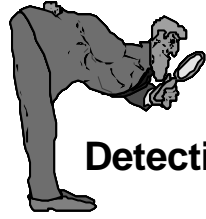


Detection Qualification and Types of Detectors in HPLC

Dr. Shulamit Levin
Medtechnica

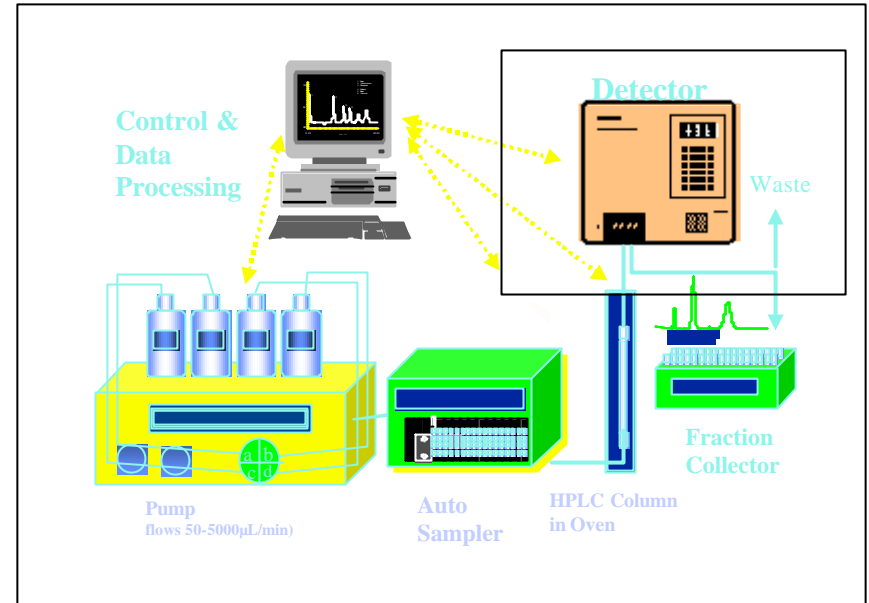
<http://www.forumsci.co.il/HPLC>
levins@medtechnica.co.il
shulal@zahav.net.il



Detection Qualification and Types of Detectors in HPLC

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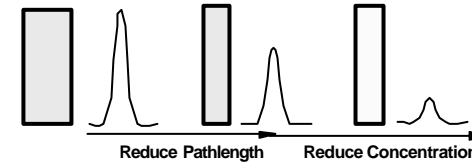
Detectors

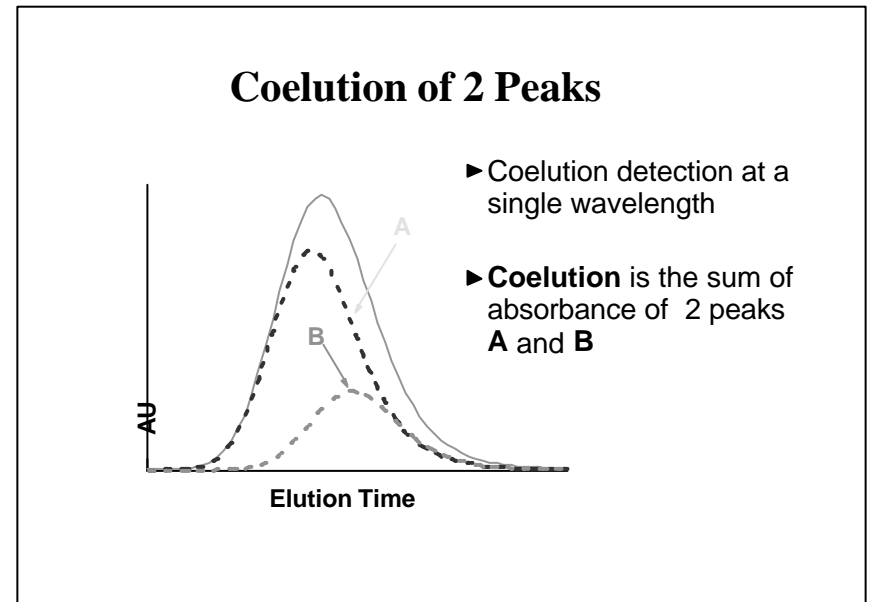
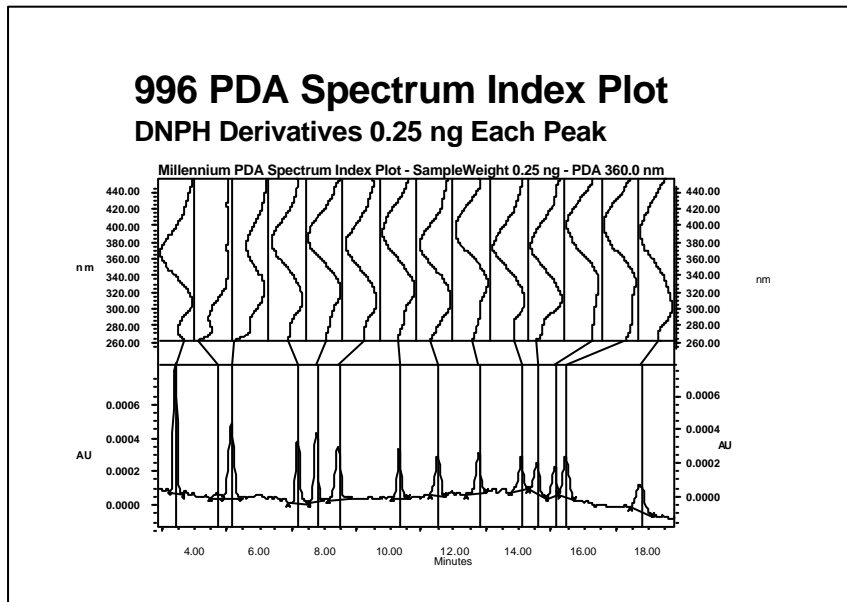
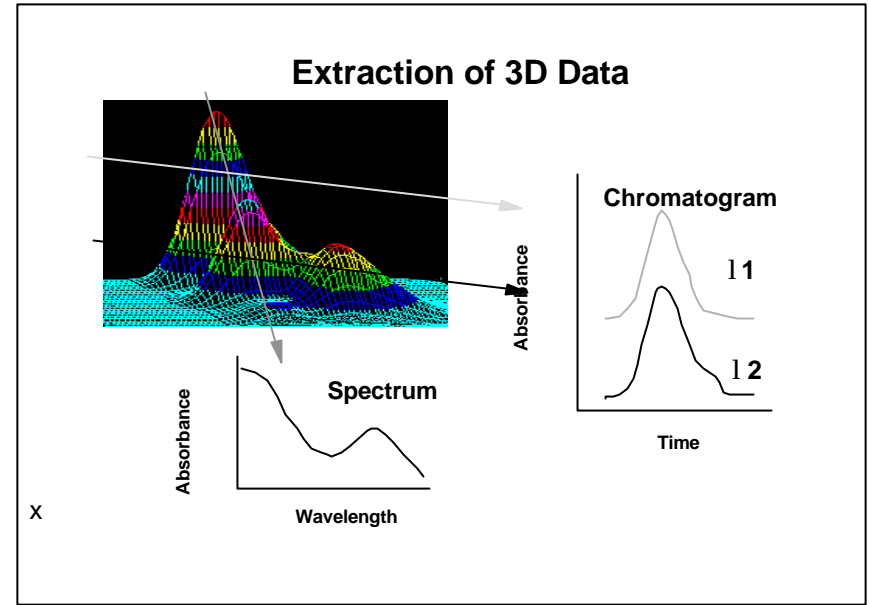
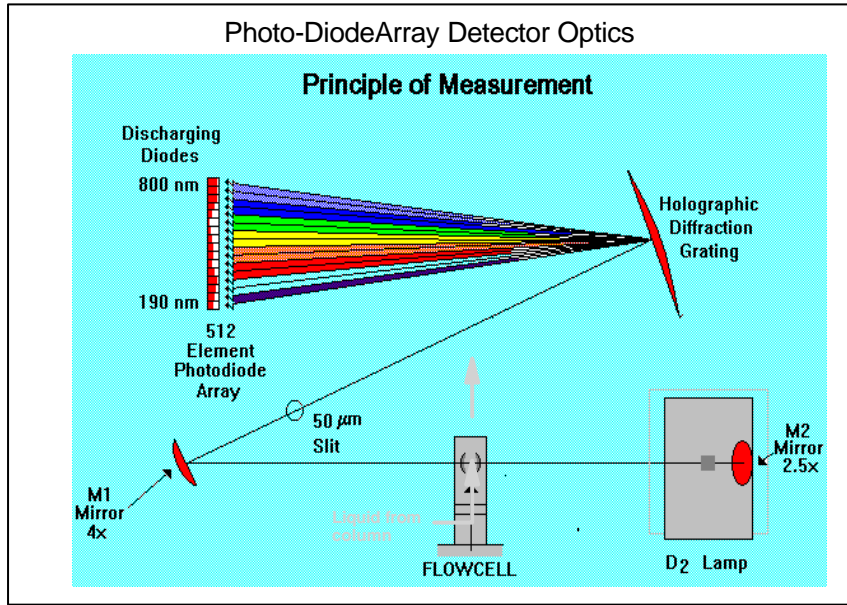
- - UV/Vis
 - Fixed wavelength
 - Variable wavelength
 - Diode array
 - Refractive index
 - Fluorescence
 - Electrochemical
- - Conductivity
 - Mass-spectrometric (LC/MS)
 - Evaporative light scattering

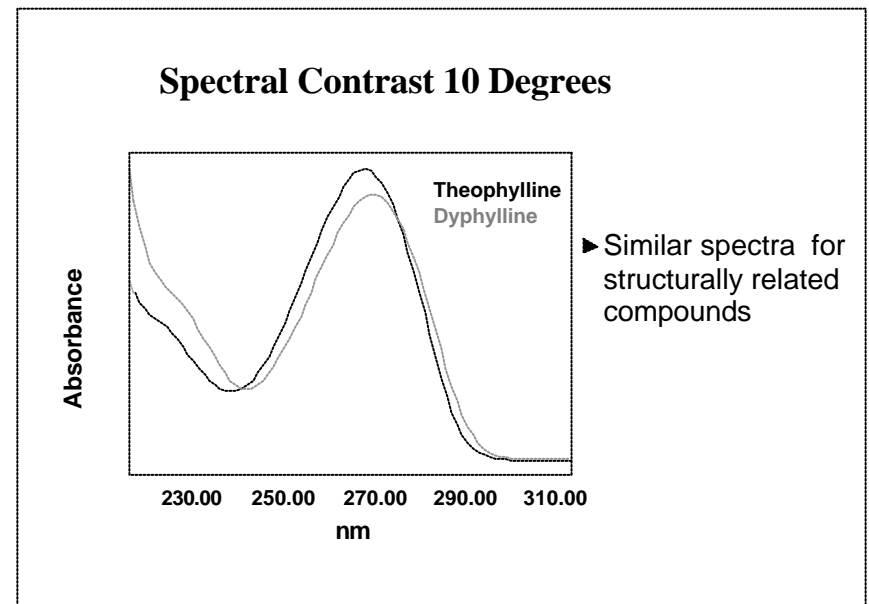
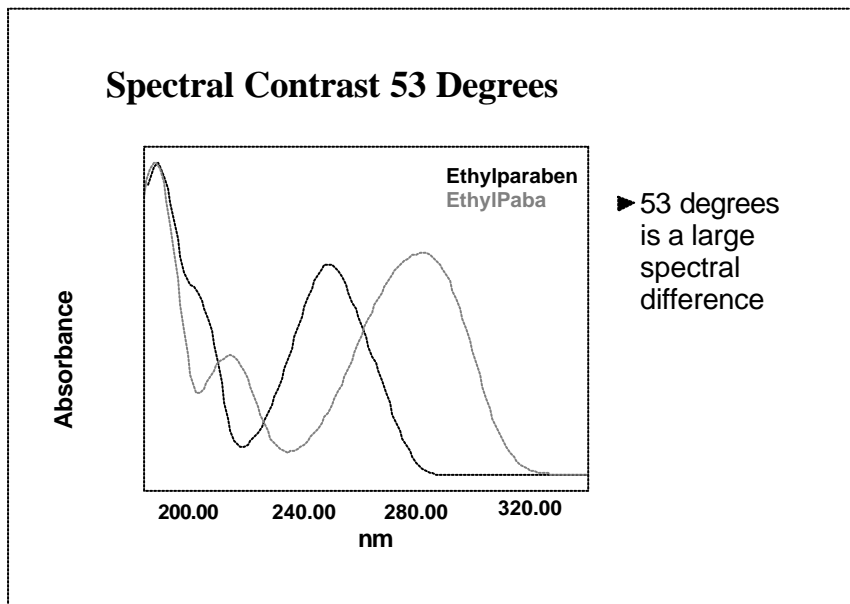
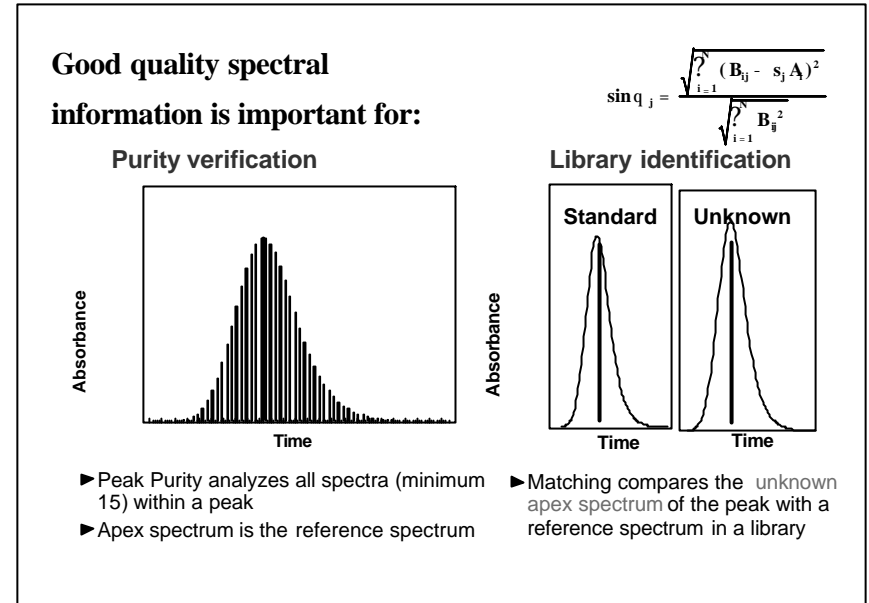
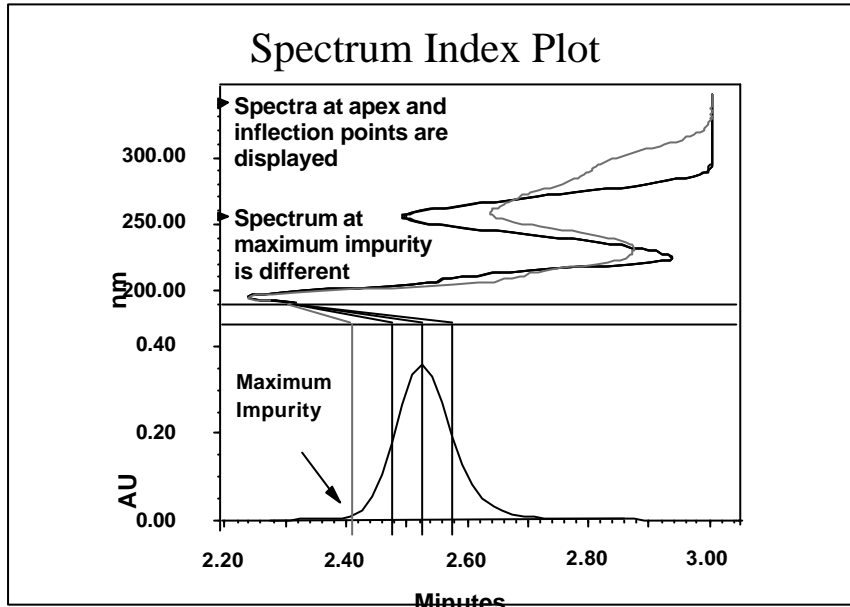
Beer's Law

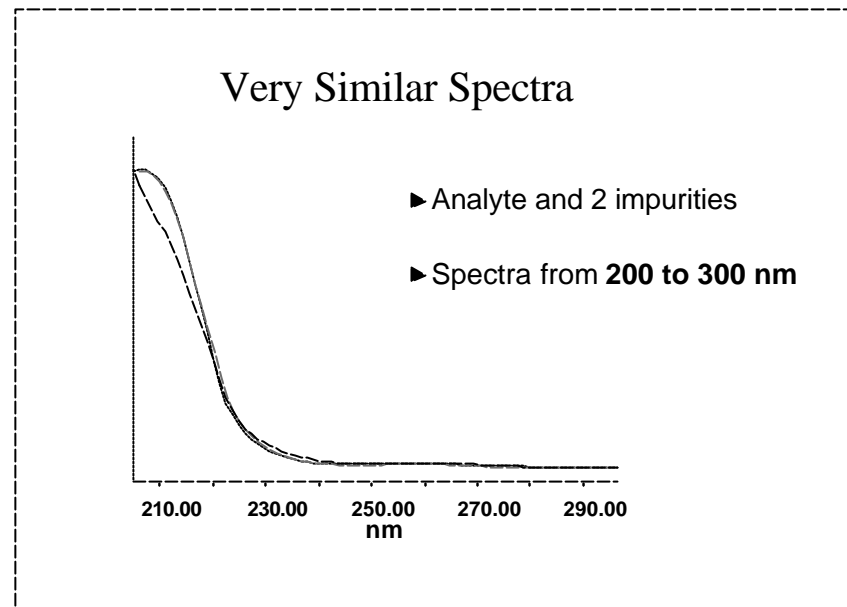
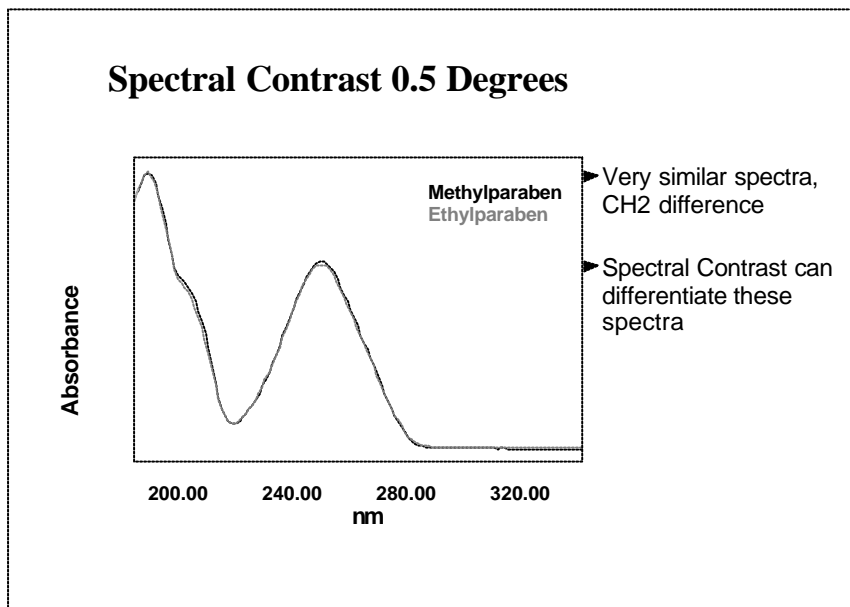
$$\text{Absorbance} = \text{Extinction Coefficient} \times \text{Pathlength} \times \text{Concentration}$$

Only for monochromatic light









- ### Detectors
- - UV/Vis
 - Fixed wavelength
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 - Evaporative light scattering

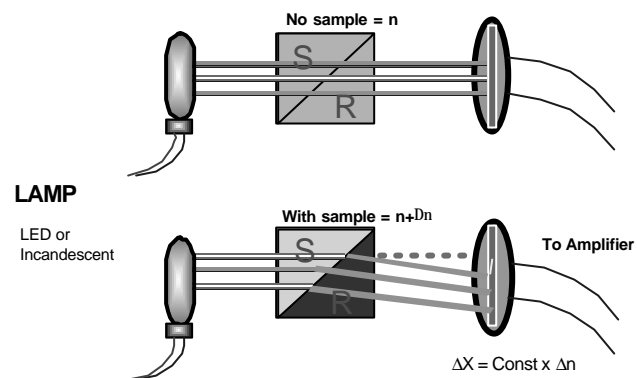
Light travels more slowly in matter, air at one atmosphere will cause light to slow down. The ratio of the speed of light in a vacuum to the speed of light in a material is the Refractive Index, n_{mat} :

$$n_{mat} = \text{Speed of Light In Vacuum} / \text{Speed of Light in Material}$$

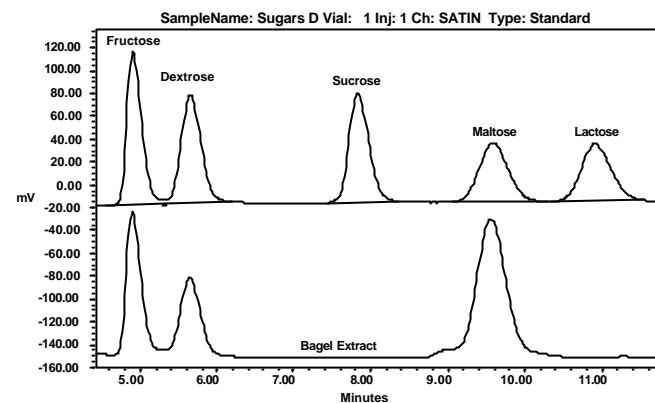
If light traveling in a medium with a refractive index of n_1 strikes the surface of a material with a refractive index of n_2 at an angle of Φ_1 to the normal the light direction in the second material is given by Φ_2 in the equation:

$$\text{Snell Low: } n_1 \sin \Phi_1 = n_2 \sin \Phi_2$$

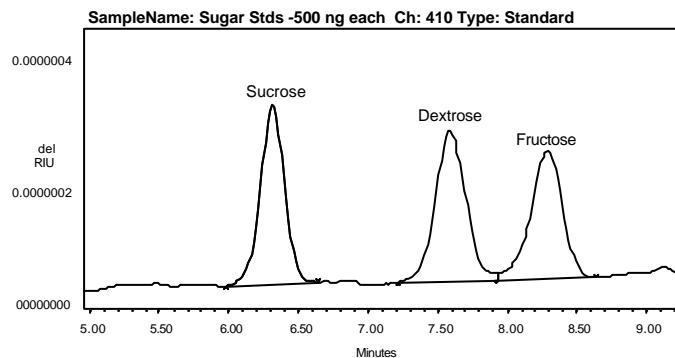
Differential Refractive Index Detector



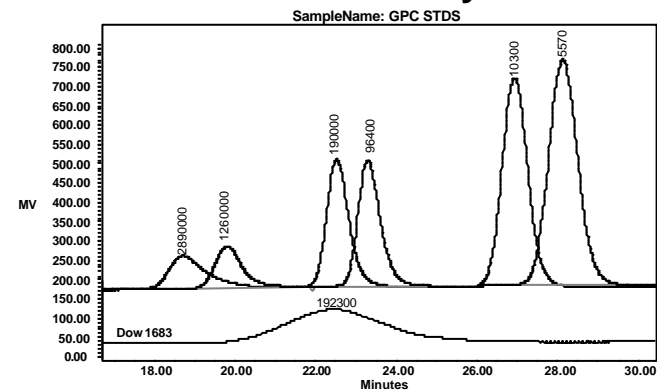
Refractive Index Detection with Differential RI - Sugars

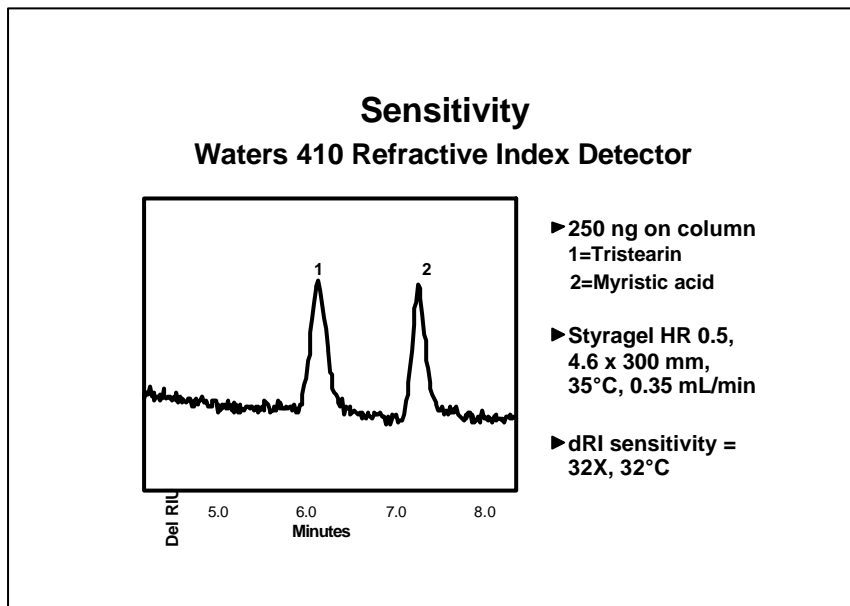


Refractive Index Detection with Differential RI - Sugars

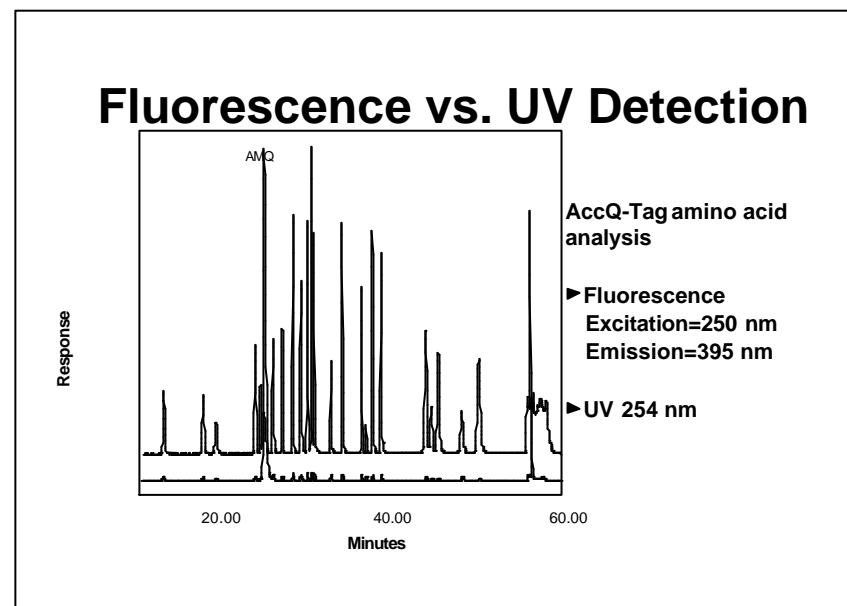
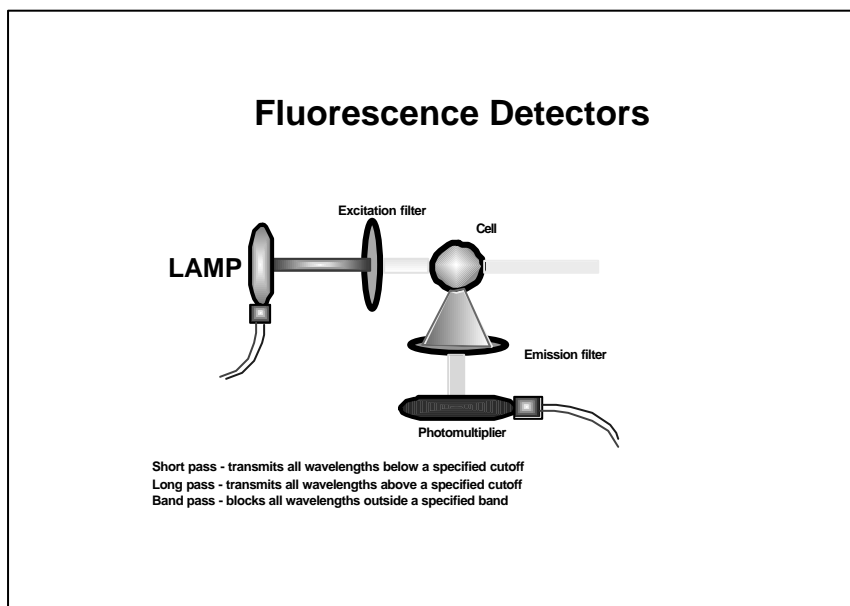


Refractive Index Detection with Differential RI - Polymers





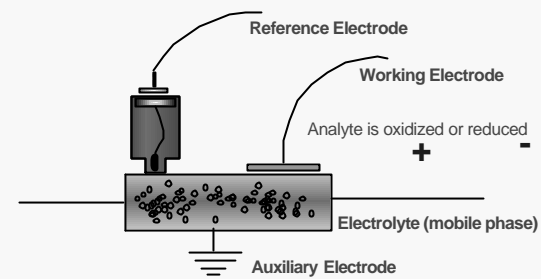
- ### Detectors
- - UV/Vis
 - Fixed wavelength
 - Variable wavelength
 - Diode array
 - Refractive index
 - Fluorescence
 - Electrochemical
 - - Conductivity
 - Mass-spectrometric (LC/MS)
 - Evaporative light scattering



Detectors

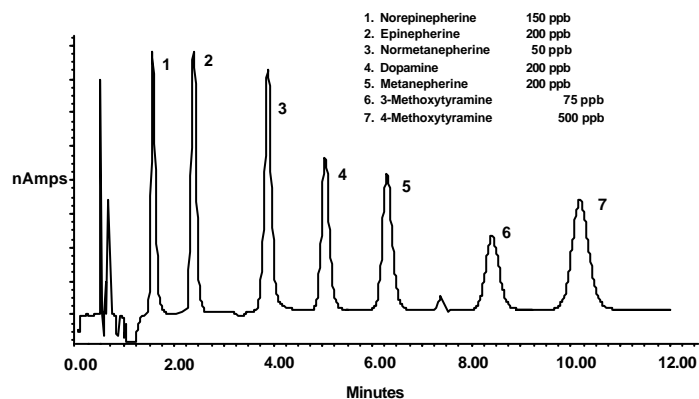
- - UV/vis
 - Fixed wavelength
 - Variable wavelength
 - Diode array
 - Refractive index
 - Fluorescence
 - **Electrochemical**
- - Conductivity
 - Mass-spectrometric (LC/MS)
 - Evaporative light scattering

Electrochemical Detector

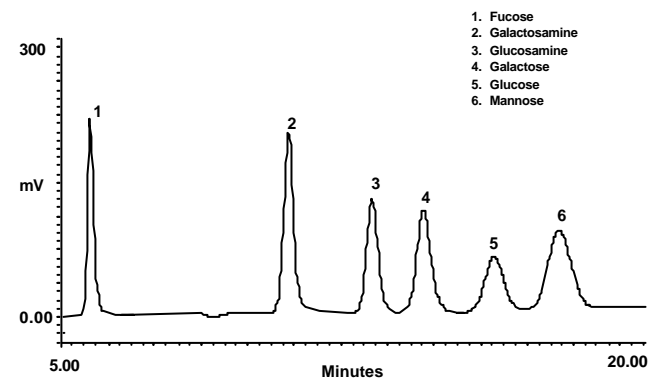


As compounds are oxidized or reduced, a current proportional to concentration is produced.

Electrochemical Detection of Catecholamines & Related Compounds



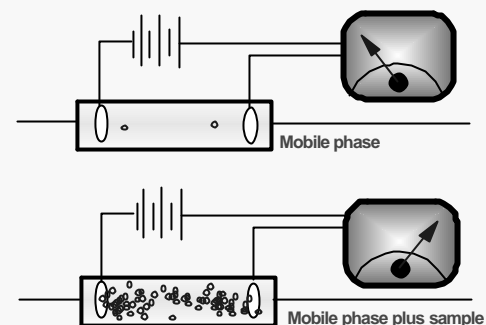
Pulsed Amperometric Detection of Monosaccharides



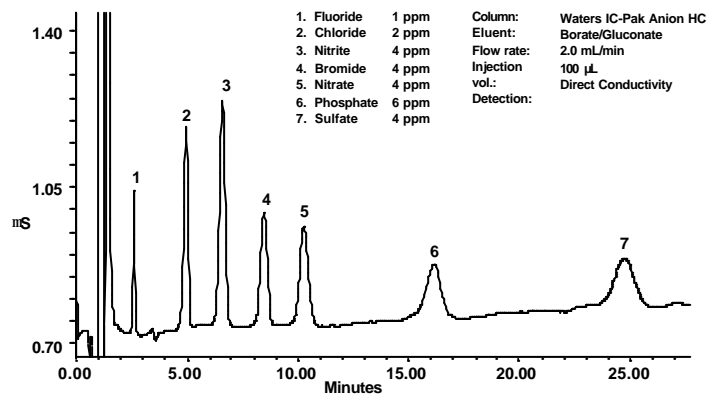
Detectors

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

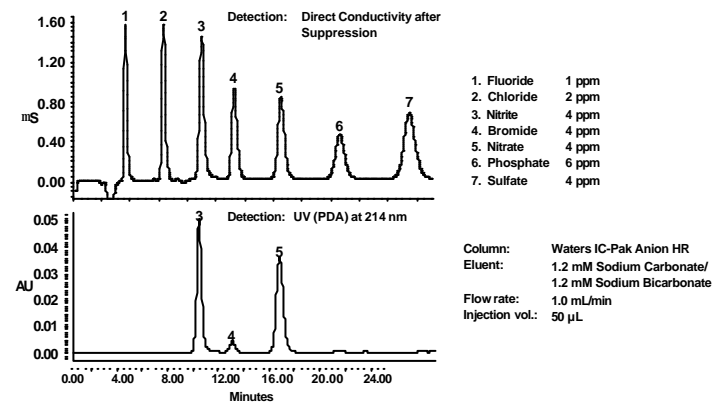
Conductivity Detector



Conductivity Detection of Seven Anion Standard



Conductivity and 996 PDA Detectors in Series



Applications

- Sensitivities for compounds such as phenol, catecholamines, nitrosamines, and organic acids are in the picomole (nanogram) range.

The mobile phase must be made electrically conductive, usually by the addition of a suitable salt:

Ion Exchange

Reversed Phase and Ion-Pair RP

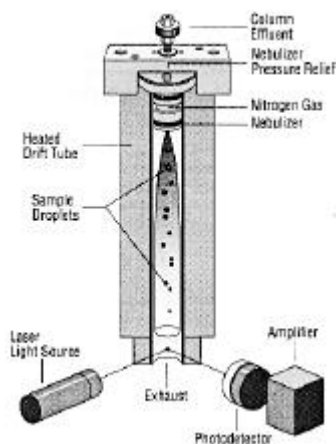
No normal phase separations

Detectors

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

EVAPORATIVE LIGHT SCATTERING

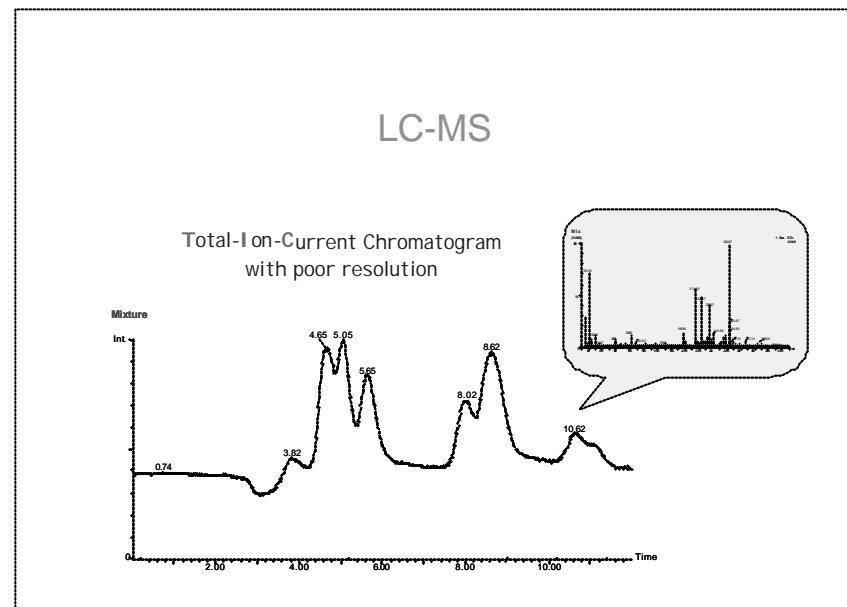
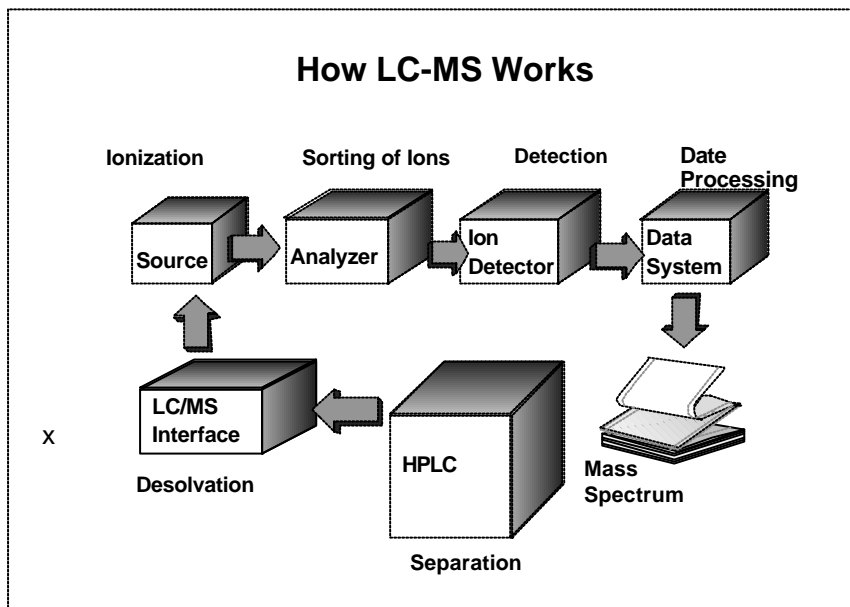
- The scattered light is detected by a silicone photodiode located at a 90° angle from the laser. The photodiode produces a signal which is sent to the analog outputs for collection. A light trap is located 180° from the laser to collect any light not scattered by particles in the aerosol stream.



Radioactive Detector

Primarily used for the measurement of ^3H , ^{14}C , and ^{32}P , beta-emitters and many soft gamma and positron emitters encountered in bio-medical research and pharmaceutical quality control.





Types of Mass Spectrometer's Analyzers

FT-ICR-Spectrometer

Time Of Flight Mass Analyzers

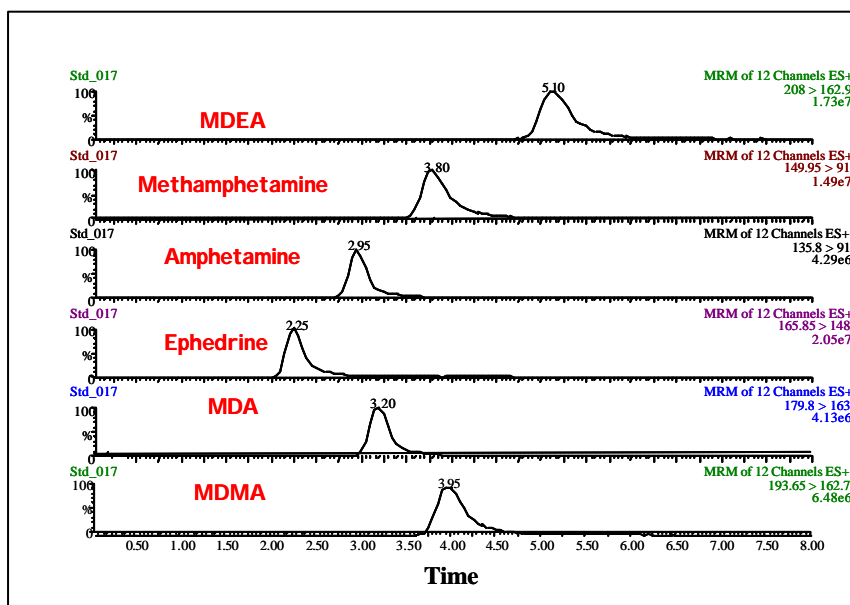
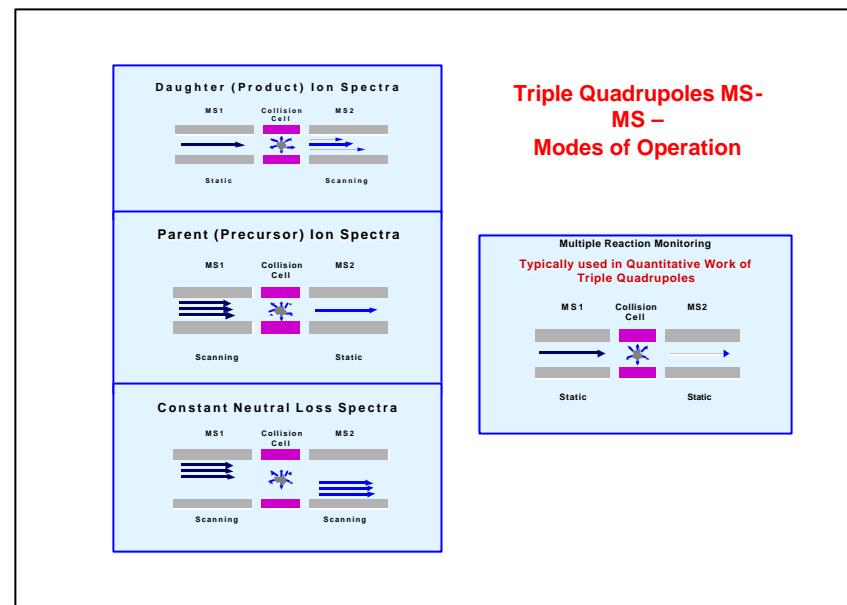
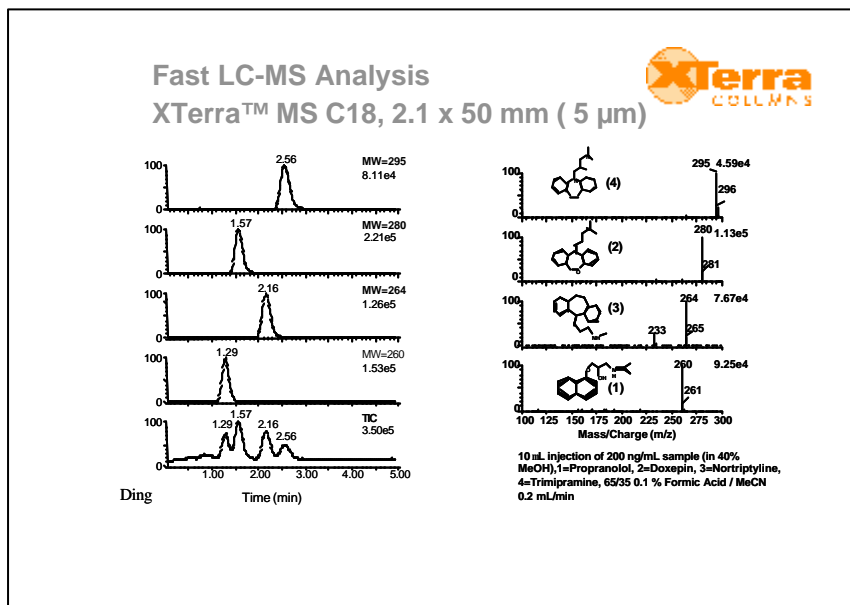
The Quadrupole Analyzator

Sector Mass Spectrometers

Ion Traps

HARDWARE - ES/APCI Ion Source

- ESI and APCI are easily interchangeable in seconds without venting the system
- ESI and APCI use a unique counter electrode to optimize sampling from the liquid spray and to aid sample desolvation
- Automatic Probe recognition



BASIC DETECTOR REQUIREMENTS

An ideal LC detector should have the following properties:

- Low drift and noise level (trace analysis).
- High sensitivity.
- Fast response for high performance systems.
- Wide linear dynamic range (quantitation).
- Low dead volume (minimal peak broadening & remixing of the separated bands).
- Insensitivity to changes in type of solvent, flow rate, and temperature.
- Operational simplicity and reliability.
- Tuneable, so that detection can be optimized for different compounds.
- Preferably non-destructive.

Detector Criteria

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

PROPERTIES OF DETECTORS

SELECTIVITY



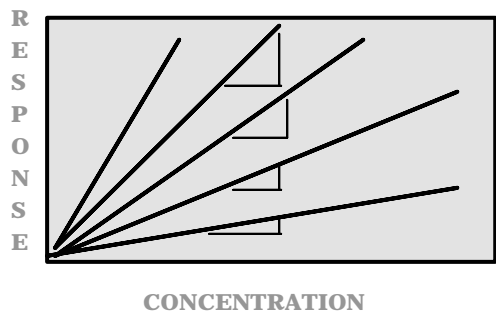
SPECIFIC

UNIVERSAL

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

PROPERTIES OF DETECTORS

SENSITIVITY

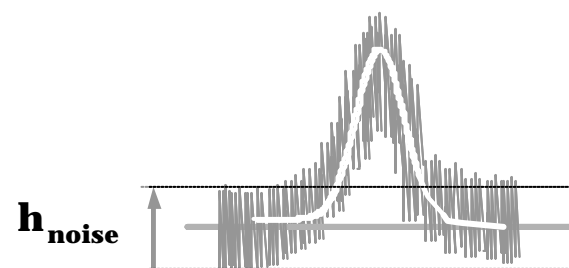


Sensitivity of a detector is the minimum amount that can be detected.

PROPERTIES OF DETECTORS

DETECTION LIMIT

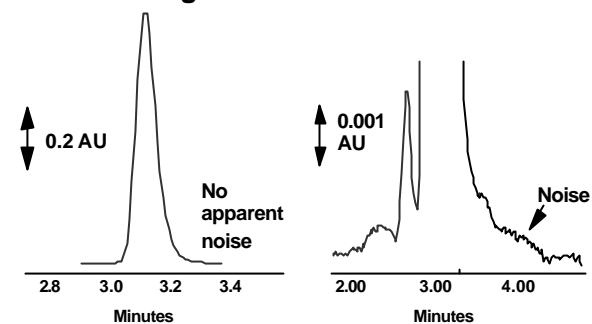
$$h_{\text{signal}} = 2 \times h_{\text{noise}}$$



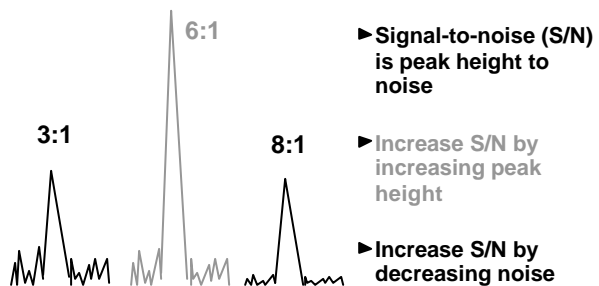
Detector Sensitivity

- **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

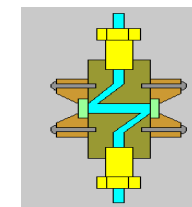


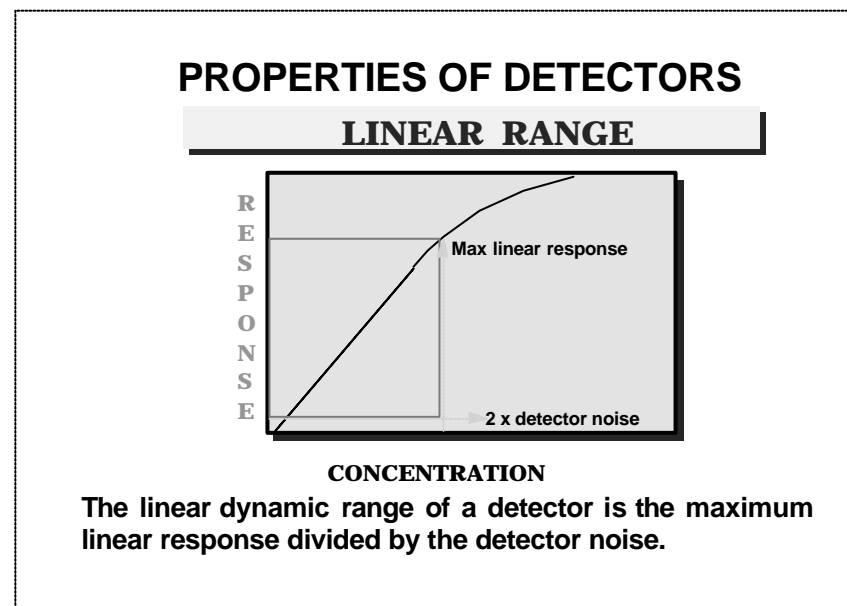
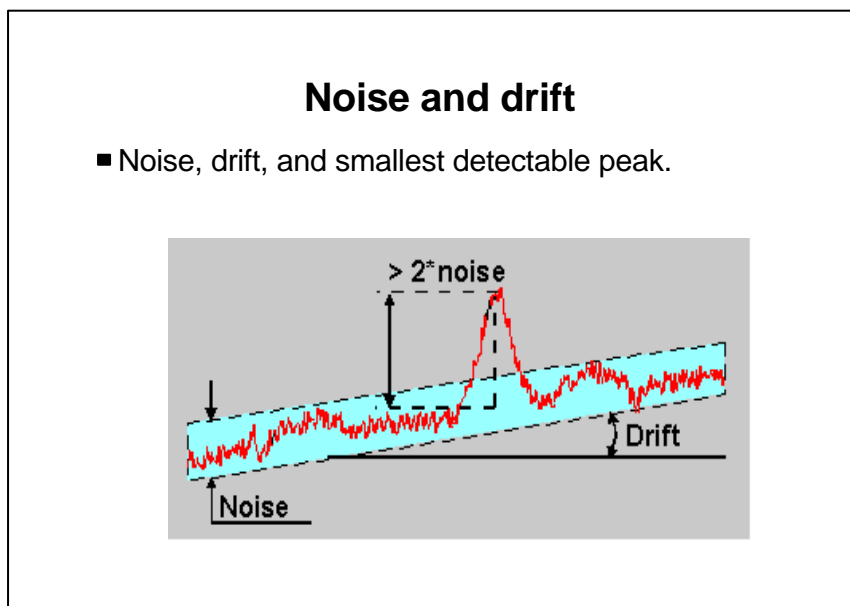
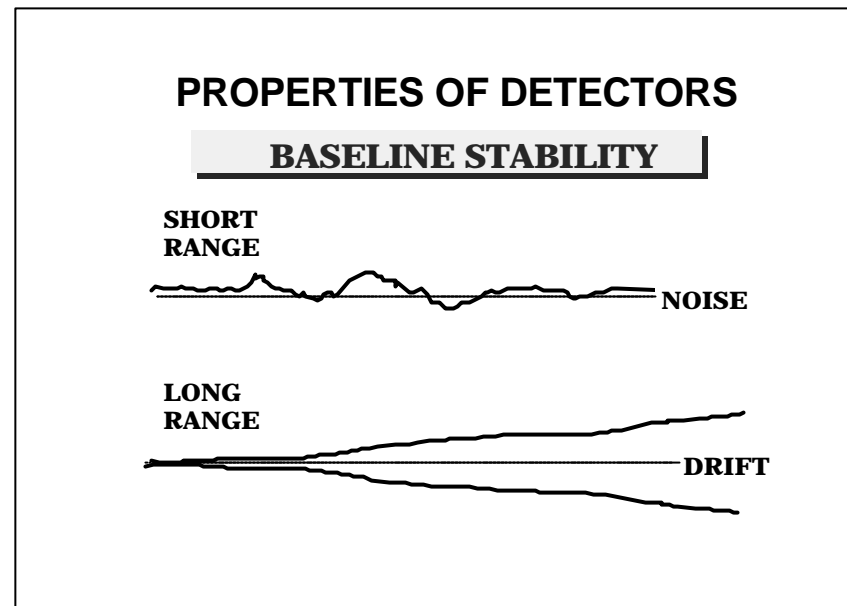
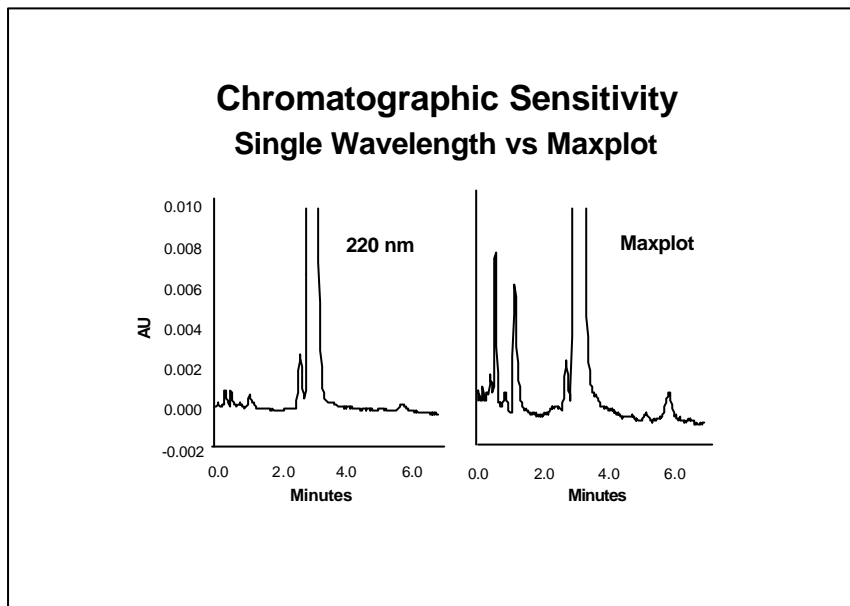
Factors Increasing UV Signal

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

Factors Affecting Noise in UV Detectors

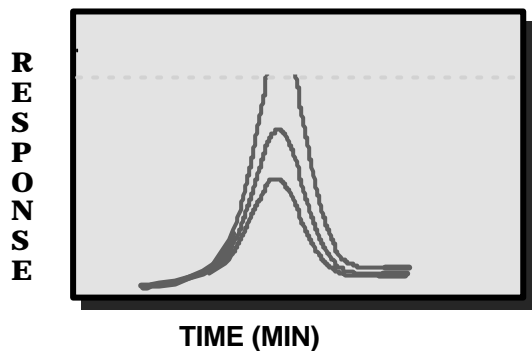
- ▶ Optics bench design
- ▶ Lamp energy
- ▶ Wavelengths
- ▶ Mobile phase composition
- ▶ Pump pulsation
- ▶ Electronics





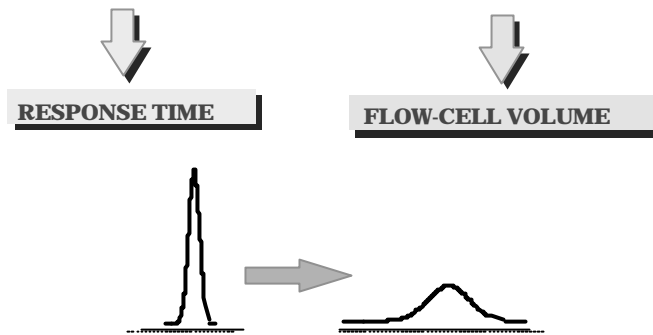
PROPERTIES OF DETECTORS

DYNAMIC RANGE



PROPERTIES OF DETECTORS

CONTRIBUTION TO BAND BROADENING



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