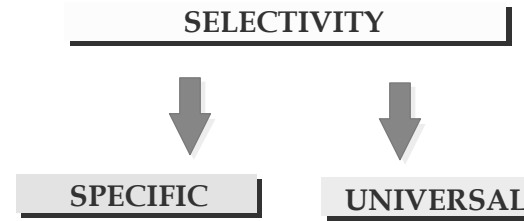


Detection in HPLC - Qualification

Qualification of Detectors

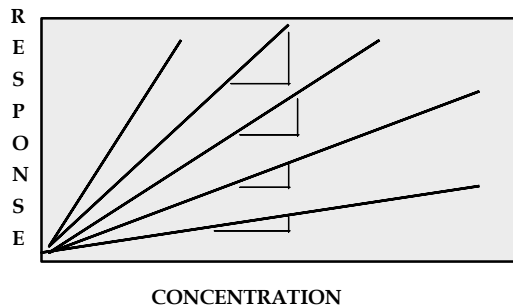
- Selectivity
- Sensitivity and detection limit
- Stability
- Linear range
- Dynamic Range
- Reproducibility
- Effect on peak shape
- Maintenance

Properties of Detectors



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

Sensitivity



Sensitivity of a detector is not the minimum amount that can be detected.

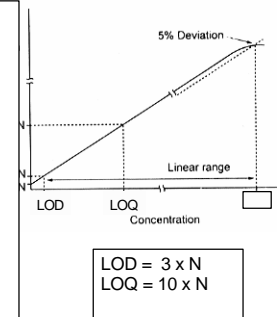
Linear Dynamic Range (LDR)

- Linear range is based upon the following equation:

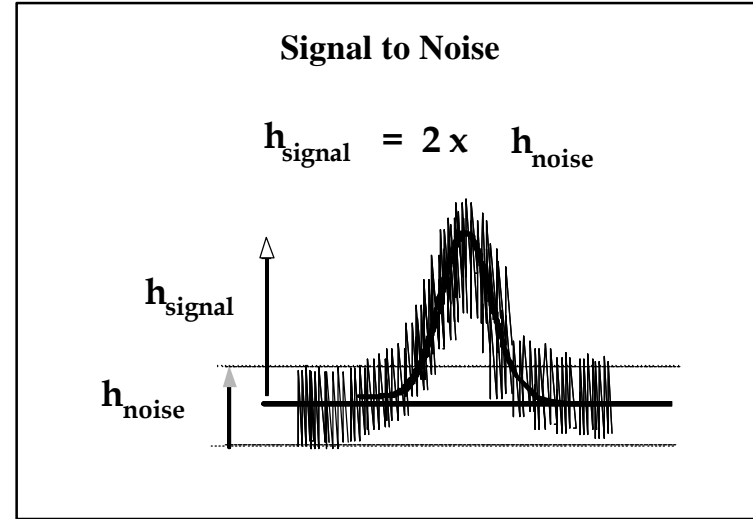
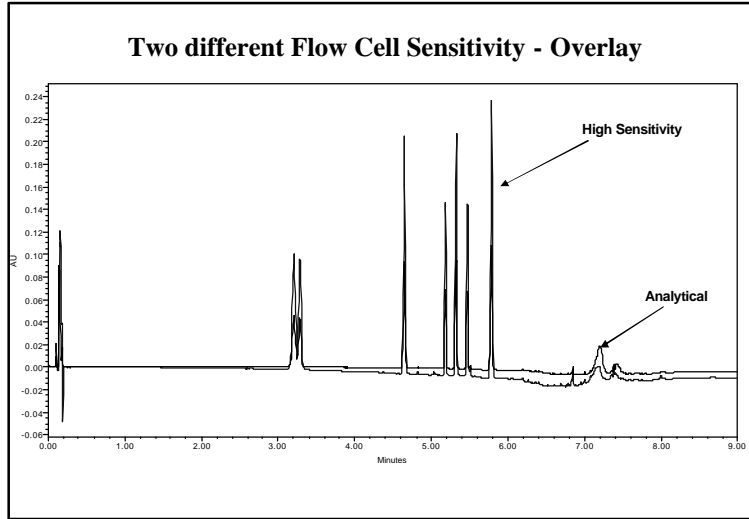
$$R = SC + N$$

R: Detector response
 S: Sensitivity
 C: Concentration
 N: Noise Signal (intercept)

- For linear detector the equation becomes $R = RC$, response is linear from the limit of detection (LOD) to the upper concentration level which produces a deviation from linearity of about 5%.



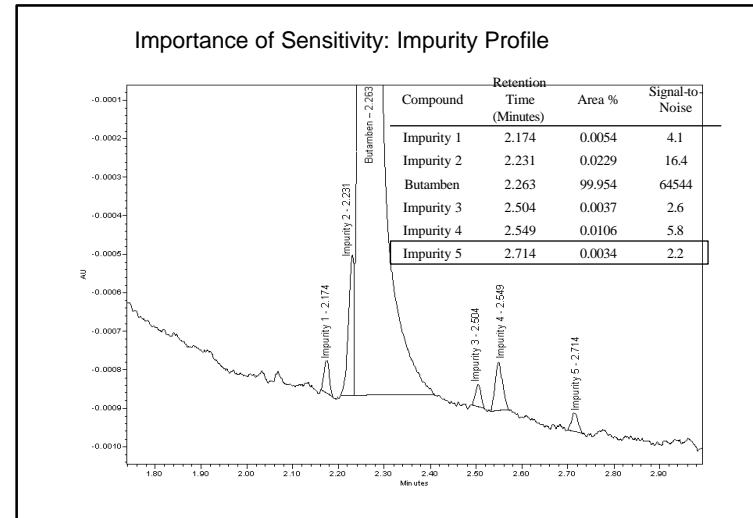
Detection in HPLC - Qualification



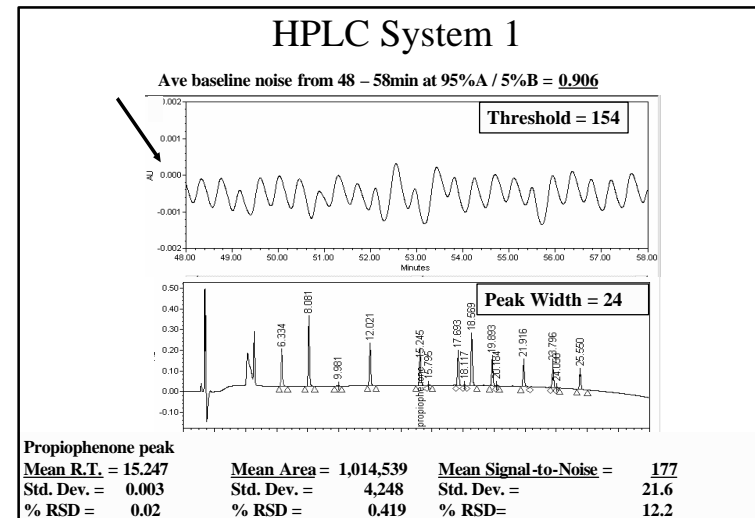
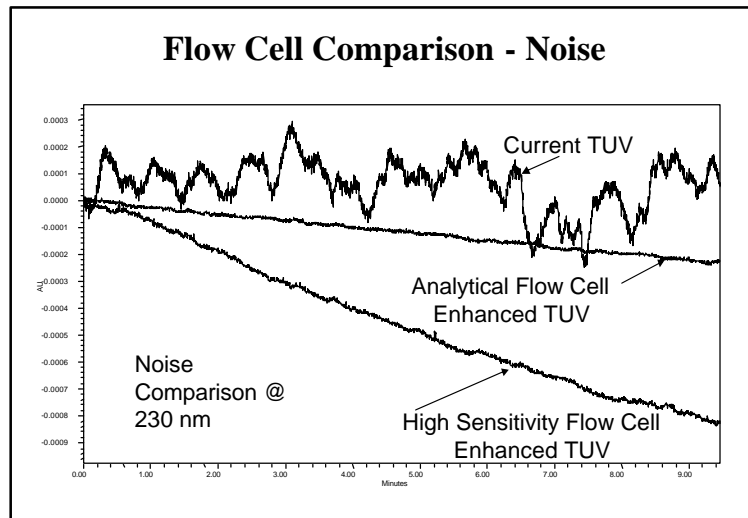
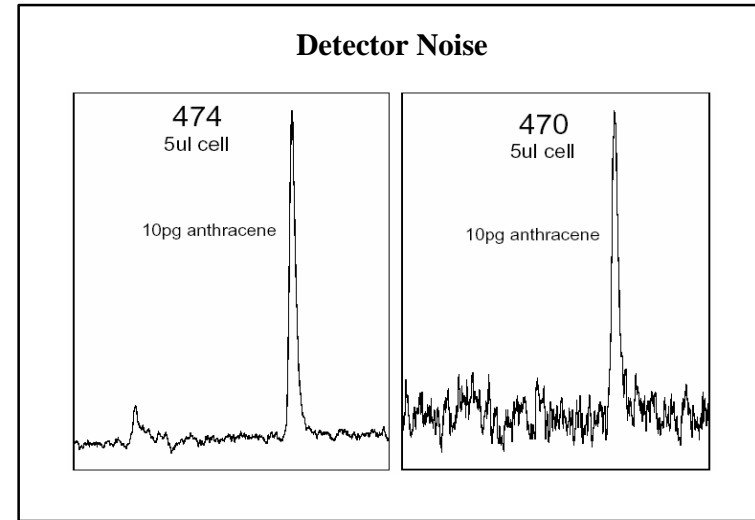
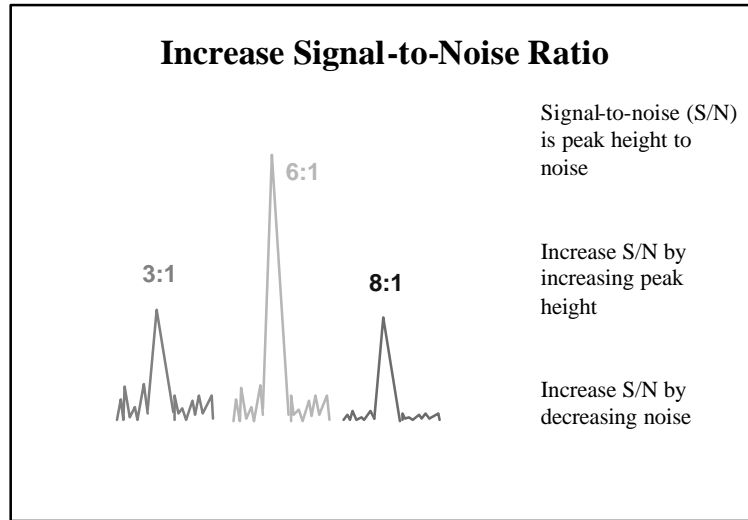
LOD & LOQ

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

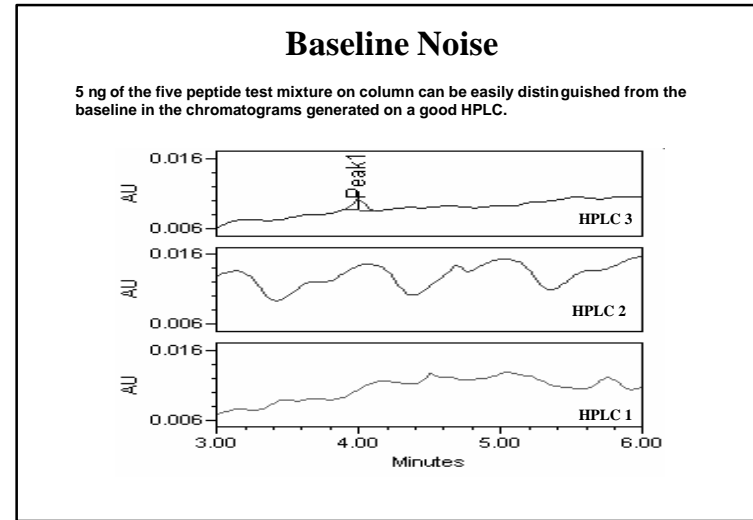
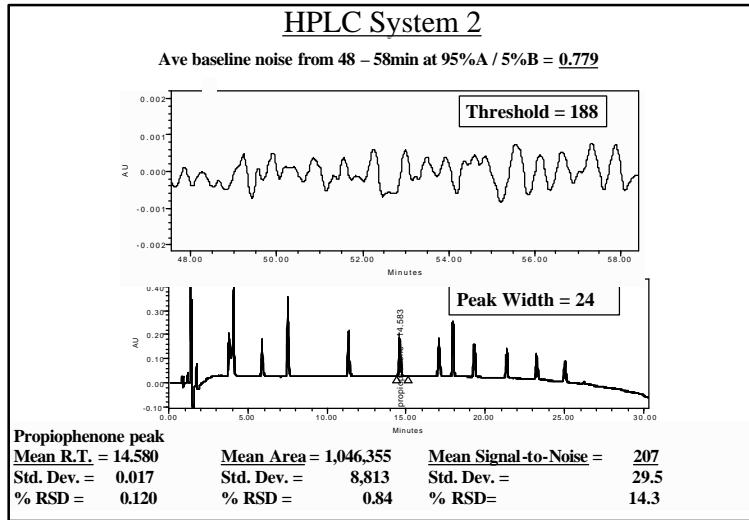
Limit of quantitation Lowest concentration that can be determined with acceptable precision. Signal-to-noise ratio of 10:1



Detection in HPLC - Qualification

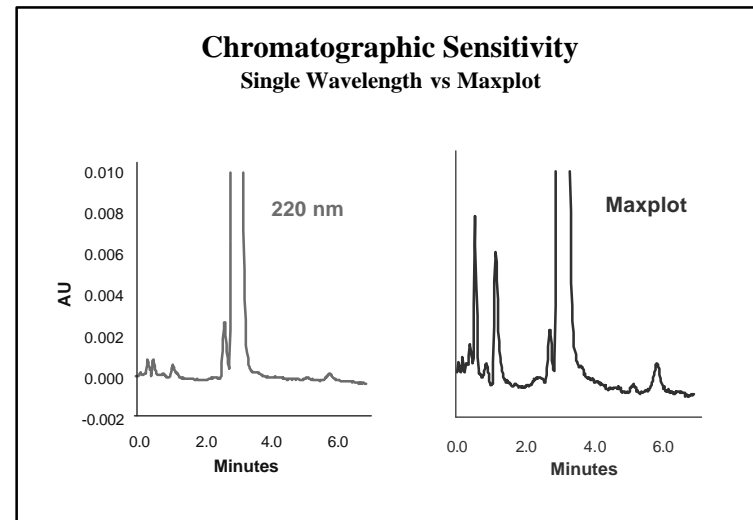


Detection in HPLC - Qualification

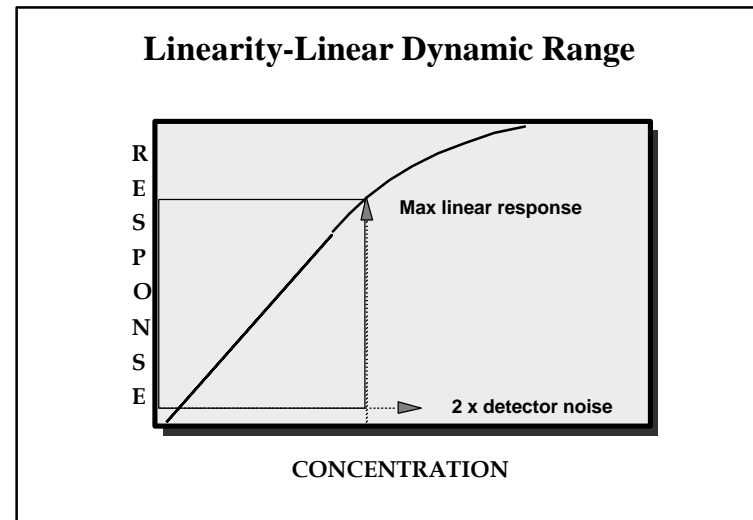
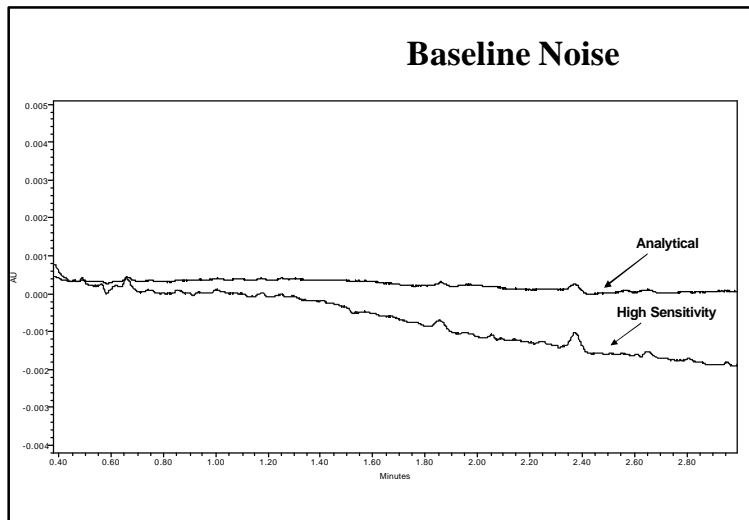
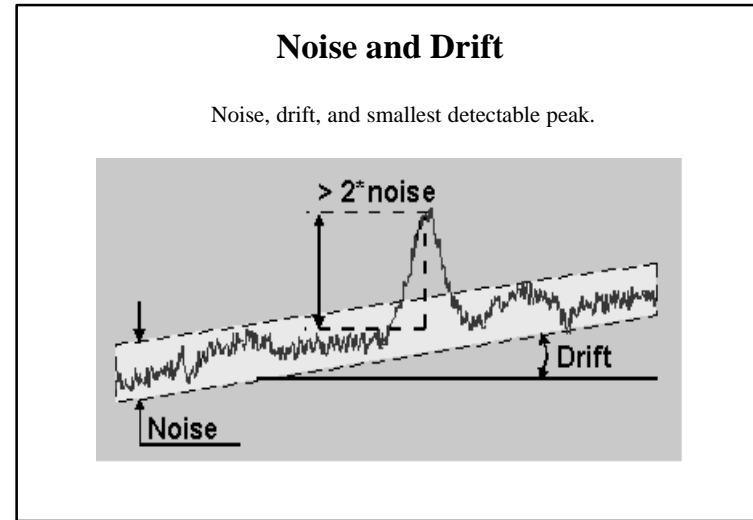
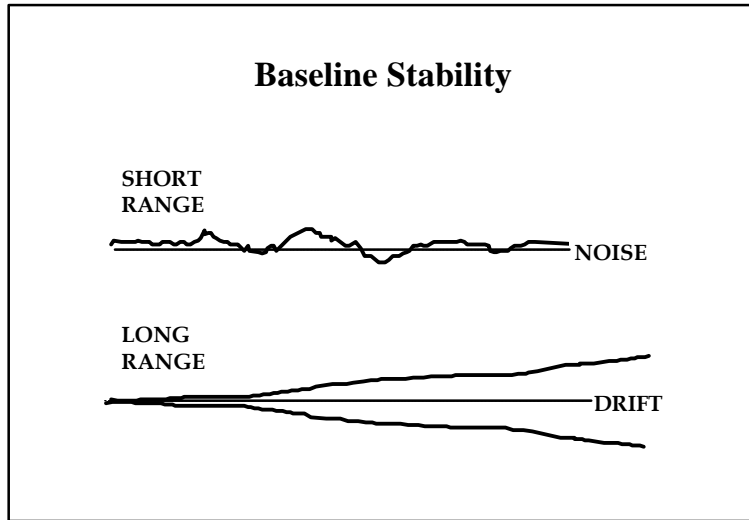


Increasing Signal to Noise

<p>Factors Increasing UV Signal</p> <ul style="list-style-type: none"> Increase sample concentration Increase injection volume Choice of wavelength (s) Low volume flow cell Flow cell pathlength 	<p>Factors Affecting Noise in UV Detectors</p> <ul style="list-style-type: none"> Optics bench design Lamp energy Wavelengths Mobile phase composition Pump pulsation Electronics
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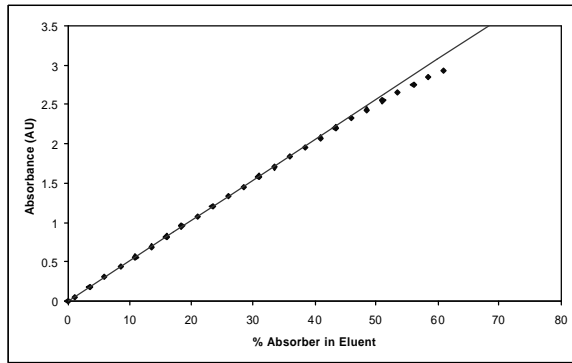


Detection in HPLC - Qualification

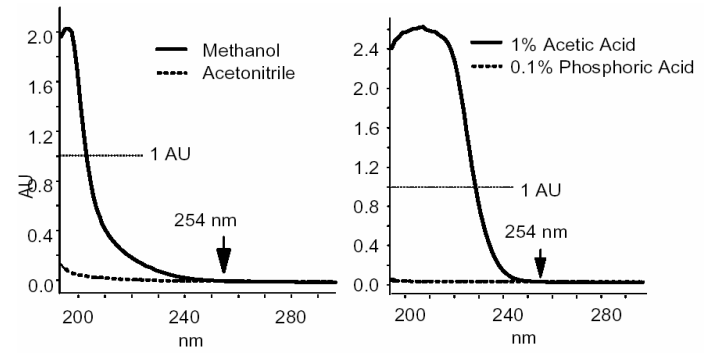


Detection in HPLC - Qualification

Linearity Test Results

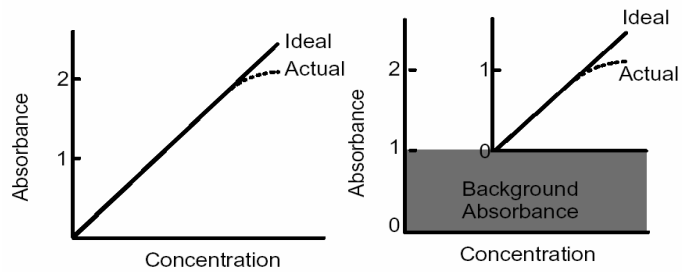


Background Response

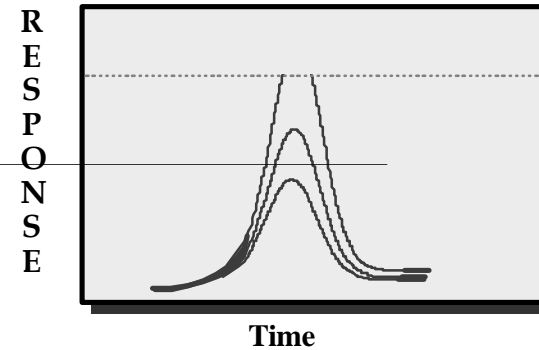


Background Absorbance

Reduces Dynamic Range

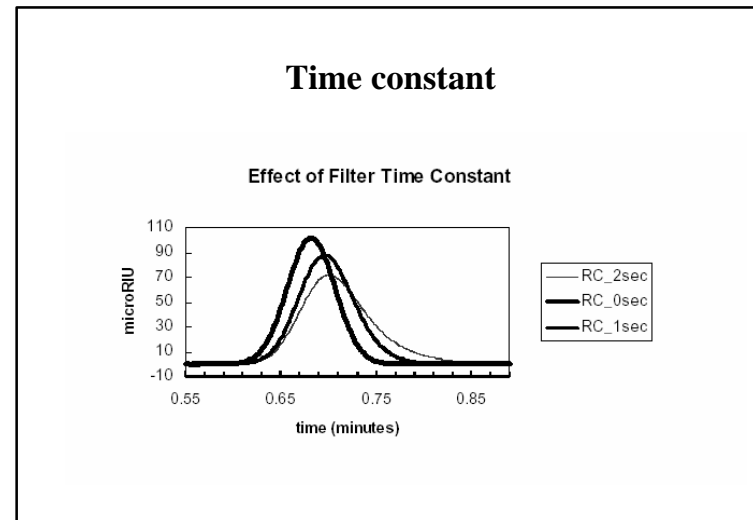
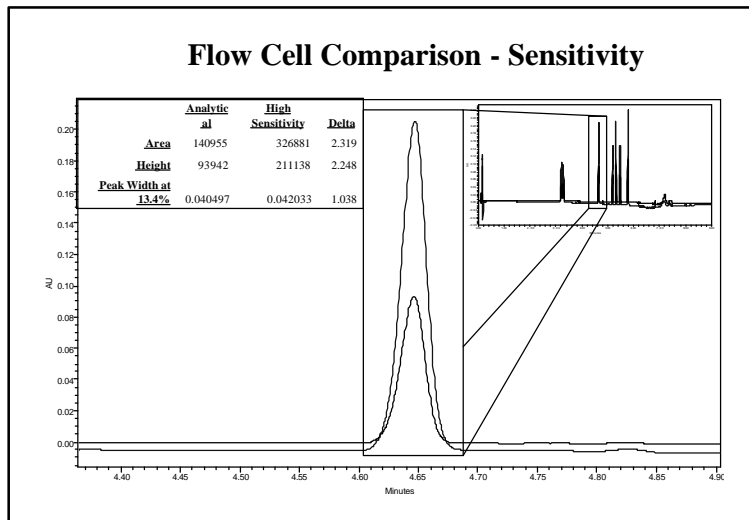
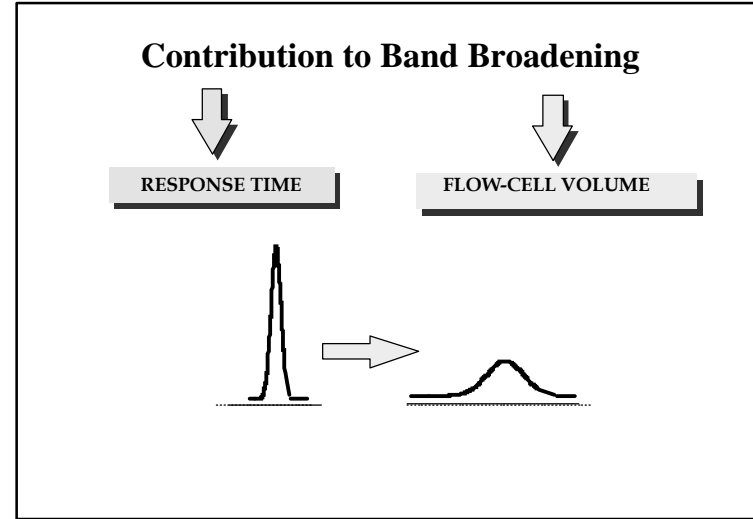
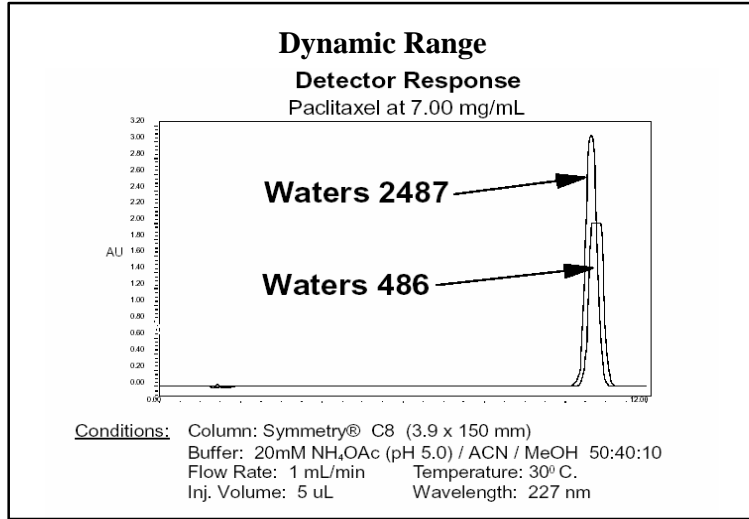


Dynamic Range



The range of solute concentration over which the detector continues to respond to changes in solute concentration

Detection in HPLC - Qualification



Detection in HPLC - Qualification

Maintenance

- Flow Cell & Optics– UV-VIS, Fluorescence, RI
- Electrode – Conductivity and Electrochemical
- Nebulizer – ELS and MS

Wavelength Accuracy Test

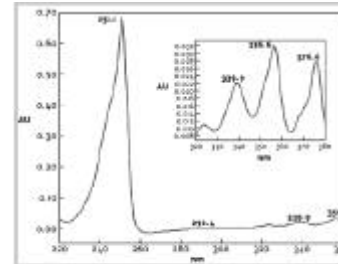


Figure 1. Spectrum of anthracene solution (1 mg/ml in acetonitrile) from Waters 996 PDA detector shows the annotations of peaks. The inset shows an expanded view of the bands centered between 300 and 360 nm.

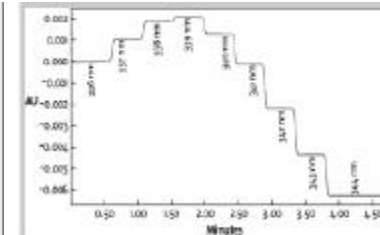


Figure 2. Calibrations of a UV-vis detector (Waters 2487) by incremental scanning from 336 to 344 nm of anthracene is determined to be at 339 nm for this. The autotune on wavelength function in the detector activated for this test.