

Detection Qualification and Types of Detectors in HPLC

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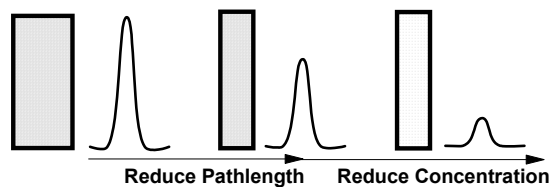
Detectors

- - UV/Vis
 - Fixed wavelength
 - Variable wavelength
 - Diode array
 - Refractive index
 - Fluorescence
 - Electrochemical
- - Conductivity
 - Mass-spectrometric (LC/MS)
 - Evaporative light scattering

Beer's Law

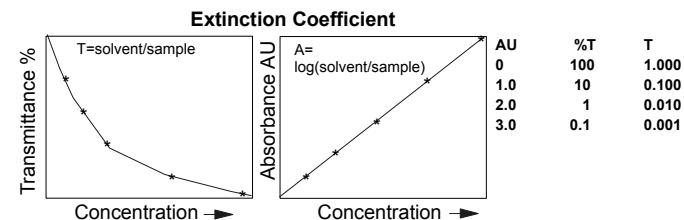
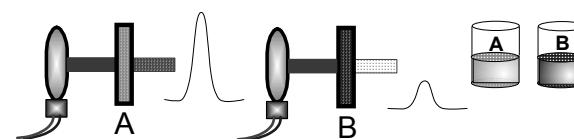
$$\text{Absorbance} = \text{Extinction Coefficient} \times \text{Pathlength} \times \text{Concentration}$$

Only for monochromatic light

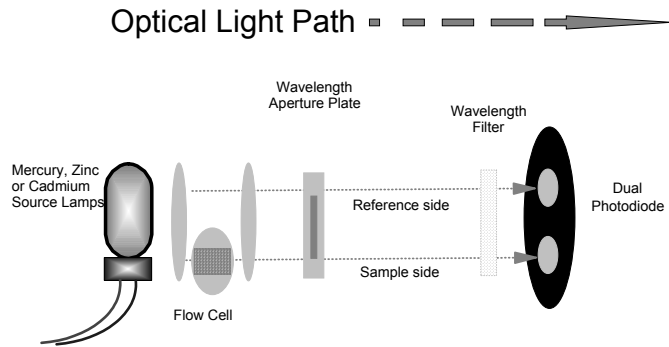


Beer's Law

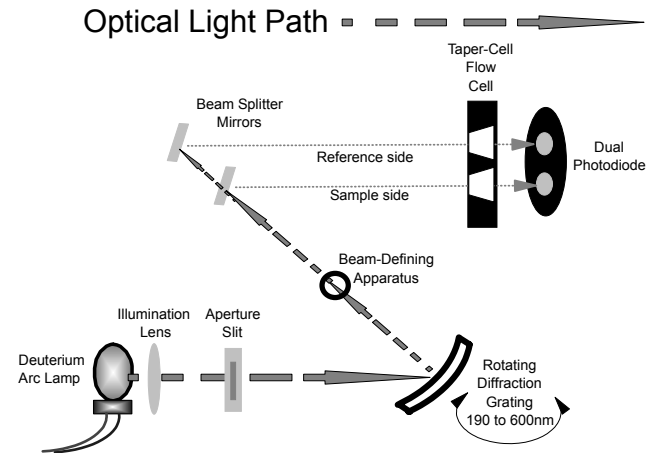
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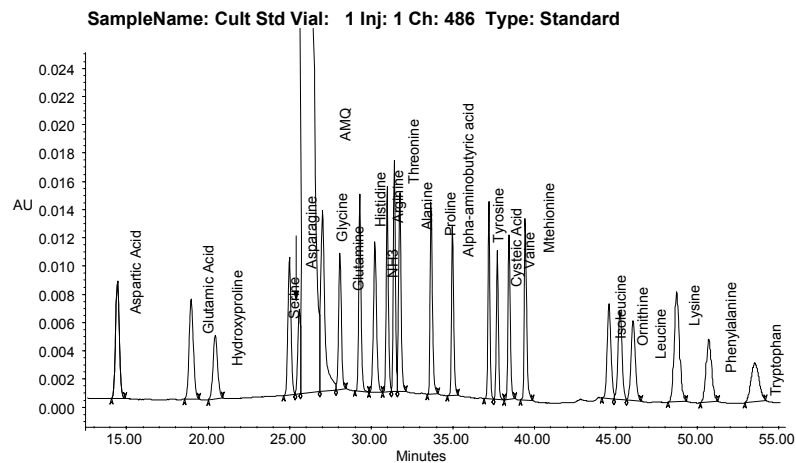
Single Wavelength UV Detector



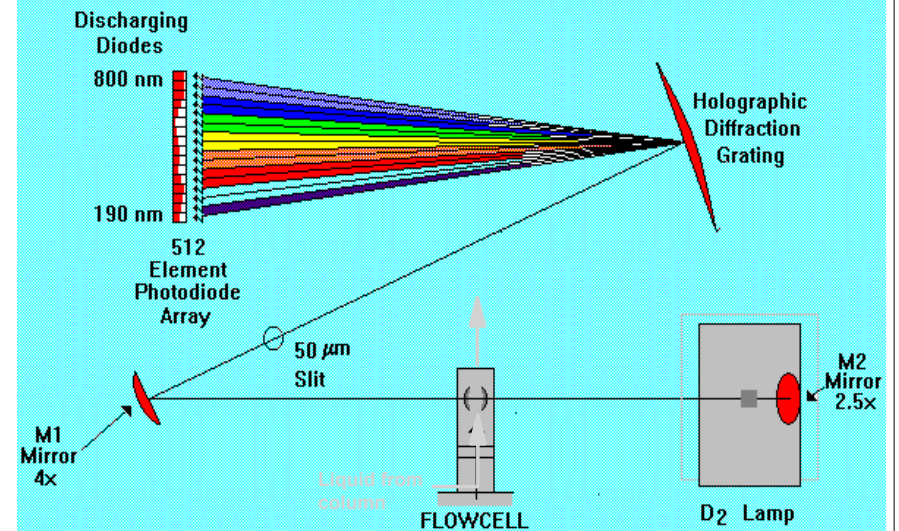
486 Detector Optical Bench



UV Detection of AccQ-Tag Amino Acid Derivatives



Principle of Measurement



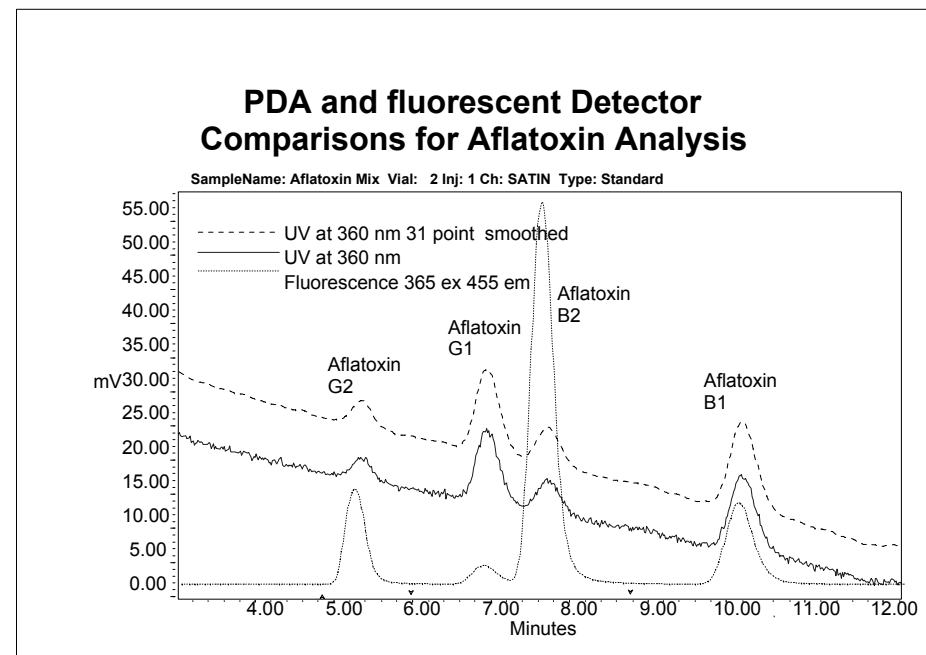
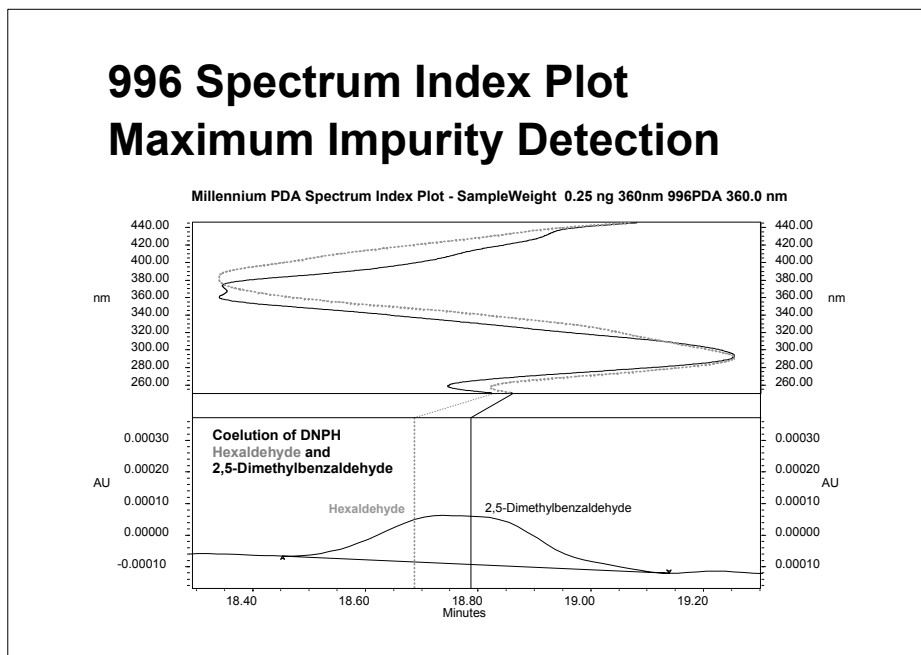
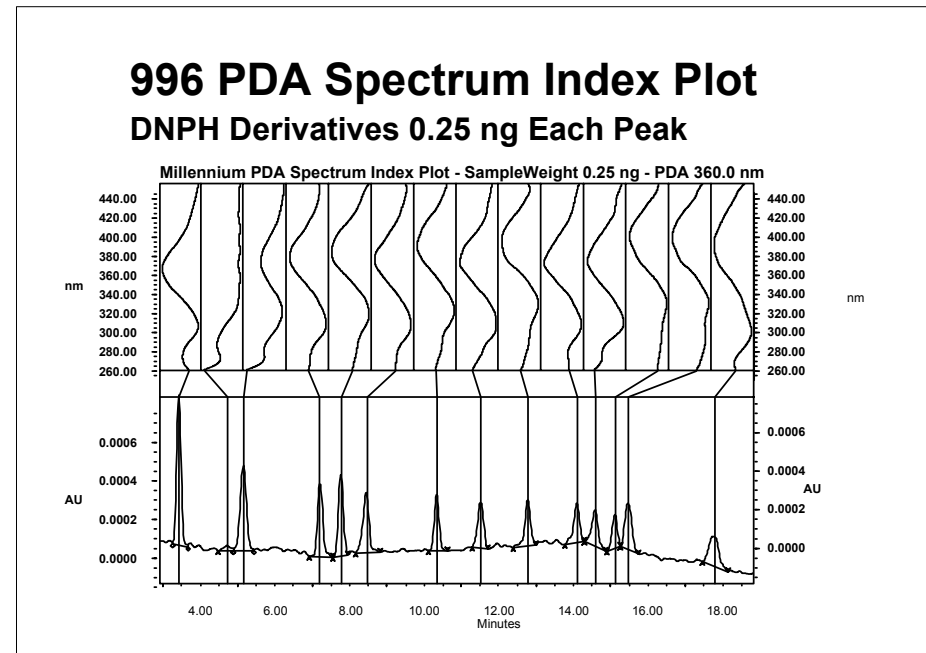
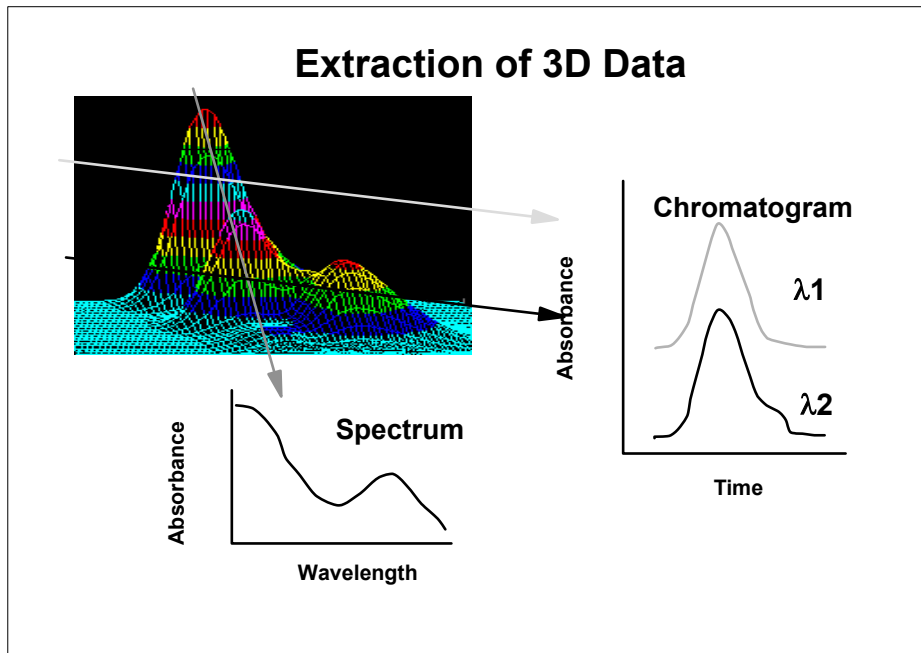
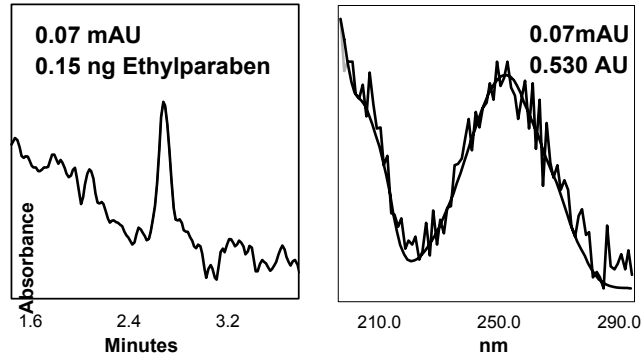


Photo-diode array Chromatographic and Spectral Sensitivity



Detector Criteria

- ▶ Selectivity
- ▶ Sensitivity and detection limit
- ▶ Stability
- ▶ Linear range
- ▶ Dynamic Range
- ▶ Reproducibility
- ▶ Effect on peak shape
- ▶ Maintenance

PROPERTIES OF DETECTORS

SELECTIVITY

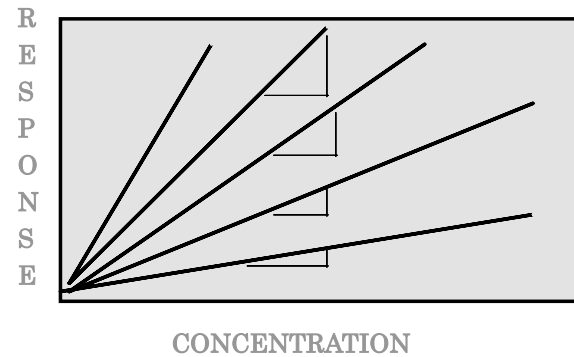
SPECIFIC

UNIVERSAL

- A selective detector allows one to see only components of interest despite of their co-elution with any others.

PROPERTIES OF DETECTORS

SENSITIVITY

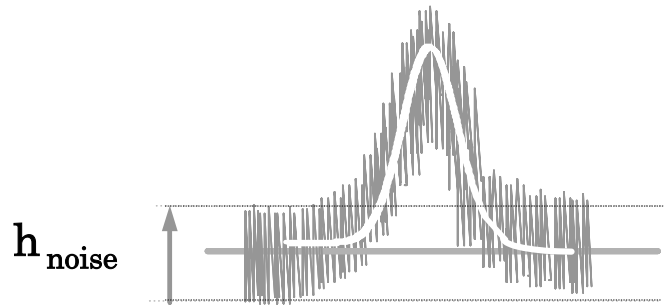


Sensitivity of a detector is the minimum amount that can be detected.

PROPERTIES OF DETECTORS

DETECTION LIMIT

$$h_{\text{signal}} = 2 \times h_{\text{noise}}$$



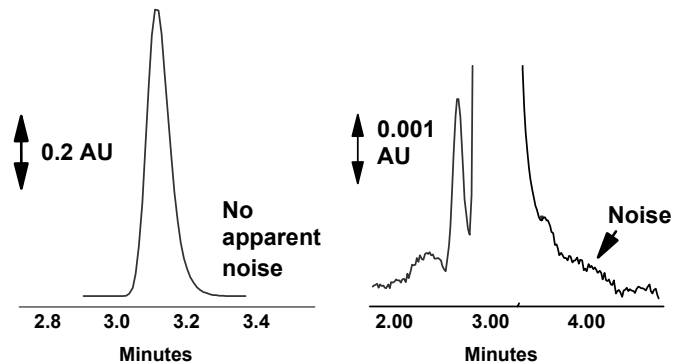
Detector Sensitivity

- **Limit of detection**
 - Lowest concentration that can be detected
 - Signal-to-noise ratio of 2:1 or 3:1

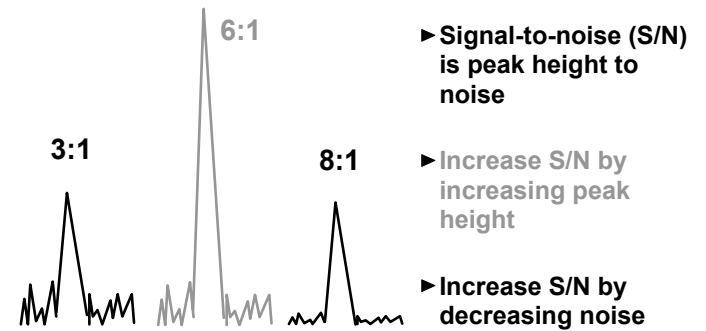
- **Limit of quantitation**
 - Lowest concentration that can be determined with acceptable precision
 - Signal-to-noise ratio of 10:1

Chromatographic Sensitivity

Signal-to-Noise Ratio



Increase Signal-to-Noise Ratio

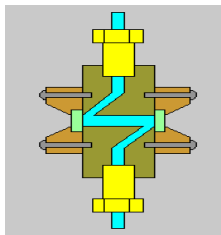


Factors Increasing Signal

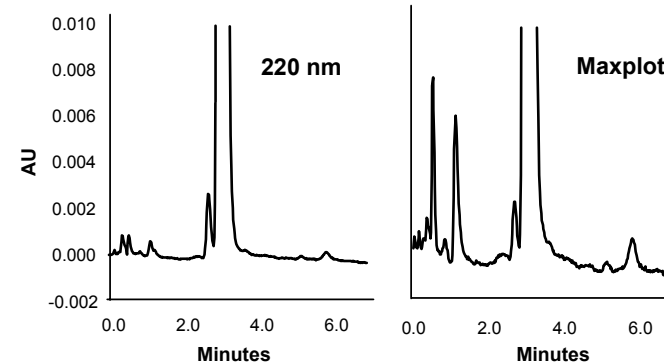
- ▶ Increase sample concentration
- ▶ Increase injection volume
- ▶ Choice of wavelength (s)
- ▶ Low volume flow cell
- ▶ Flow cell pathlength

Factors Affecting Noise in HPLC Detectors

- ▶ Optics bench design
- ▶ Lamp energy
- ▶ Wavelengths
- ▶ Mobile phase composition
- ▶ Pump pulsation
- ▶ Electronics



Chromatographic Sensitivity Single Wavelength vs Maxplot



PROPERTIES OF DETECTORS

BASELINE STABILITY

SHORT RANGE

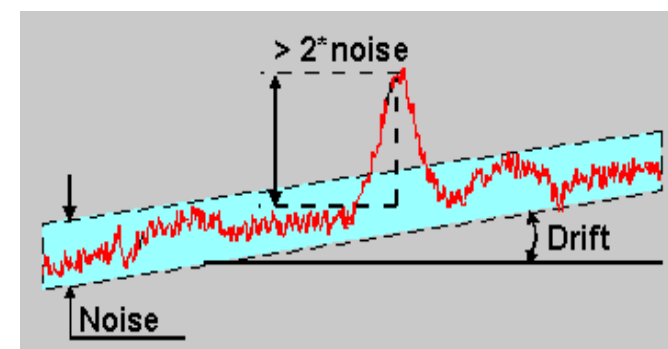


LONG RANGE



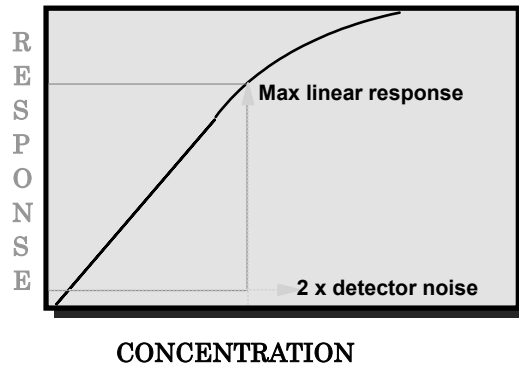
Noise and drift

- Noise, drift, and smallest detectable peak.



PROPERTIES OF DETECTORS

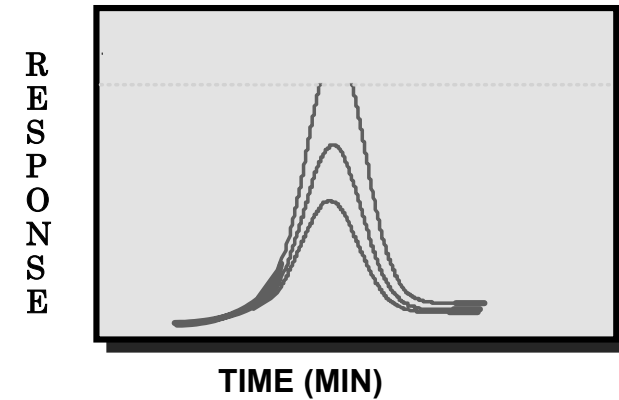
LINEAR RANGE



The linear dynamic range of a detector is the maximum linear response divided by the detector noise.

PROPERTIES OF DETECTORS

DYNAMIC RANGE



PROPERTIES OF DETECTORS

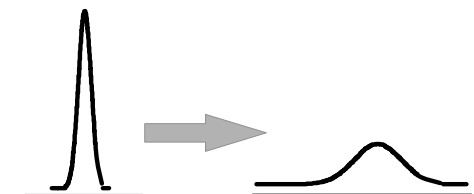
CONTRIBUTION TO BAND BROADENING



RESPONSE TIME



FLOW-CELL VOLUME



PROPERTIES OF DETECTORS

REPEATABILITY OF RESPONSE



TEMPERATURE, FLOW RATE, ELECTRONICS

PROPERTIES OF DETECTORS

MAINTENANCE AND COST



EASY HANDLING OF FLOW-CELL

EASY A/D CONVERSION

SAFETY

Detector Qualification

Wavelength Accuracy

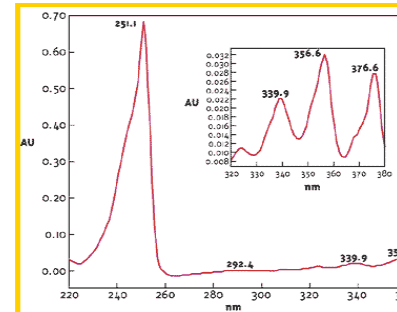


Figure 1. Spectrum of anthracene solution (1 mg/mL in acetonitrile) from Waters 996 PDA detector shows the annotations of λ_{max} . The inset shows an expanded view of the bands centered between 320 and 380 nm.

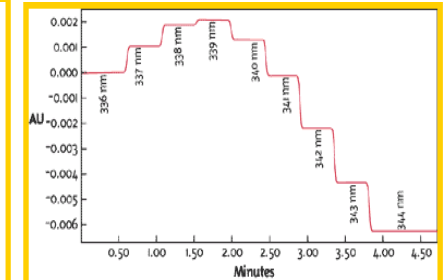


Figure 2. Calibrations of a UV-vis detector (Waters 2487) nm by incremental scanning from 336 to 344 nm. of anthracene is determined to be at 339 nm for this. The autozero on wavelength function in the detector nactivated for this test.