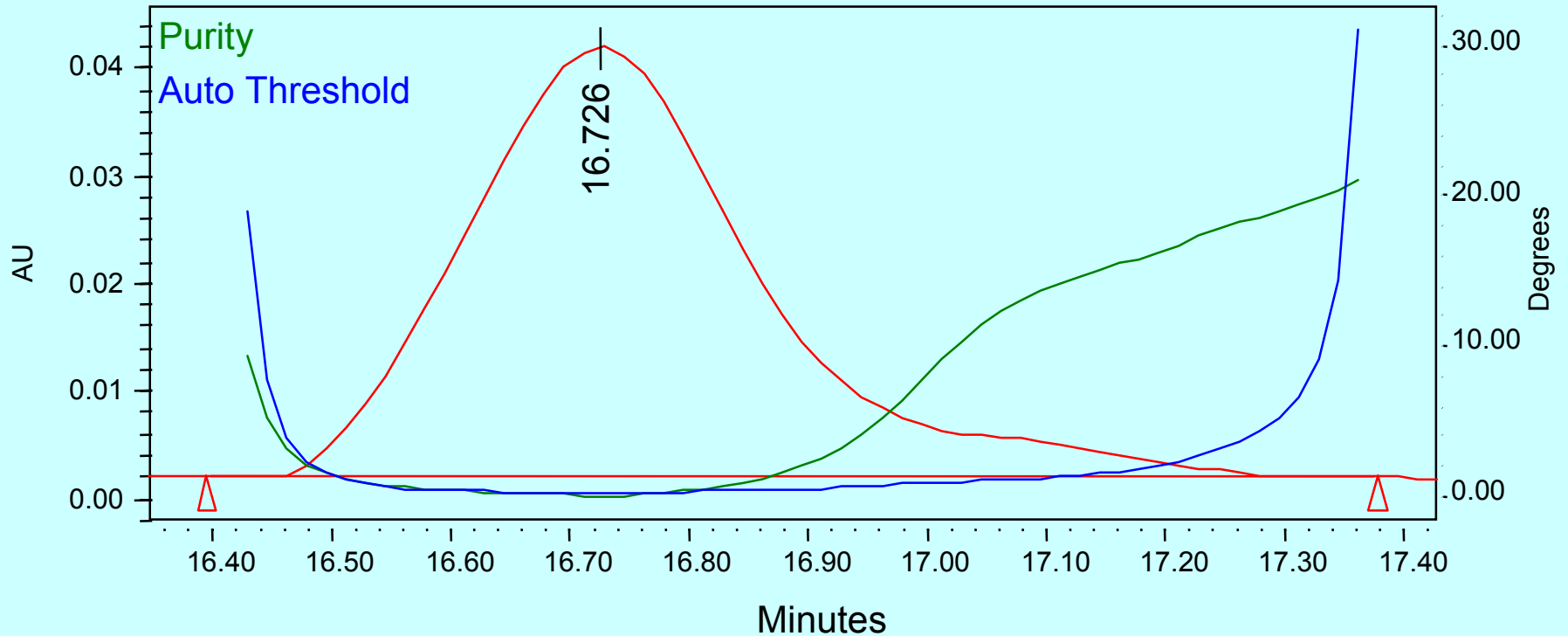


Purity Angle	Purity Threshold	Purity Flag
0.284	0.551	No

# An Example for non Pure Peak

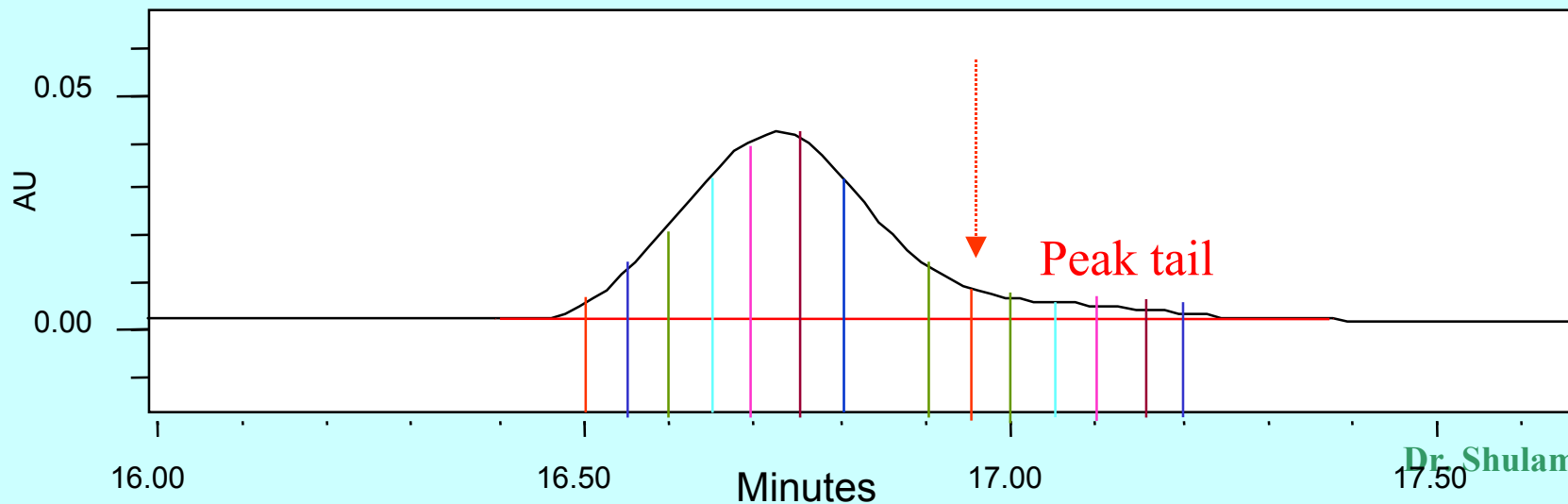
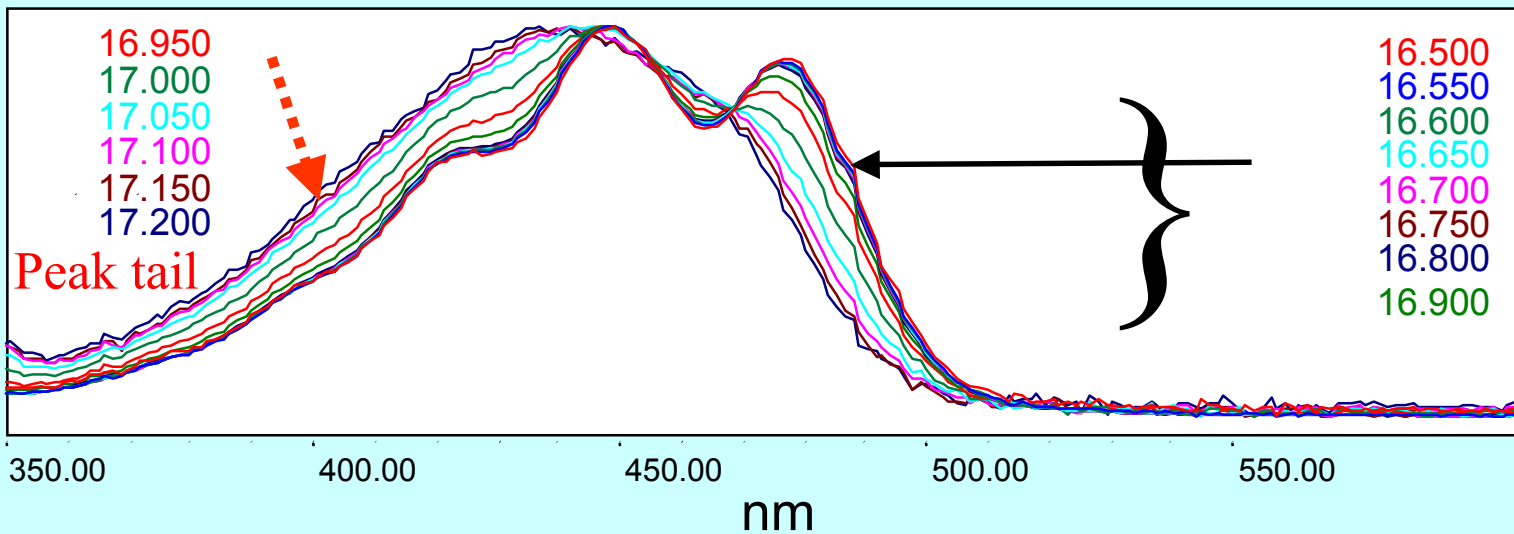
## Purity Plot of Peak 4 - Not Pure

Purity Angle 1.885	Purity Threshold 0.404	Maximum Impurity 17.078	Purity Flag Yes
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# An Example for non Pure Peak

## Spectra Selected from Peak 4

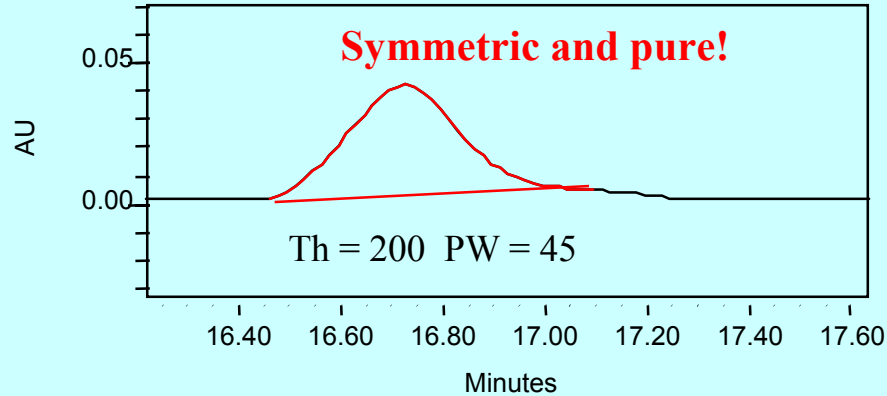




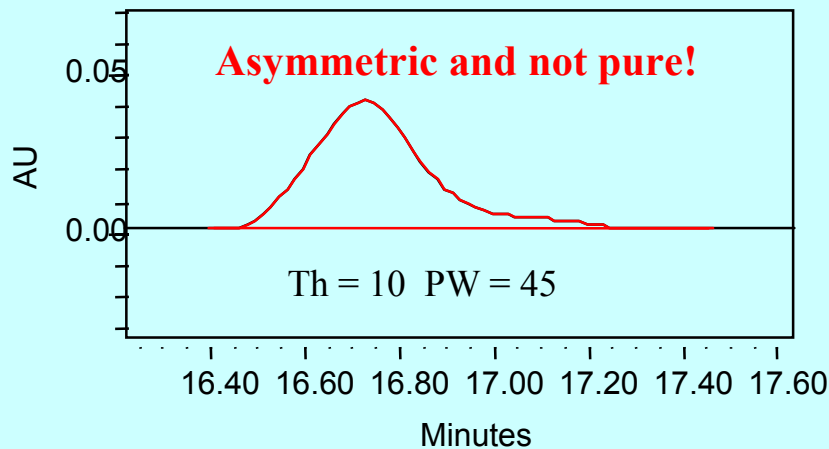
# Beware of Peak Integration- where the peak starts or ends!

## Effect of Integration Events on Peak Purity Results

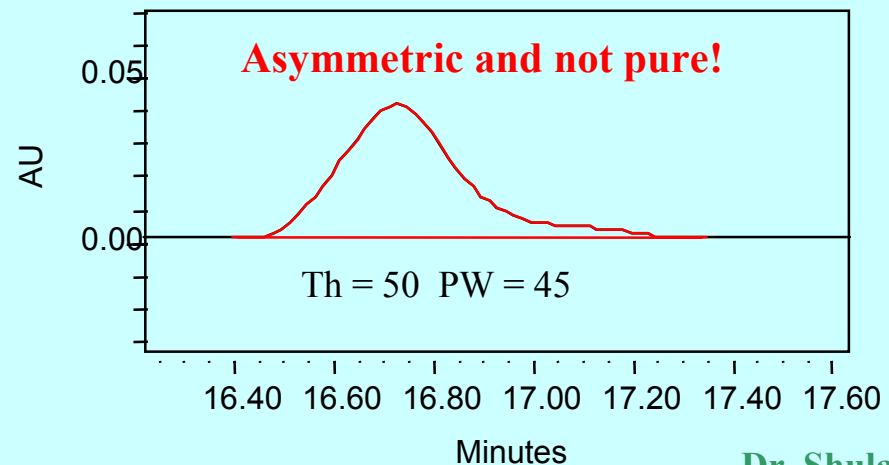
Purity Angle	Purity Threshold	USP Tailing
0.297	0.380	1.057



Purity Angle	Purity Threshold	USP Tailing
2.259	0.410	1.438

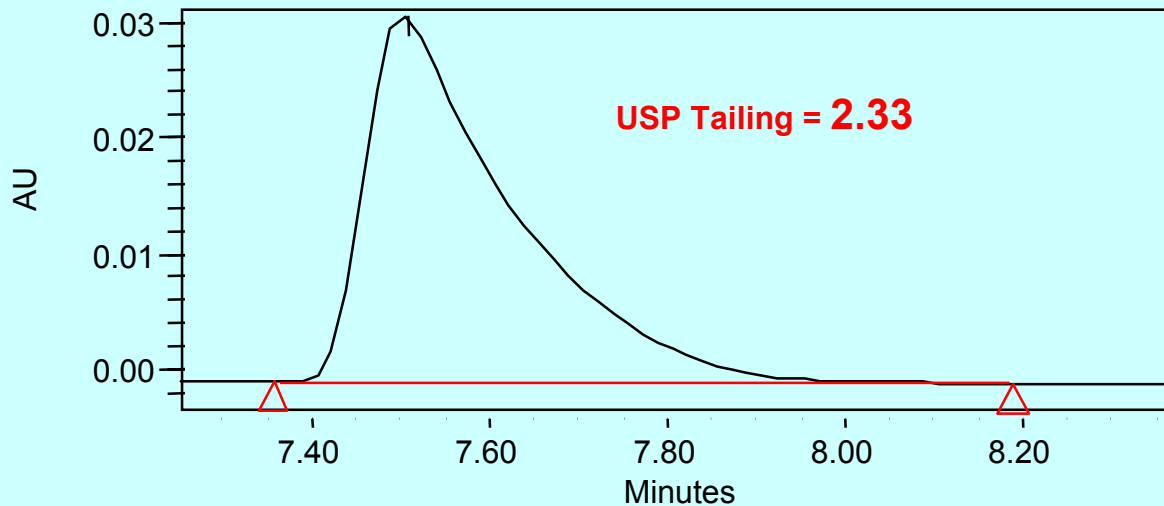
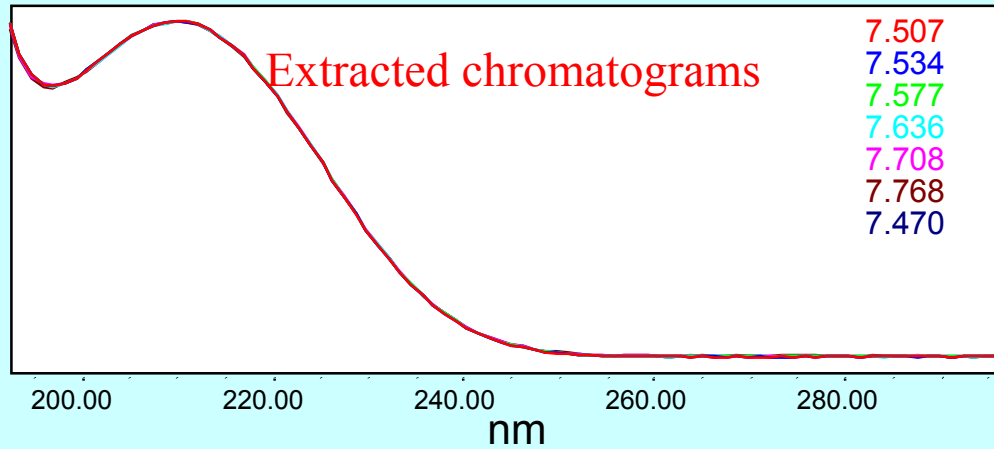


Purity Angle	Purity Threshold	USP Tailing
1.682	0.401	1.415



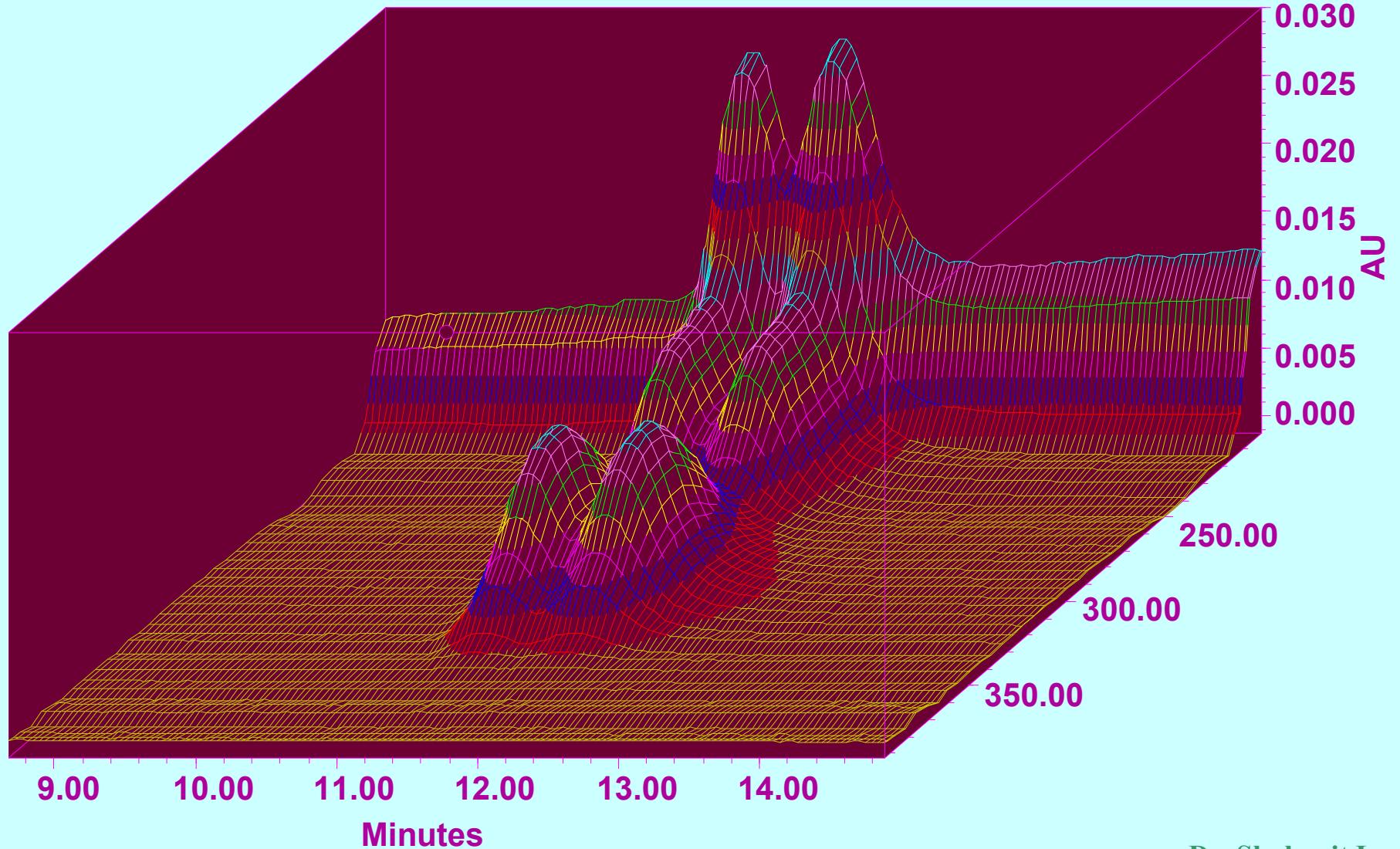
# Peak is asymmetric but pure!

Name	Purity Angle	Purity Threshold
GBPN	0.217	0.383



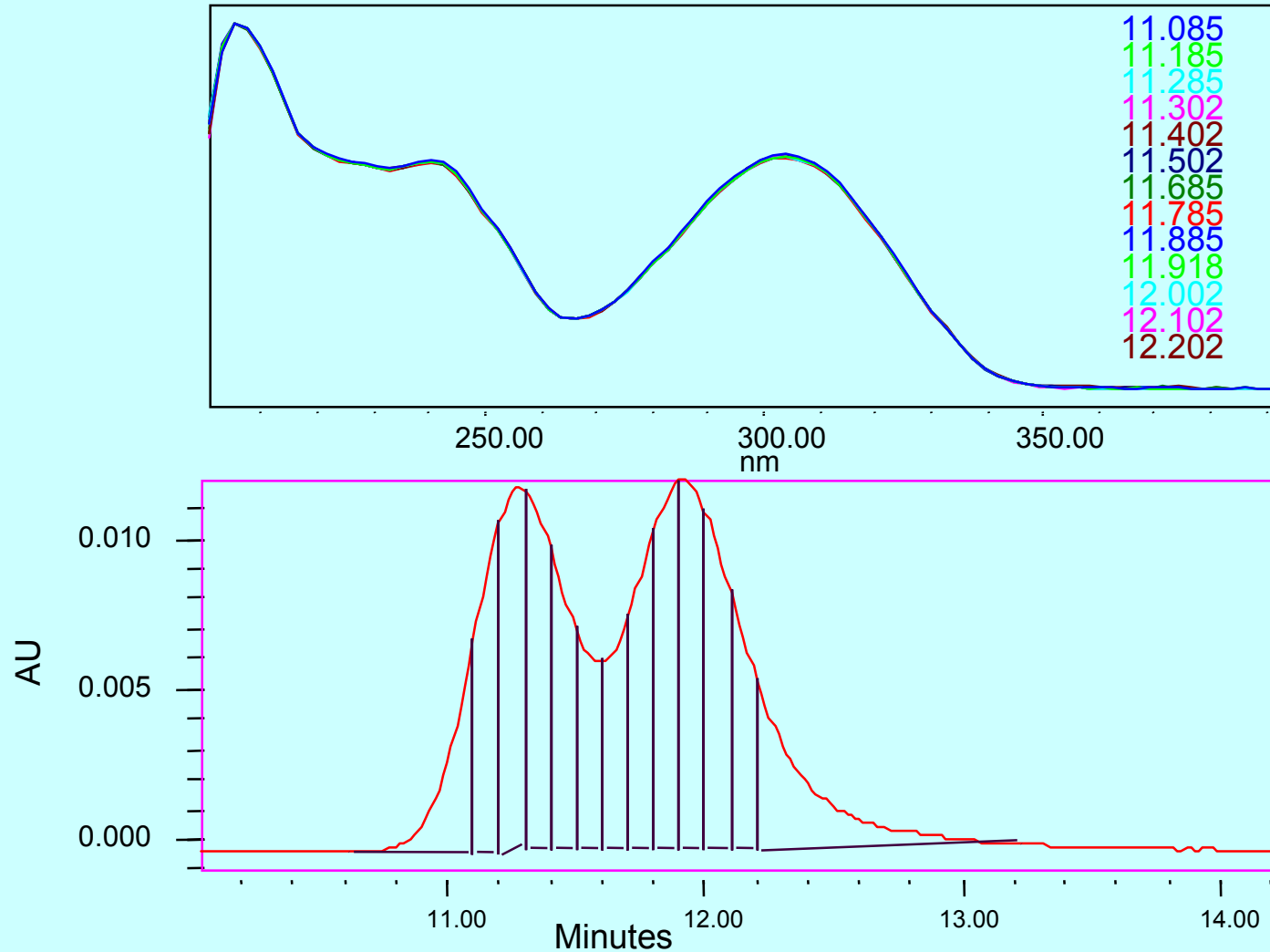
# Nucleoside analog's Enantiomers - Identical UV Spectra

## 3D Plot



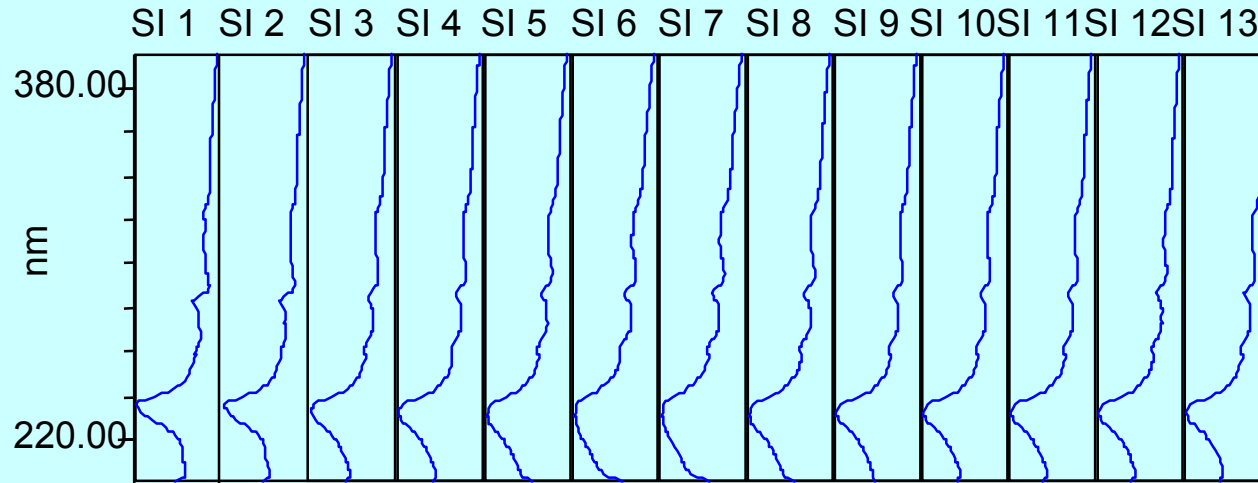
# Nucleoside analog' Enantiomers - Identical UV Spectra

Spectra collected from the two peaks

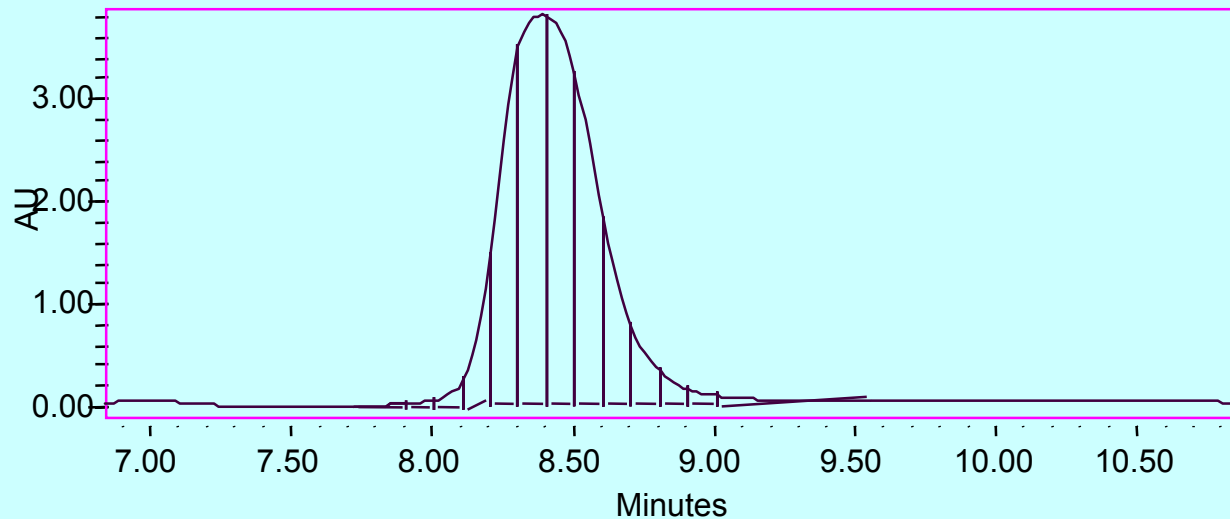


# Non-Linearity Effects

**Spectra collected at low portions of the peak are different than those at around the apex**

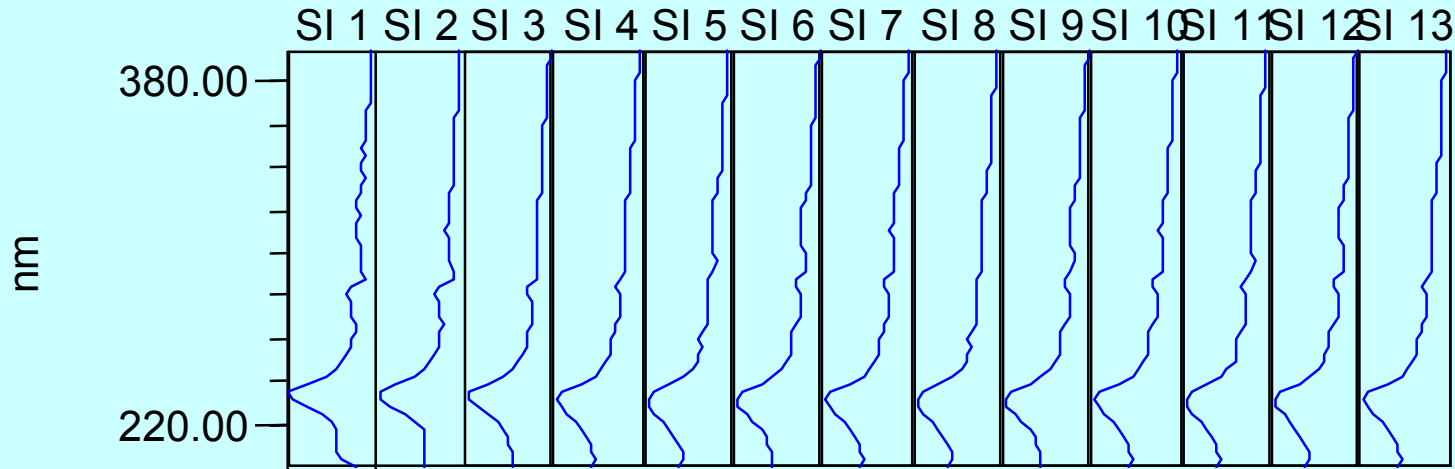


**High Conc.  
Non Linear**



# Linear Range of Concentration

**Spectra collected at low portions of the peak are identical to those around the apex**



**Low Conc.  
Linear**

