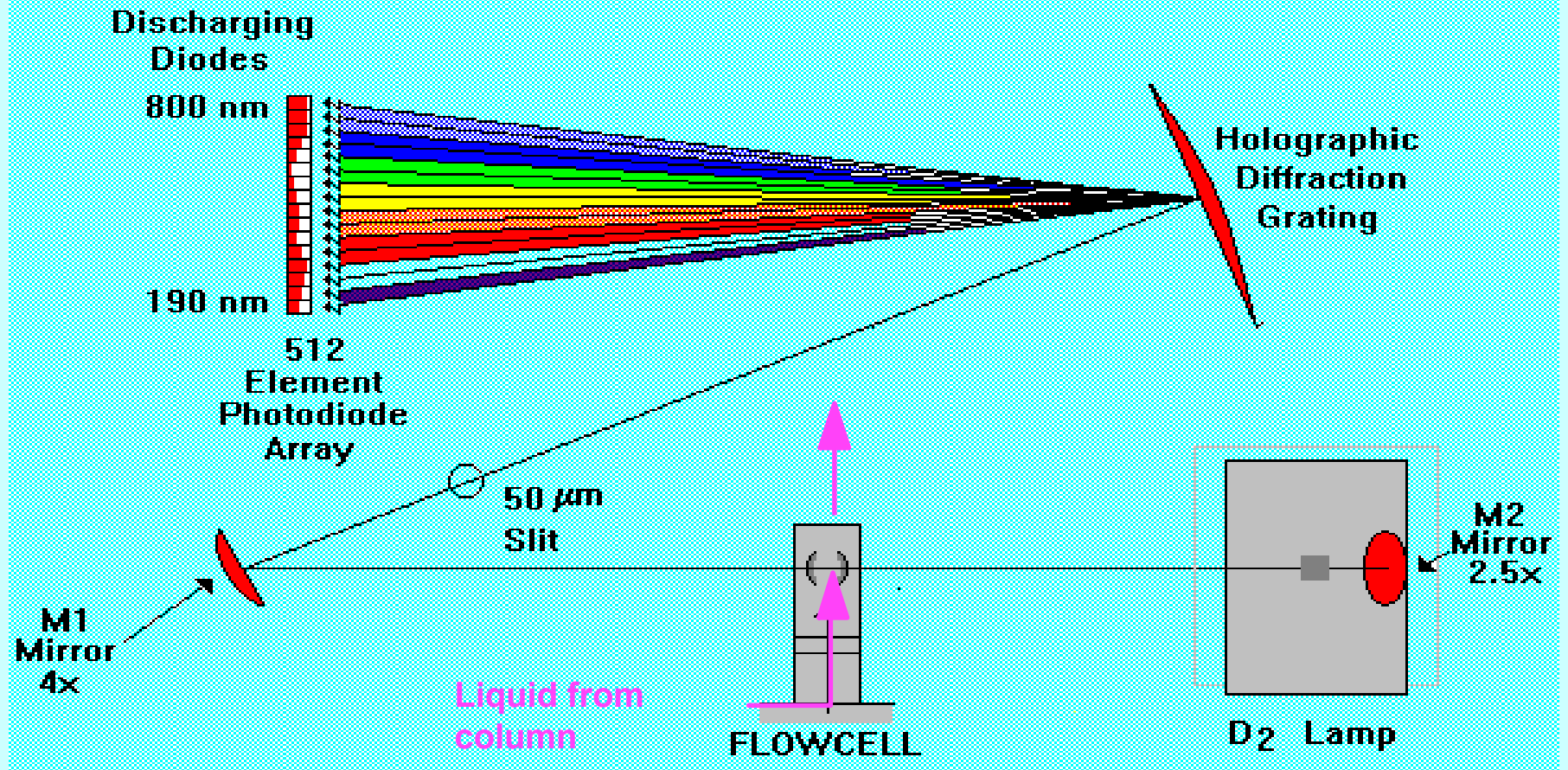


Considerations in Peak Purity Measurements

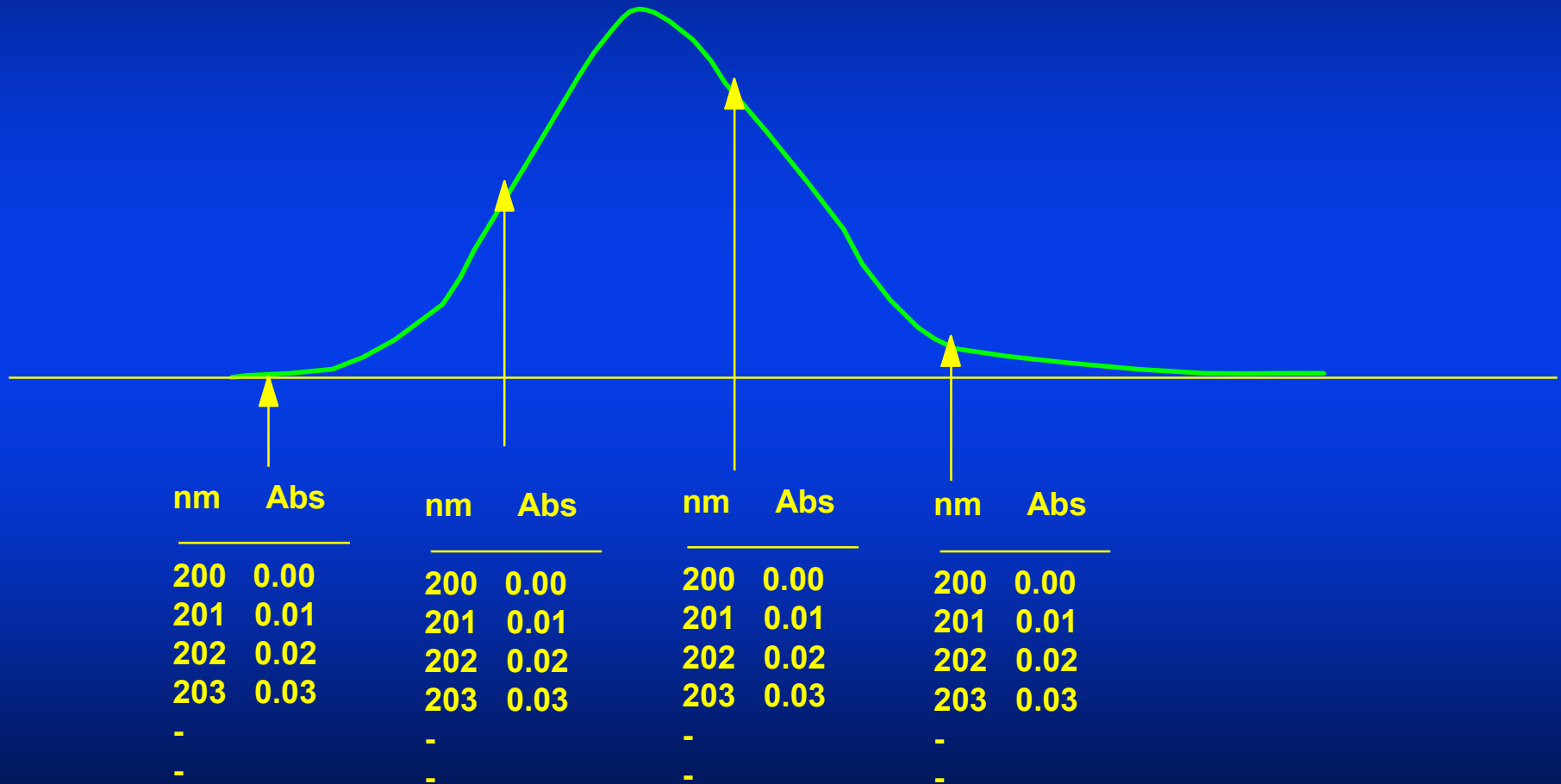
Shulamit Levin
Analytical Chemistry Department
Medtechnica

Photodiode array Detector

Principle of Measurement

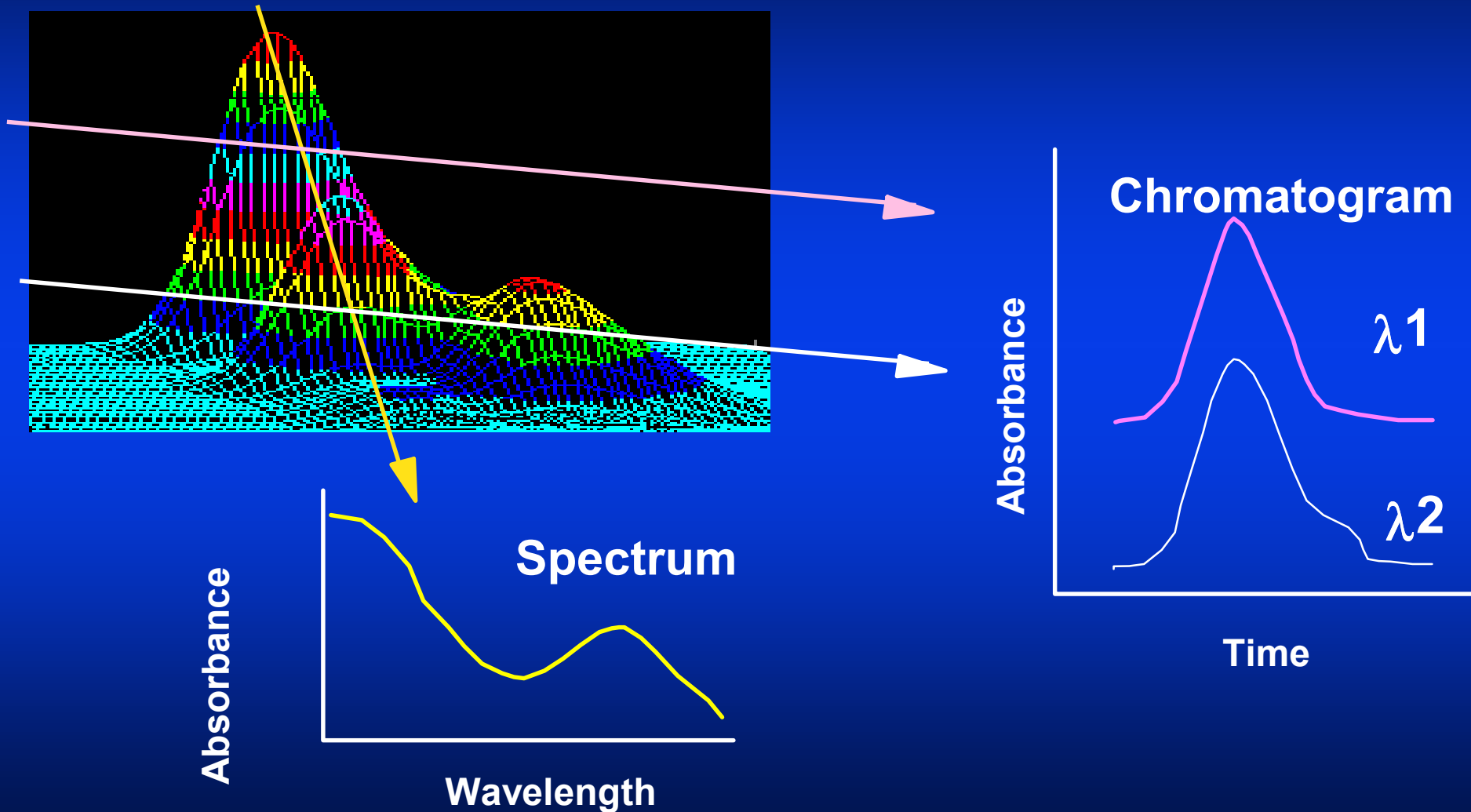


The Data is 3D – behind every point in the chromatogram hides a spectrum!

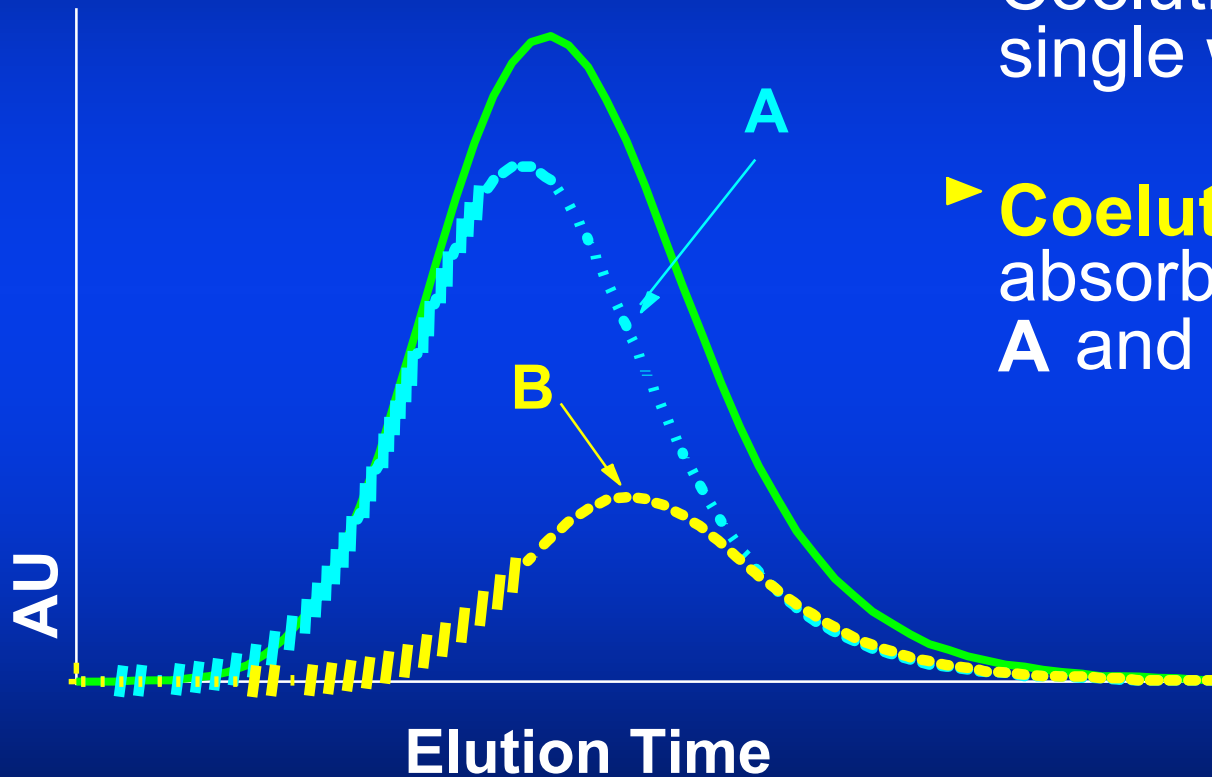


Extraction of 3D Data

XY plane = Chromatogram ; ZY plane = spectrum

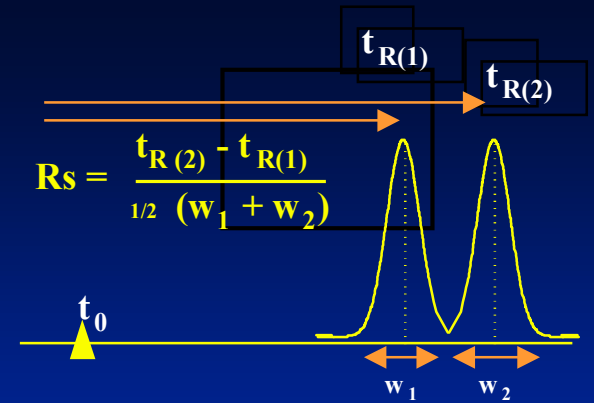
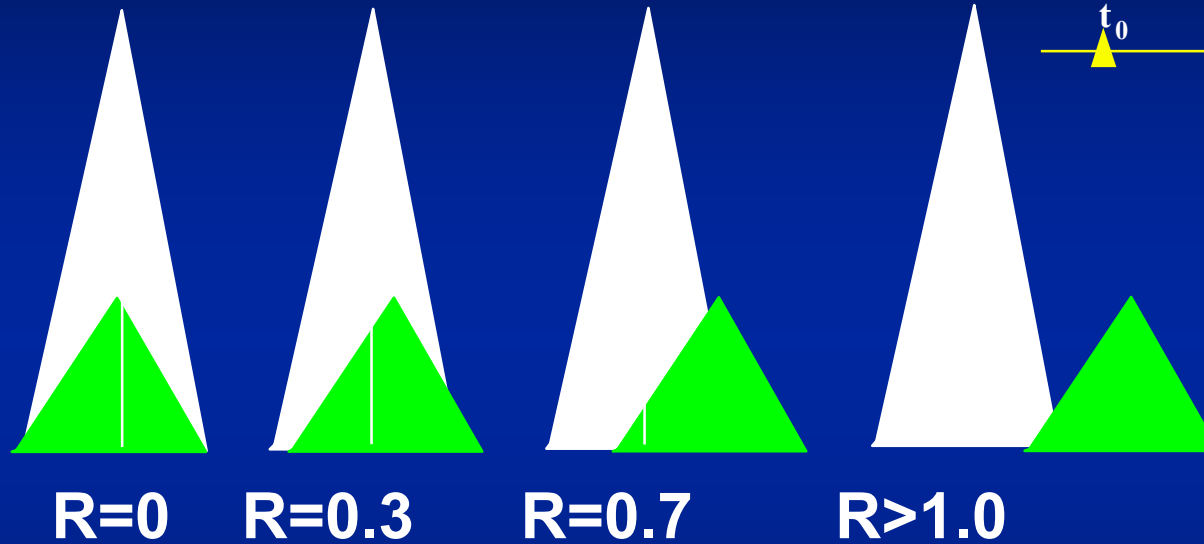


Coelution of 2 Peaks



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

Chromatographic Resolution & Coelution Detection

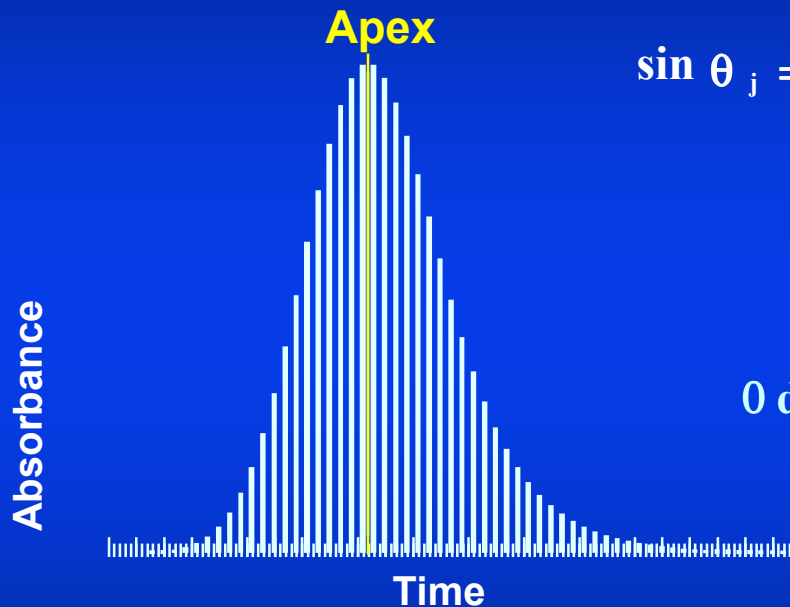


- ▶ $R=0$ Purity Angle not effective; Match Angle useful
- ▶ $R=0.3$ to $R=0.7$ Purity & Match Angle useful
- ▶ $R>0.7$ Match Angle not useful

Peak Purity and Spectral Matching Principles:

Spectral contrast angle:

Purity verification



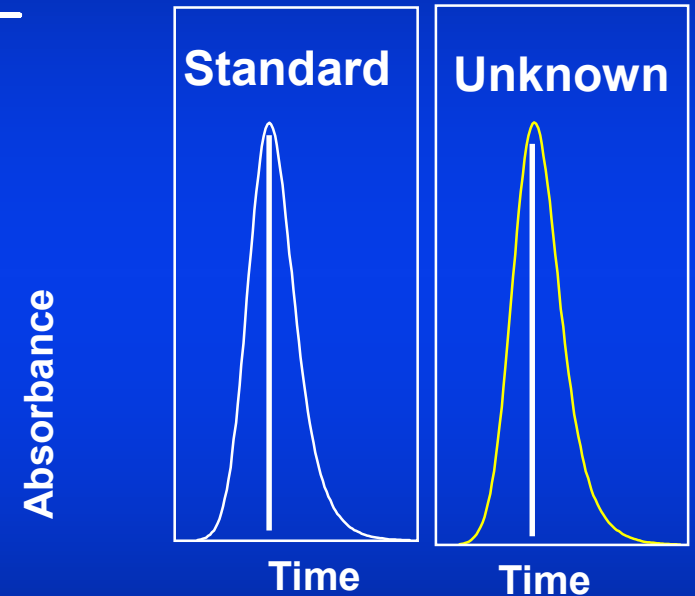
$$\sin \theta_j = \frac{\sqrt{\sum_{i=1}^N (B_{ij} - s_j A_i)^2}}{\sqrt{\sum_{i=1}^N B_{ij}^2}}$$

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

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

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

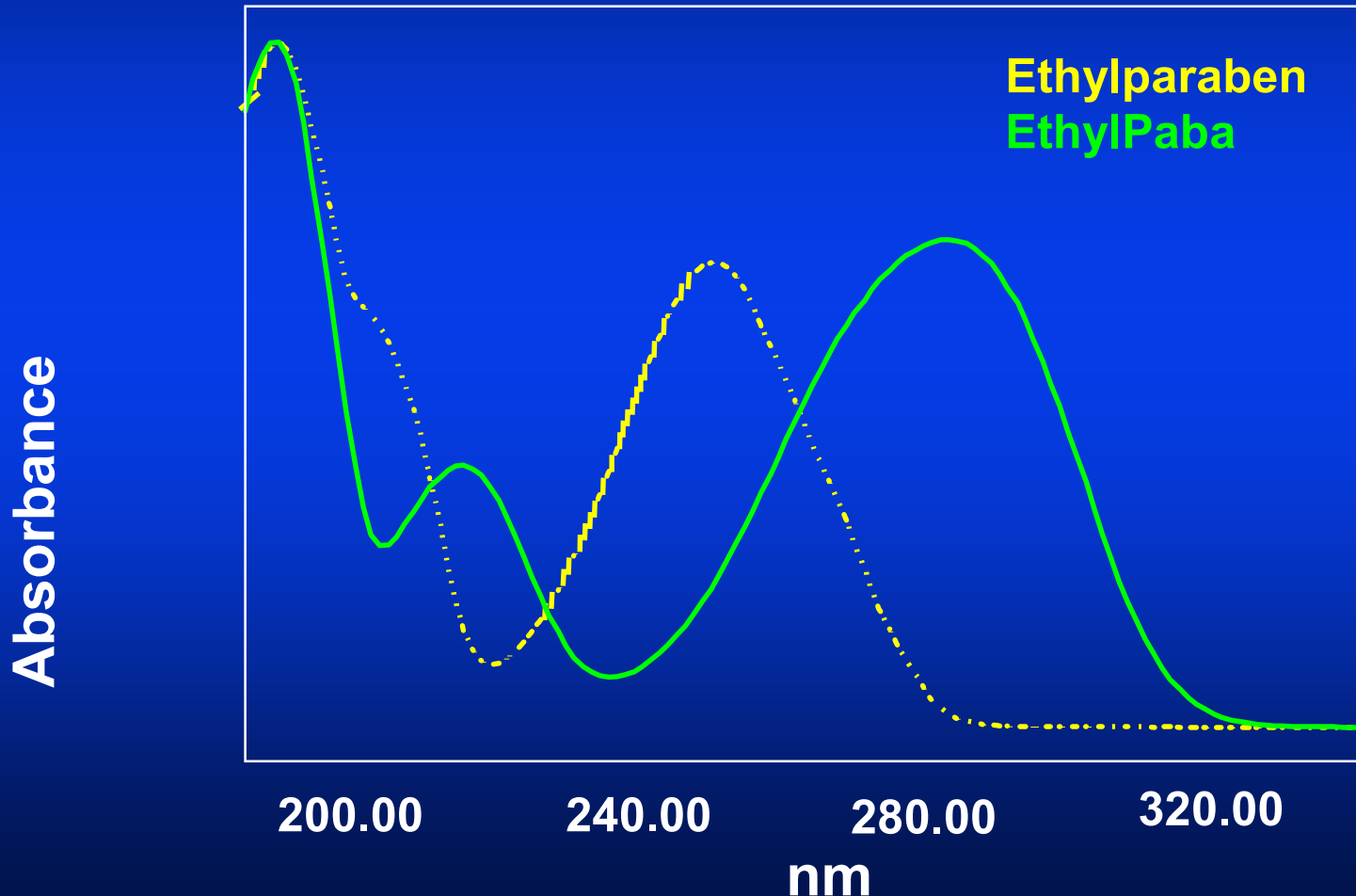
Library identification



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

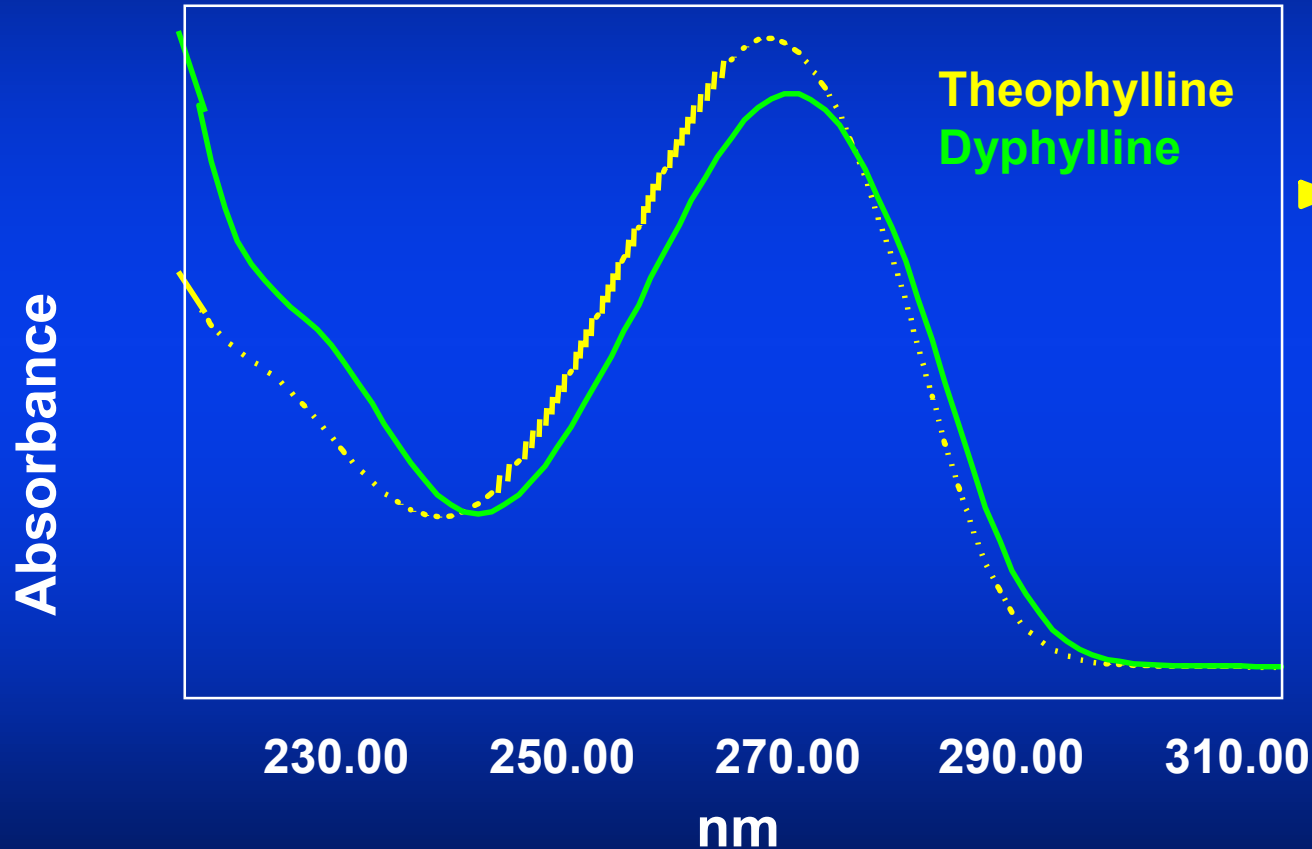
Spectral Contrast Angle = 53 Degrees

Very large difference



► 53 degrees is a large spectral difference

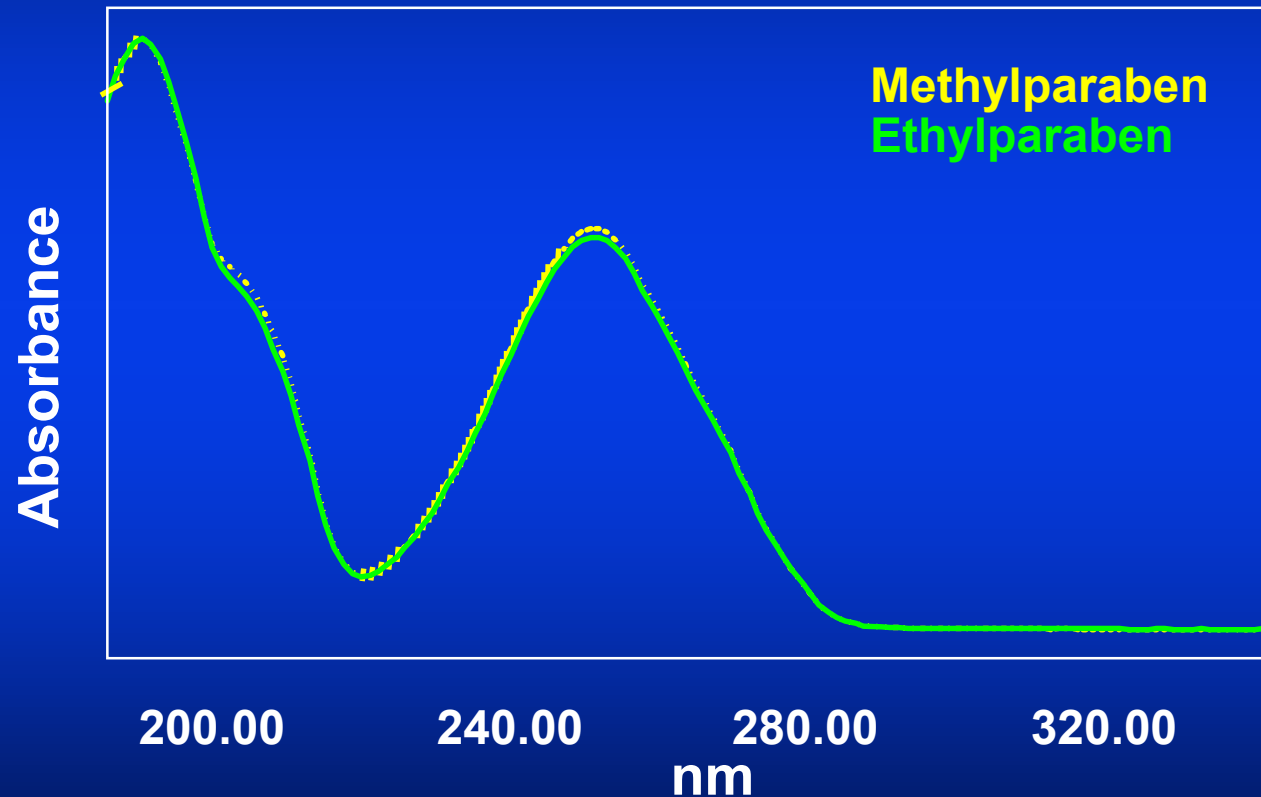
Spectral Contrast 10 Degrees



Theophylline
Dyphylline

▶ Similar spectra for structurally related compounds

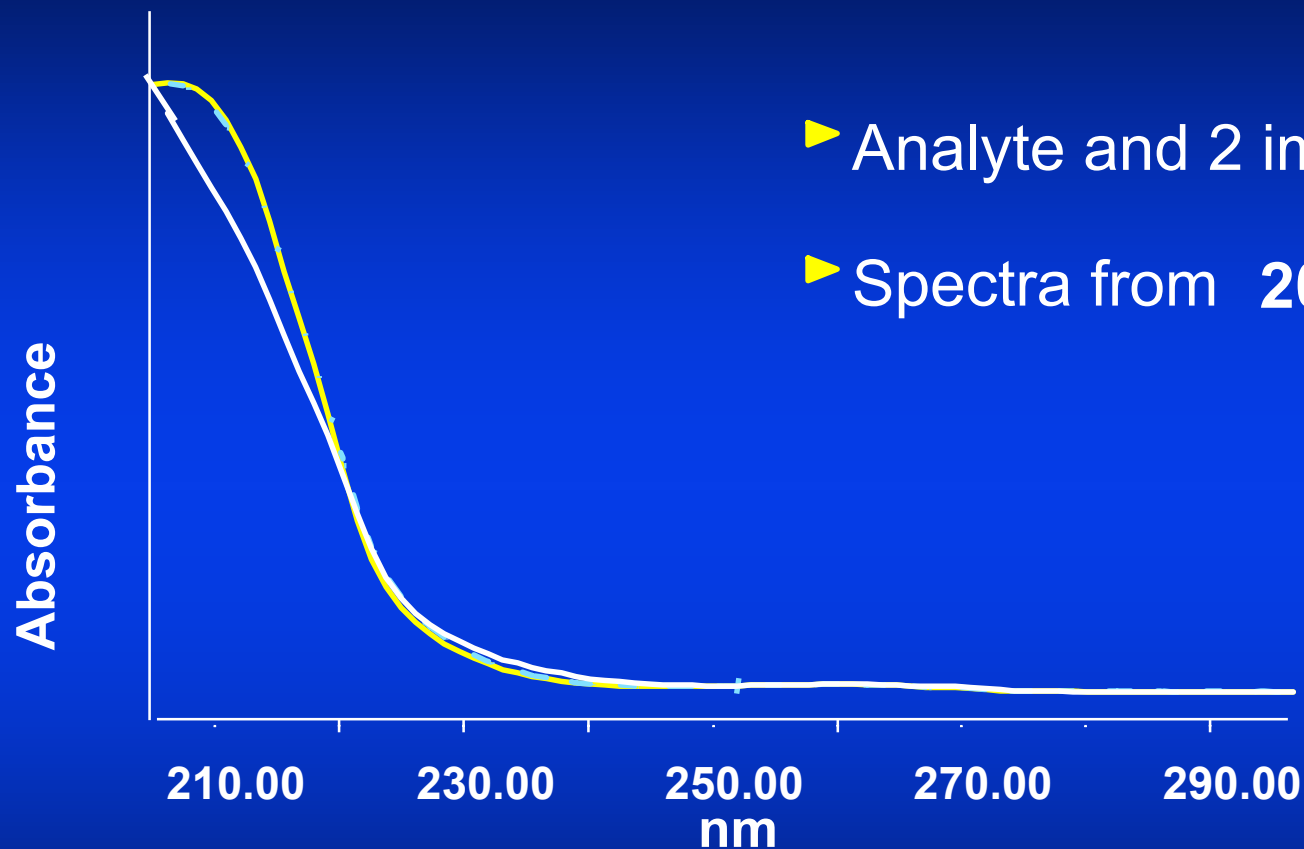
Spectral Contrast 0.5 Degrees



▶ Very similar spectra, CH₂ difference

▶ Spectral Contrast can differentiate these spectra

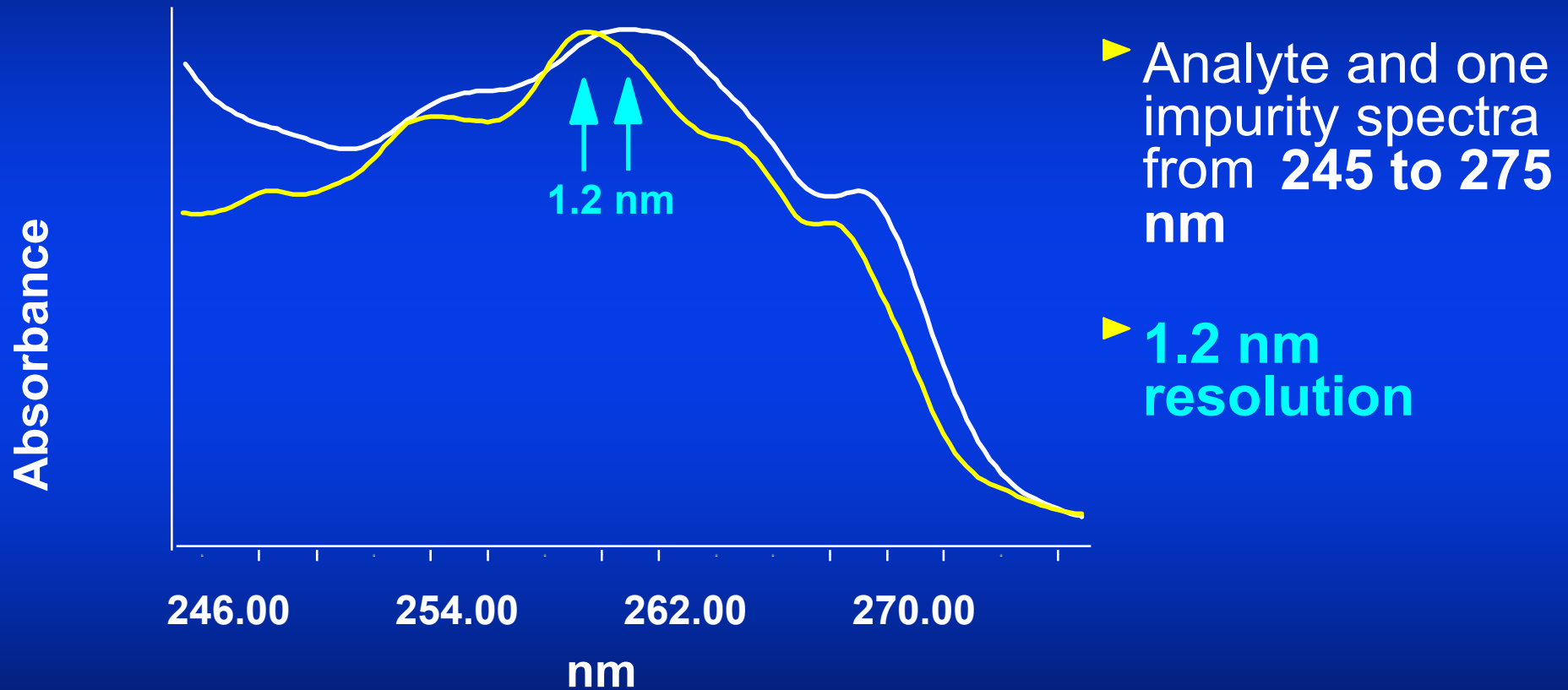
Very Similar Spectra



▶ Analyte and 2 impurities

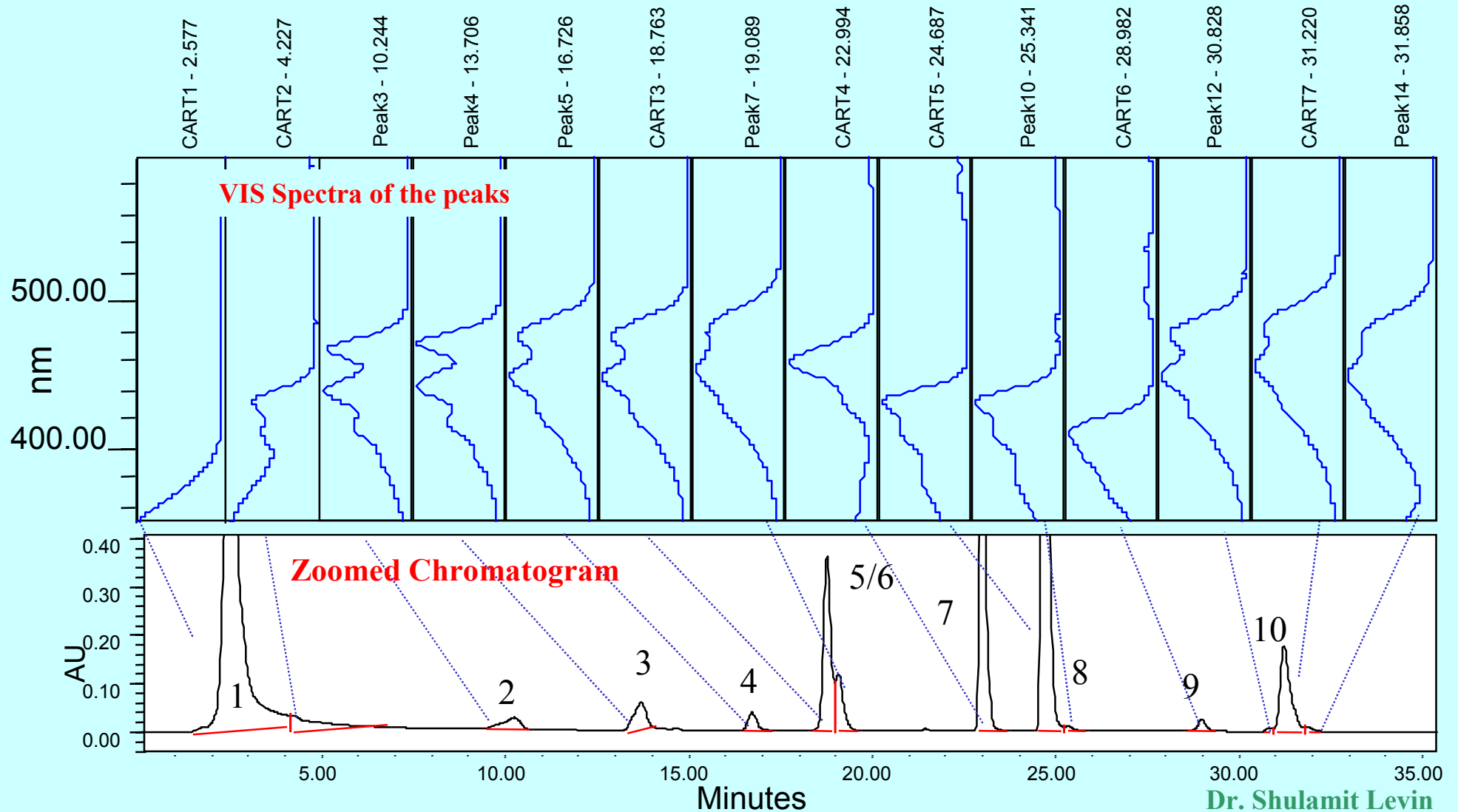
▶ Spectra from 200 to 300 nm

Detection of Spectral Fine Structure Requires 1.2 nm Resolution



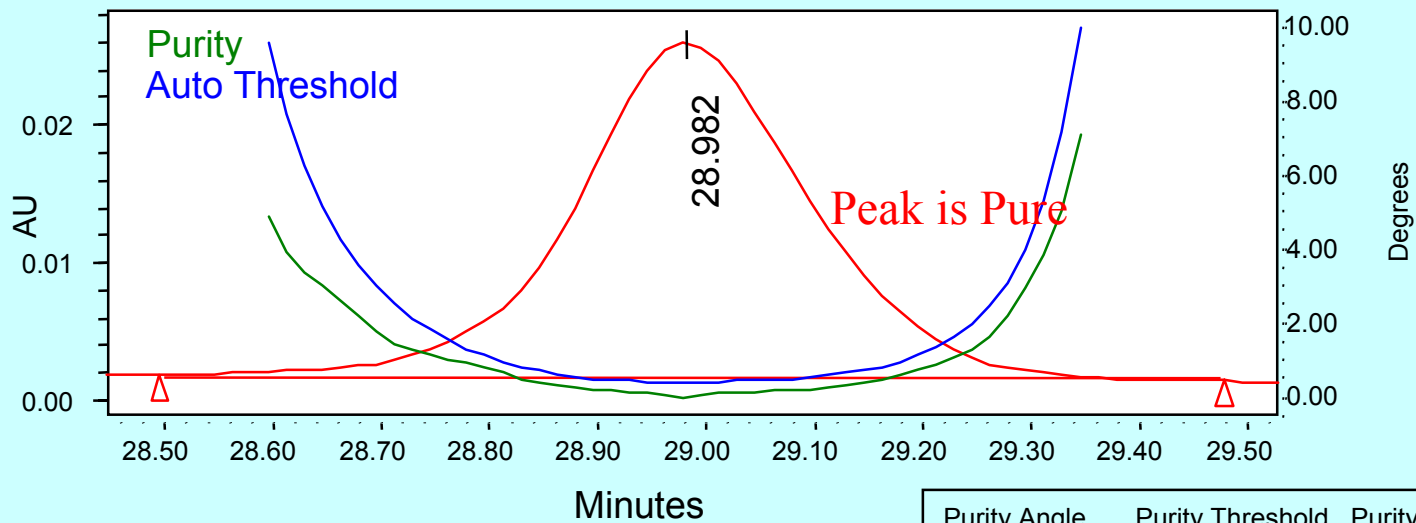
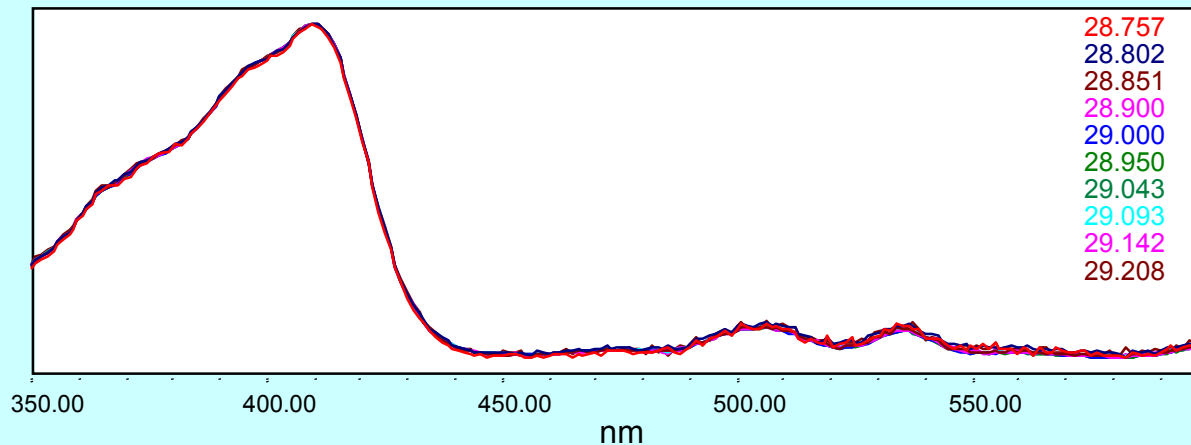
CAROTENOIDS - Extracted from leaves

Spectrum Index presentation



An Example for Pure Peak

Spectra collected from Peak 9

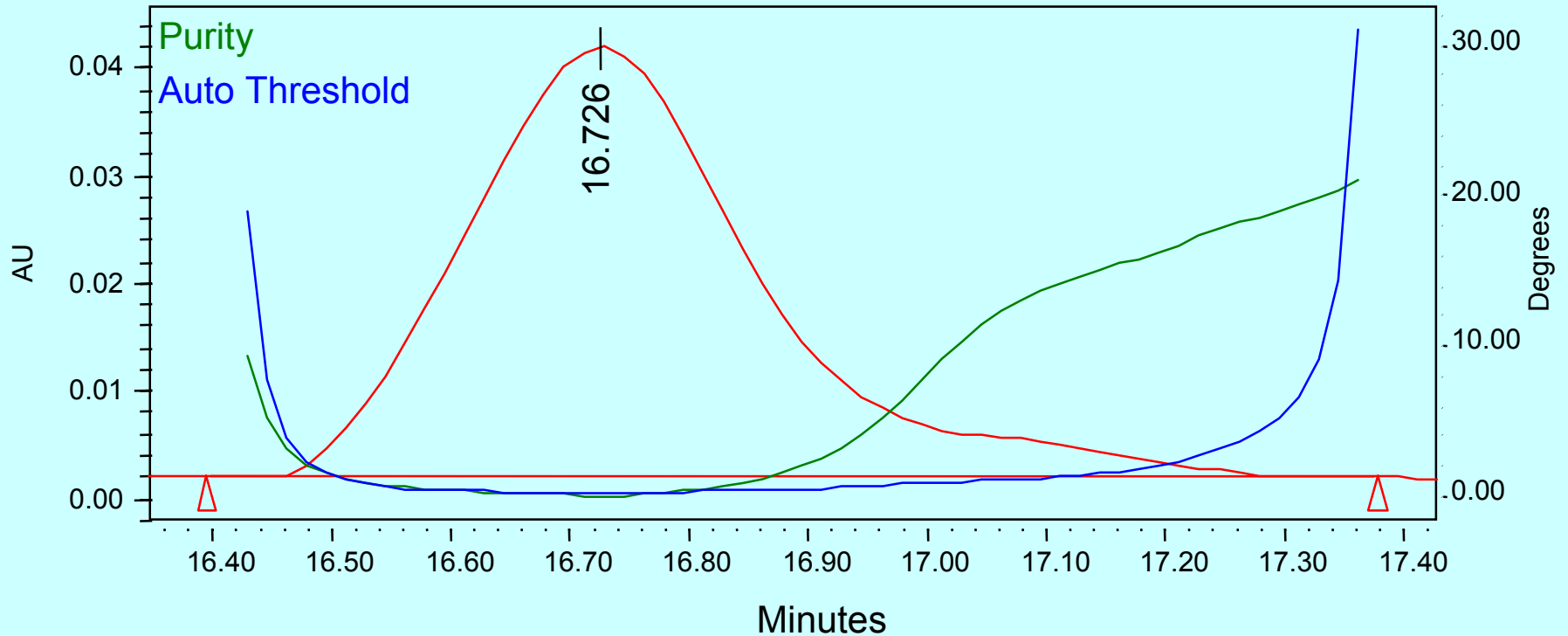


Purity Angle	Purity Threshold	Purity Flag
0.284	0.551	No

An Example for non Pure Peak

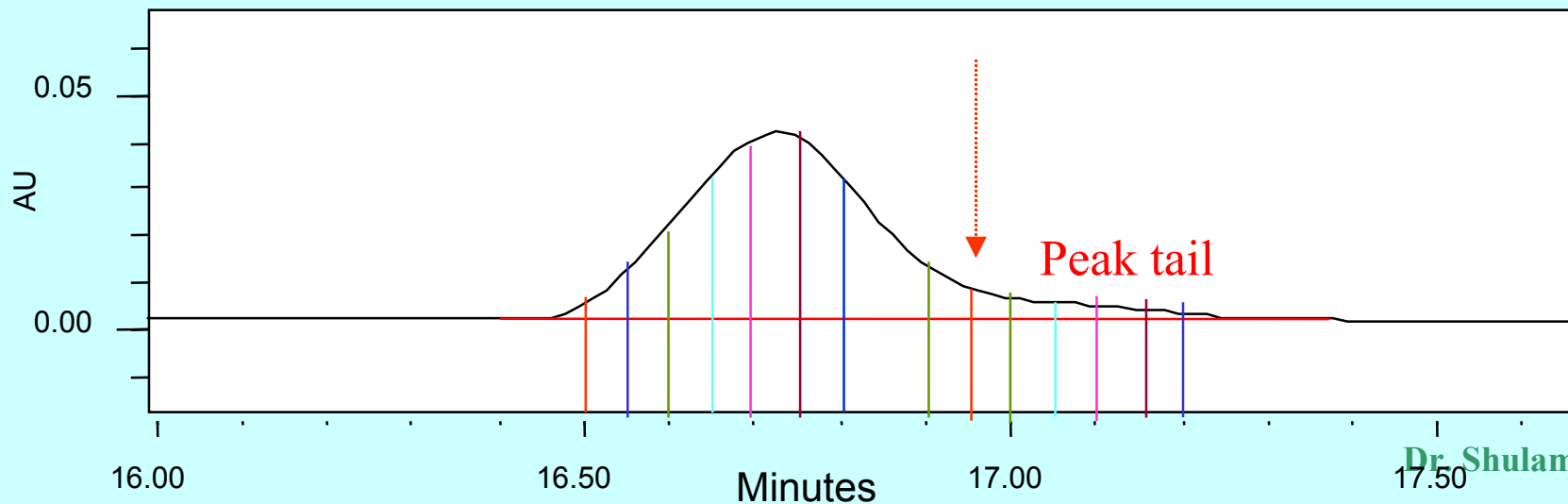
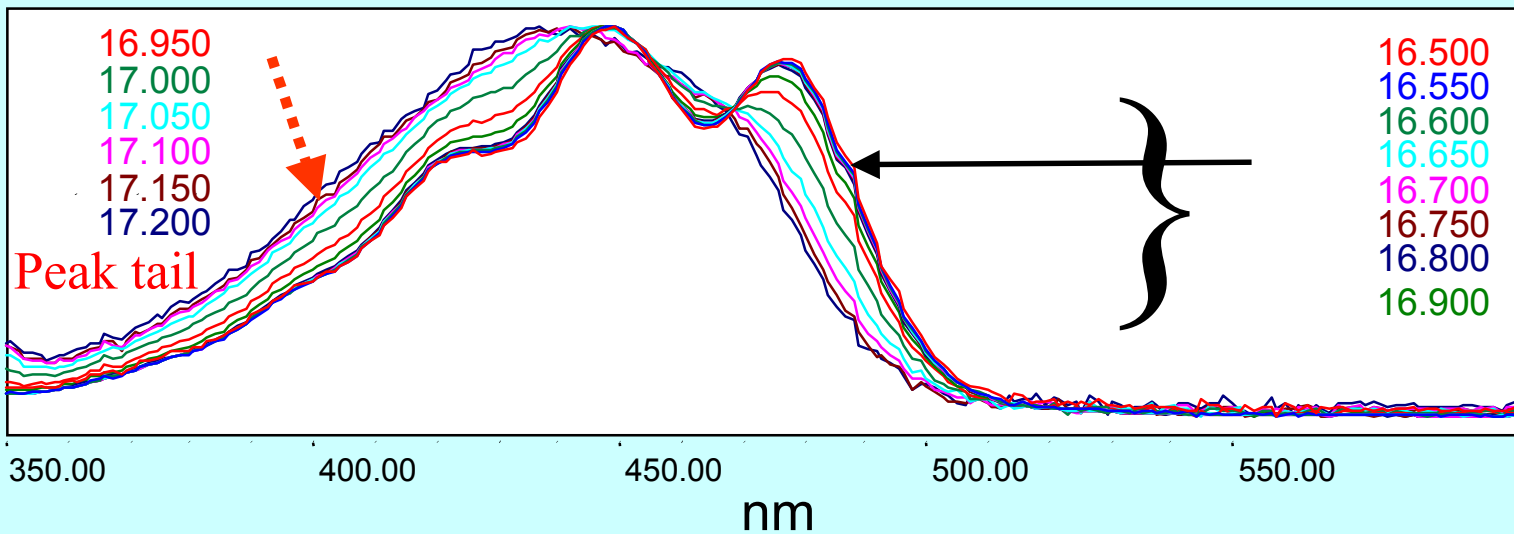
Purity Plot of Peak 4 - Not Pure

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



An Example for non Pure Peak

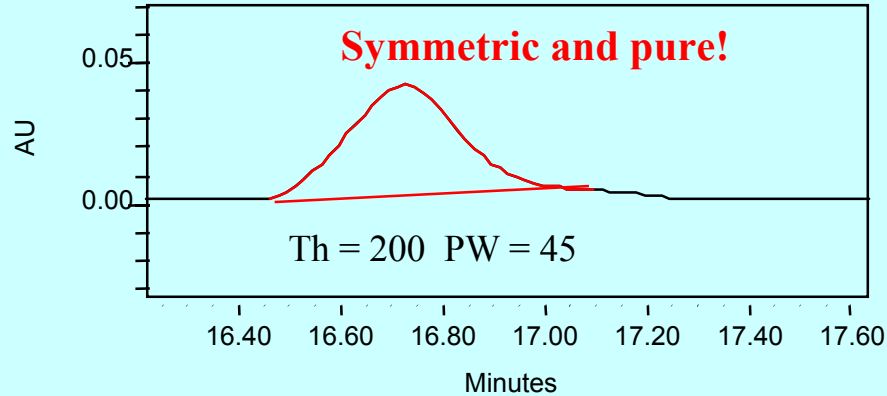
Spectra Selected from Peak 4



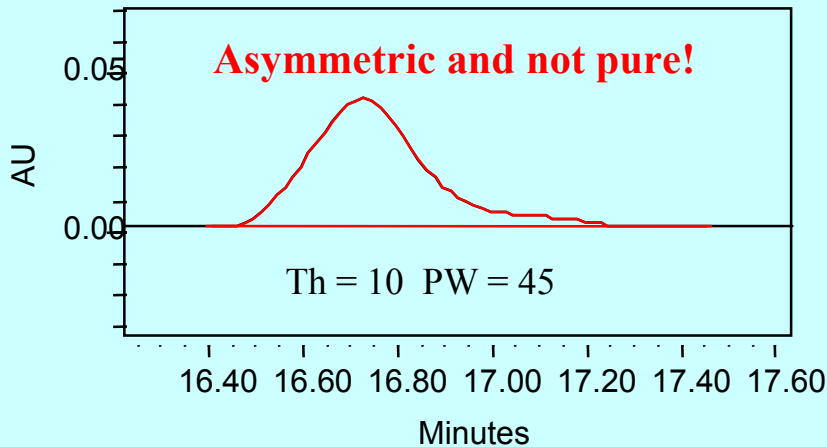
Beware of Peak Integration- where the peak starts or ends!

Effect of Integration Events on Peak Purity Results

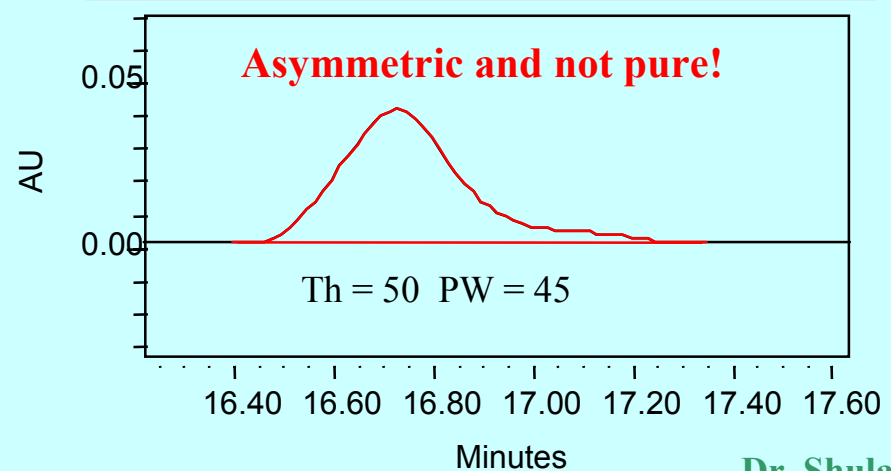
Purity Angle	Purity Threshold	USP Tailing
0.297	0.380	1.057



Purity Angle	Purity Threshold	USP Tailing
2.259	0.410	1.438

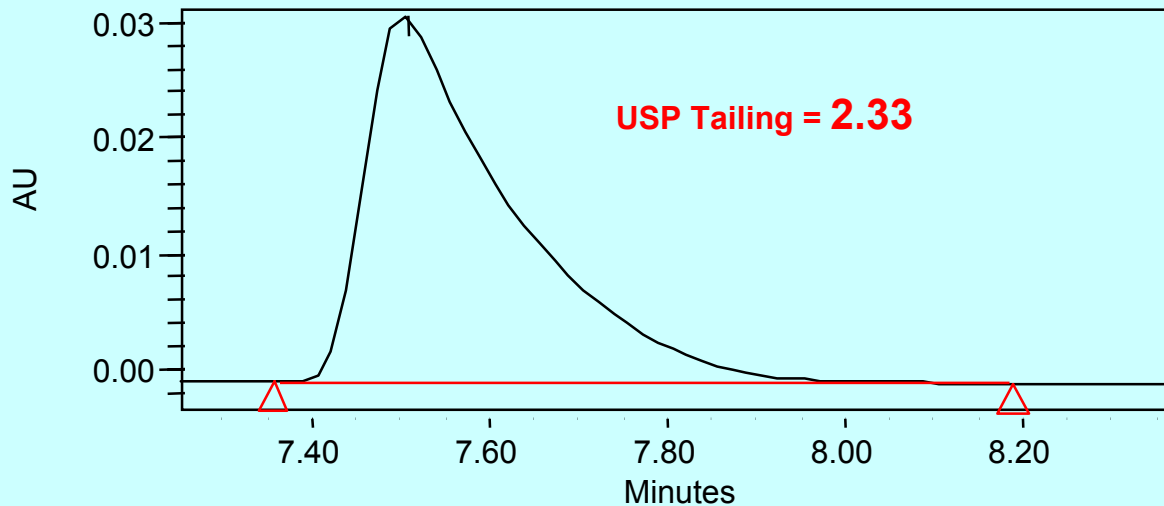
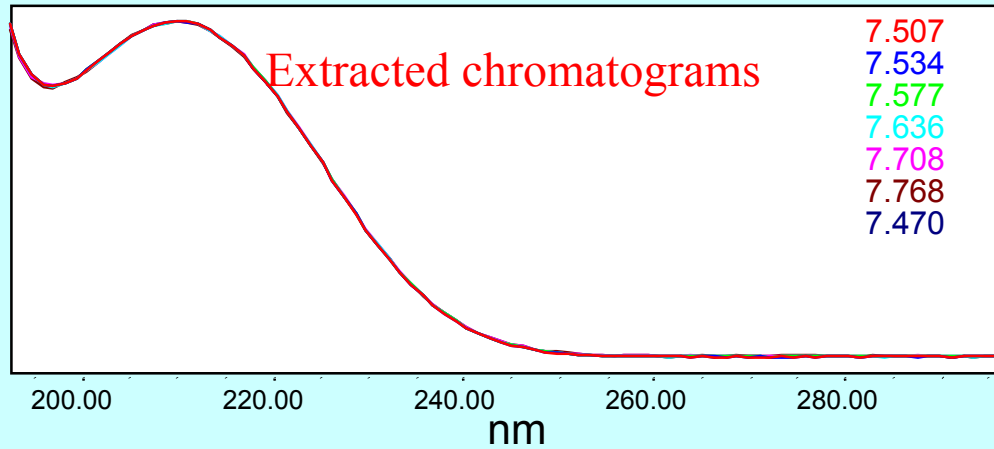


Purity Angle	Purity Threshold	USP Tailing
1.682	0.401	1.415



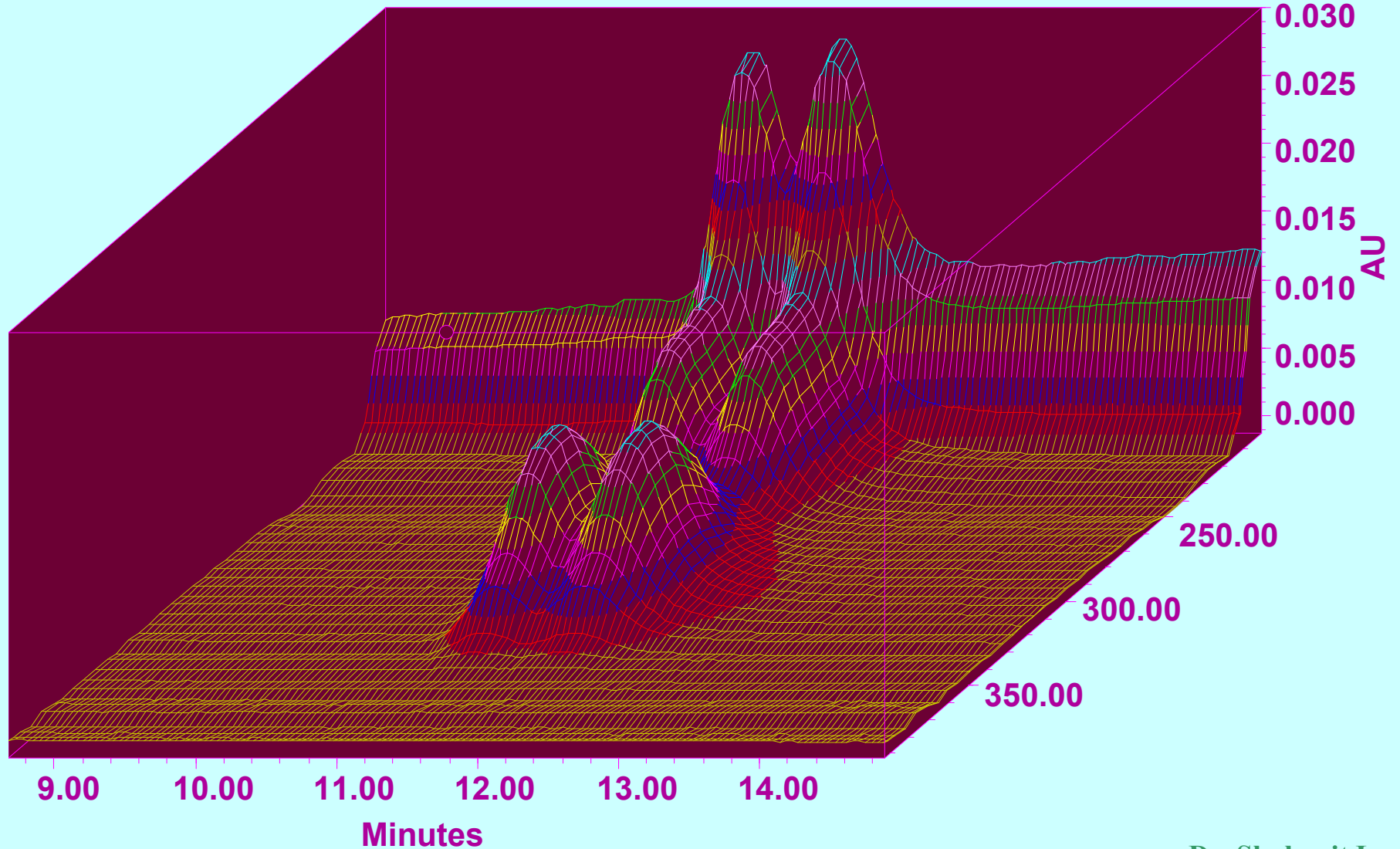
Peak is asymmetric but pure!

Name	Purity Angle	Purity Threshold
GBPN	0.217	0.383



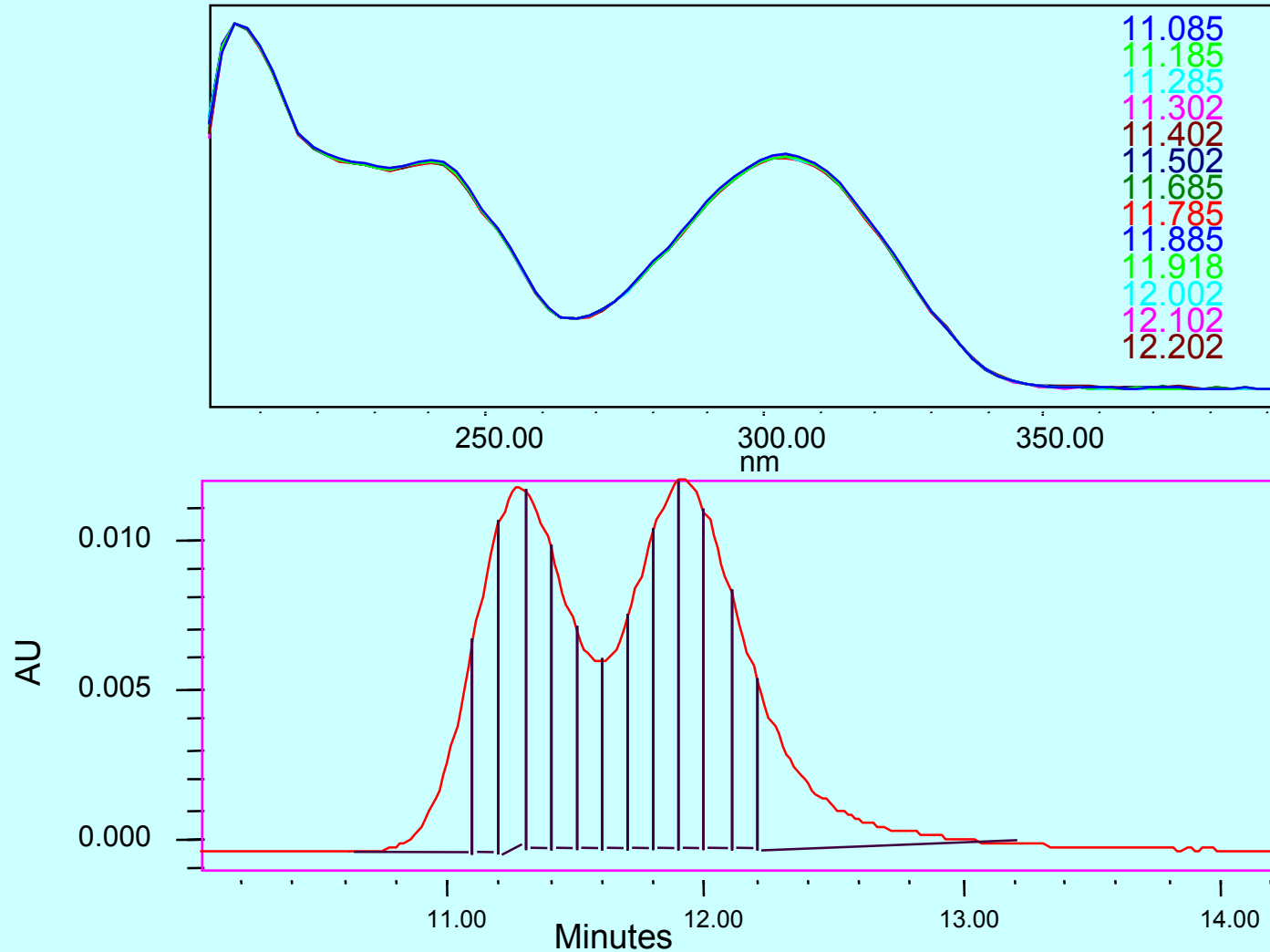
Nucleoside analog's Enantiomers - Identical UV Spectra

3D Plot



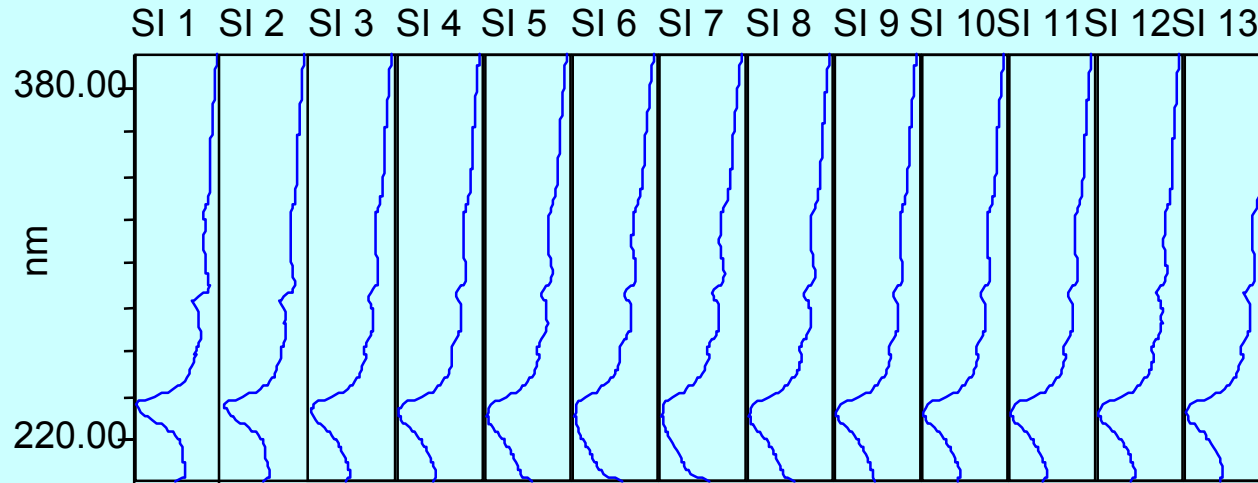
Nucleoside analog' Enantiomers - Identical UV Spectra

Spectra collected from the two peaks

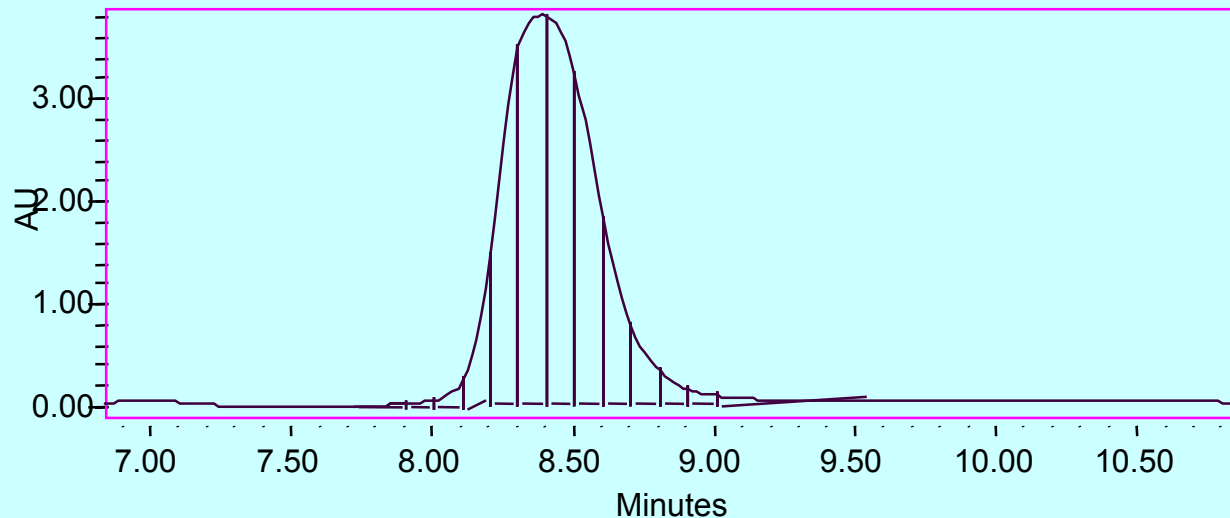


Non-Linearity Effects

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

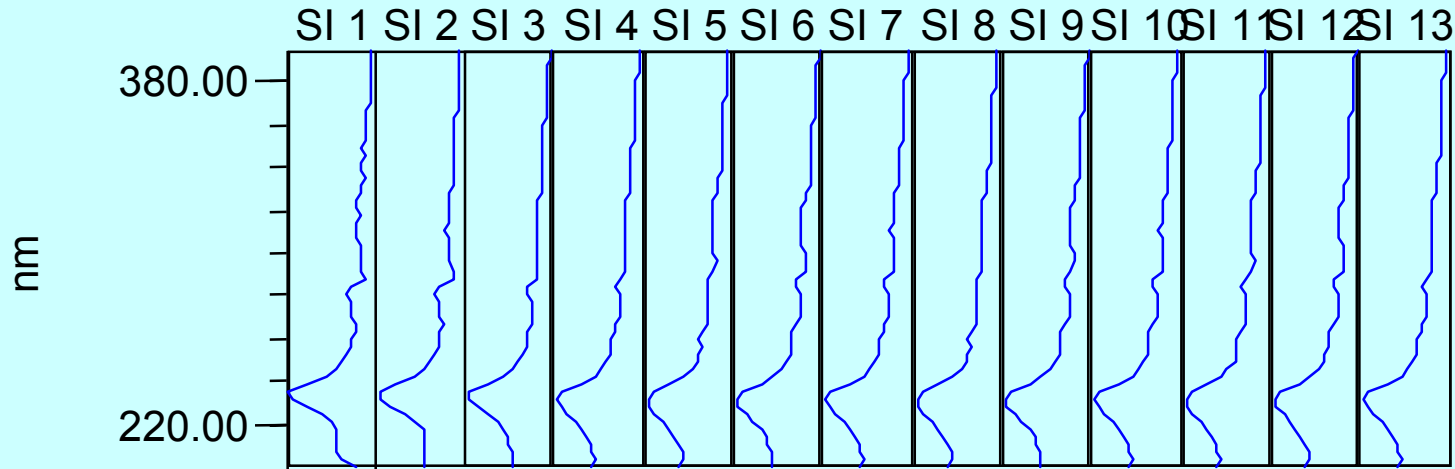


**High Conc.
Non Linear**



Linear Range of Concentration

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



**Low Conc.
Linear**

