

Detection Qualification and Types of Detectors in HPLC

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Medtechnica

<http://www.forumsci.co.il/HPLC>

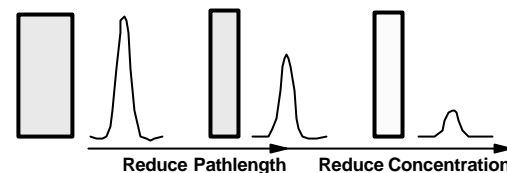
Detectors

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

Beer's Law

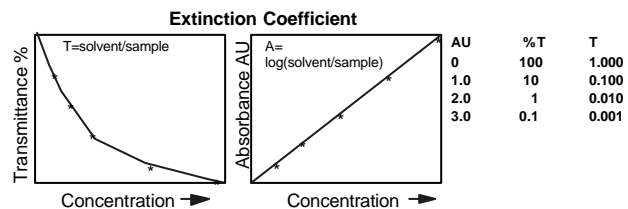
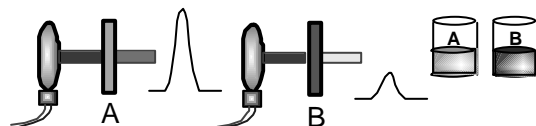
$$\text{Absorbance} = \text{Extinction Coefficient} \times \text{Pathlength} \times \text{Concentration}$$

Only for monochromatic light



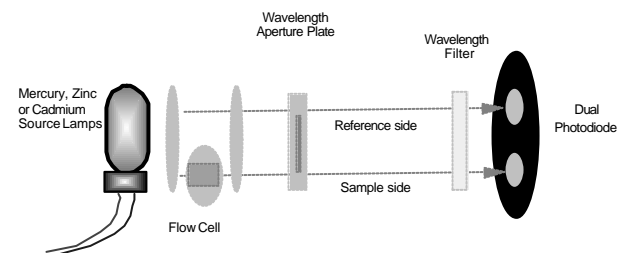
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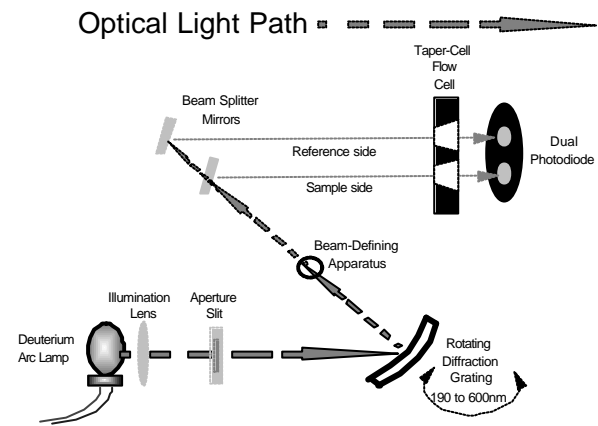


Single Wavelength UV Detector

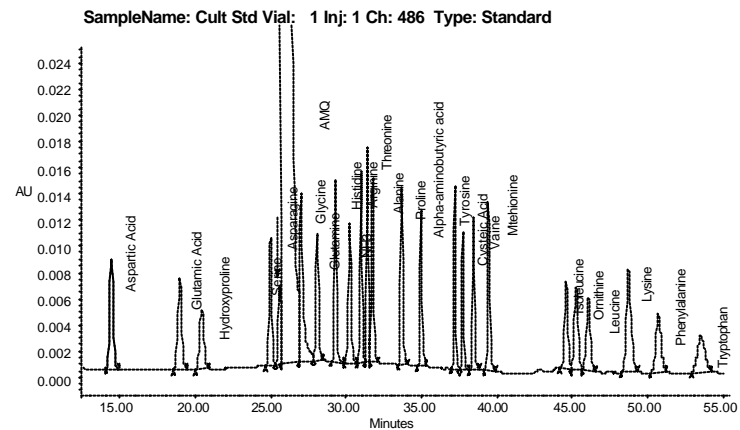
Optical Light Path →



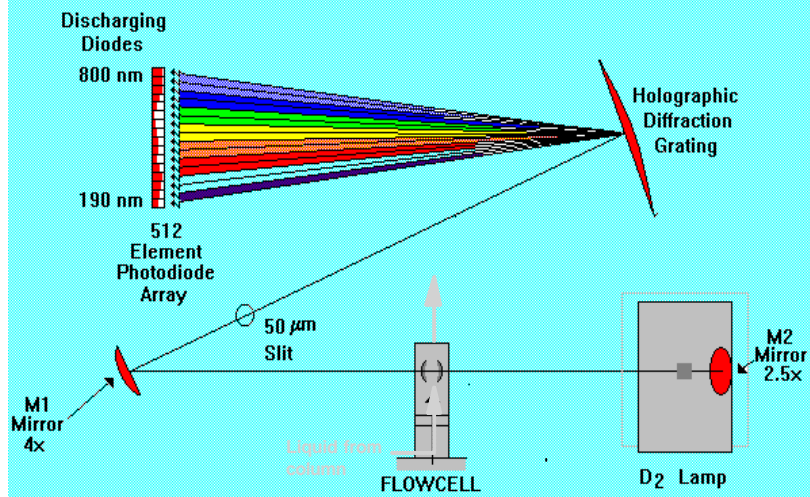
UV-VIS Detector Optical Bench



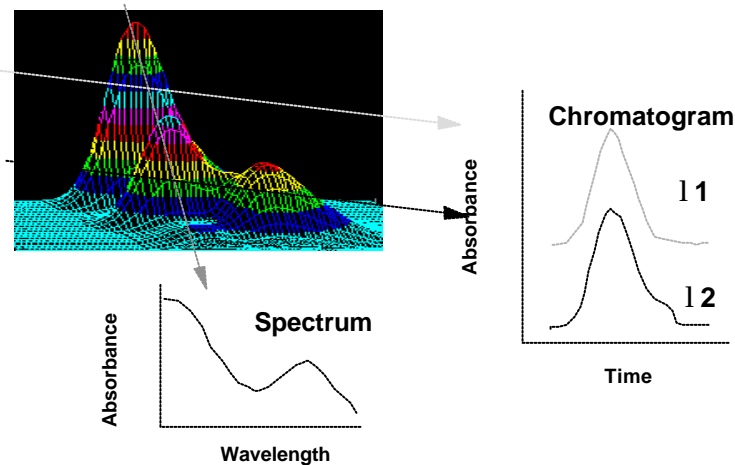
UV Detection of AccQ-Tag Amino Acid Derivatives



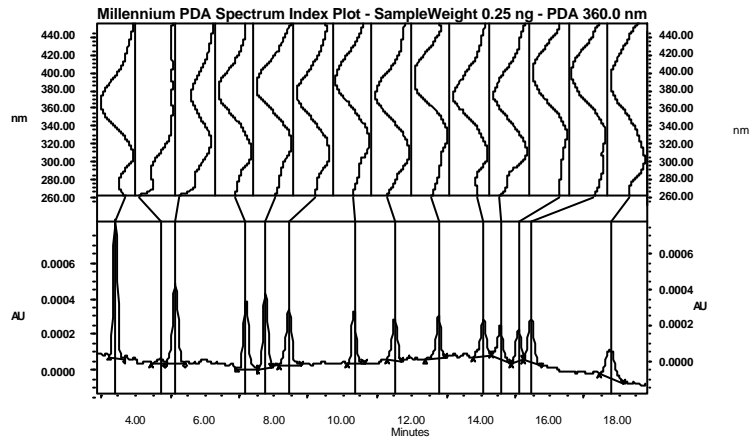
Principle of Measurement



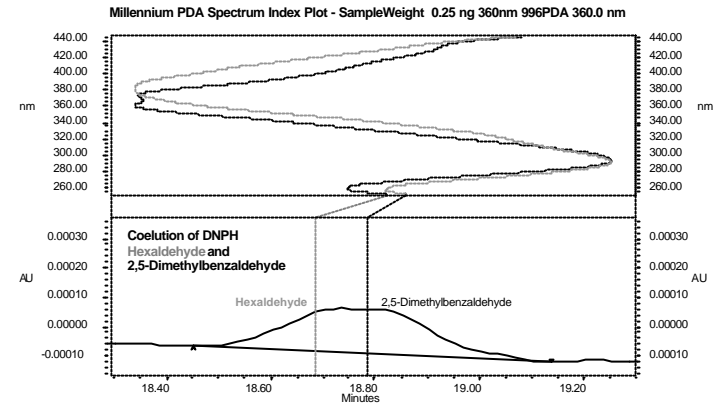
Extraction of 3D Data



PDA Spectrum Index Plot DNPH Derivatives 0.25 ng Each Peak



Maximum Impurity Detection



PDA and fluorescent Detector Comparisons for Aflatoxin Analysis

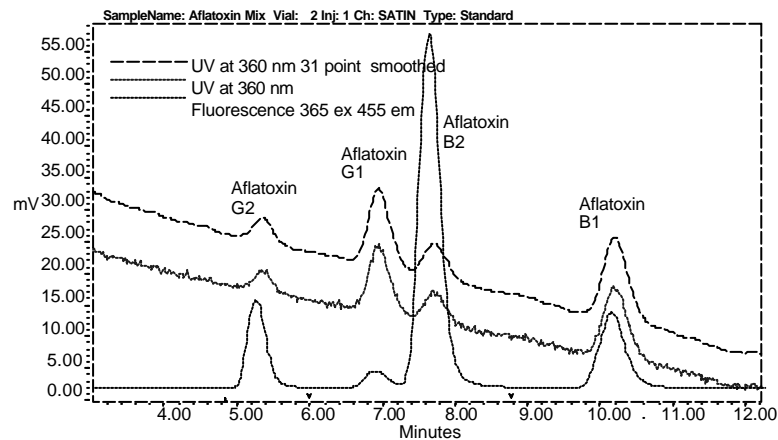
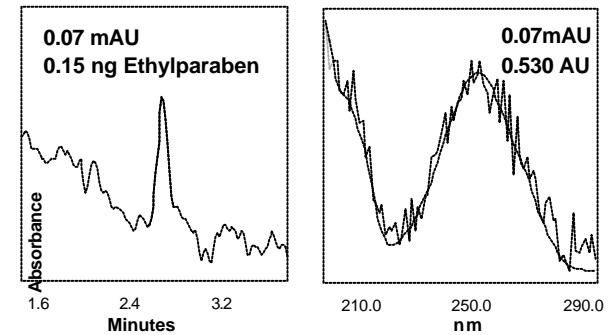


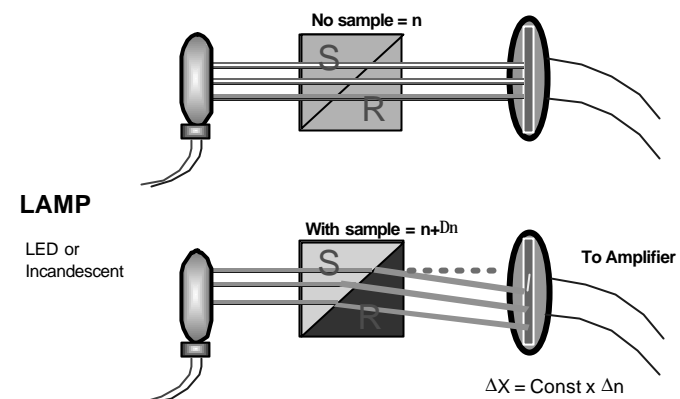
Photo-diode array Chromatographic and Spectral Sensitivity



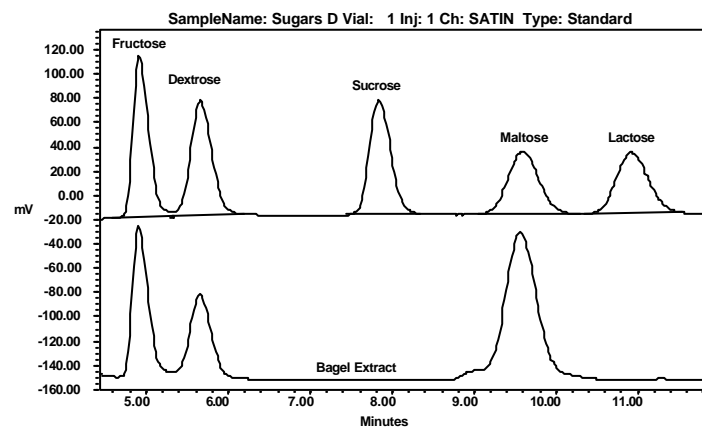
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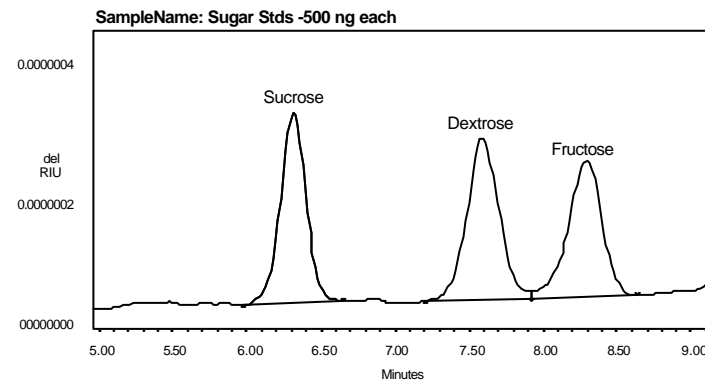
Differential Refractive Index Detector



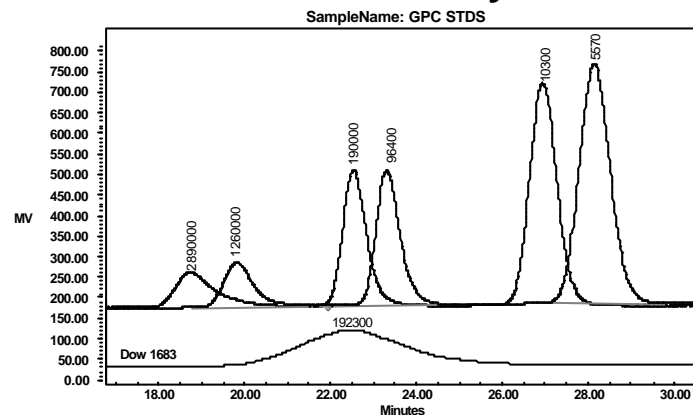
Refractive Index Detection with Differential RI - Sugars



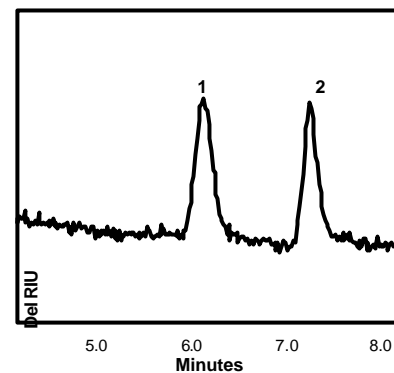
Refractive Index Detection with Differential RI - Sugars



Refractive Index Detection with Differential RI - Polymers



Sensitivity Refractive Index Detector

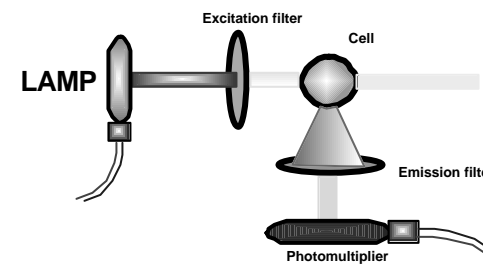


- ▶ 250 ng on column
- 1=Tristearin
- 2=Myristic acid
- ▶ Styragel HR 0.5,
4.6 x 300 mm,
35°C, 0.35 mL/min
- ▶ dRI sensitivity =
32X, 32°C

Detectors

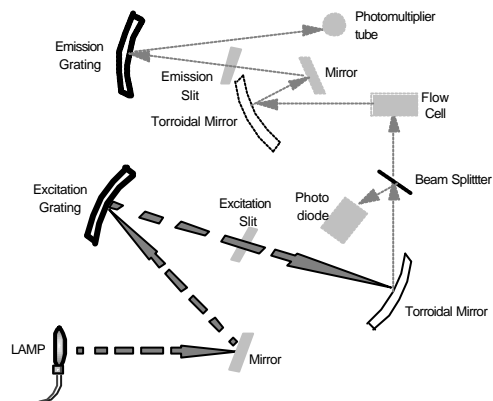
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Fluorescence Detectors

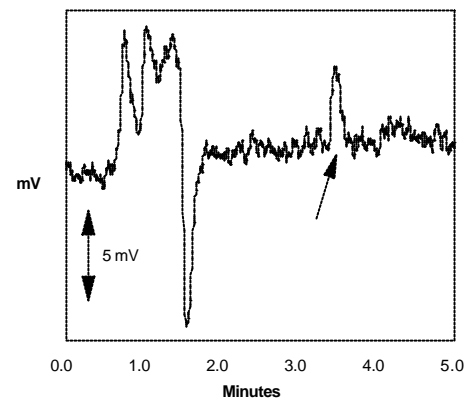


Short pass - transmits all wavelengths below a specified cutoff
 Long pass - transmits all wavelengths above a specified cutoff
 Band pass - blocks all wavelengths outside a specified band

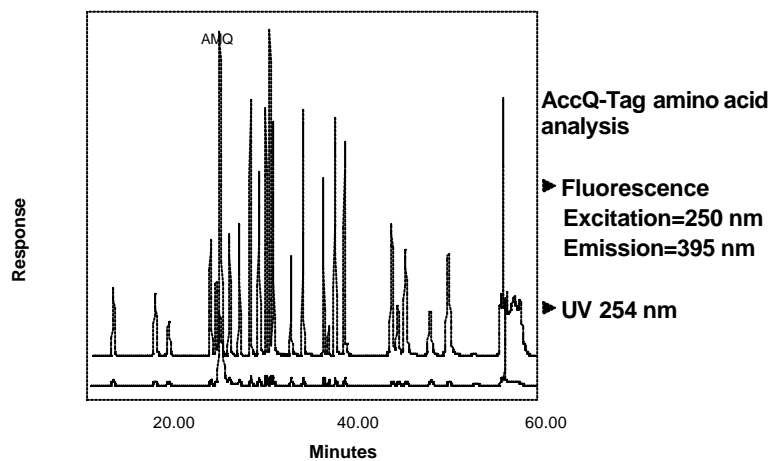
Fluorescence Detector Optical Bench



Sensitivity Fluorescence Detector



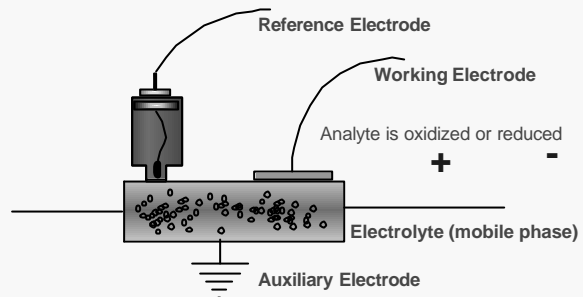
Fluorescence vs. UV Detection



Detectors

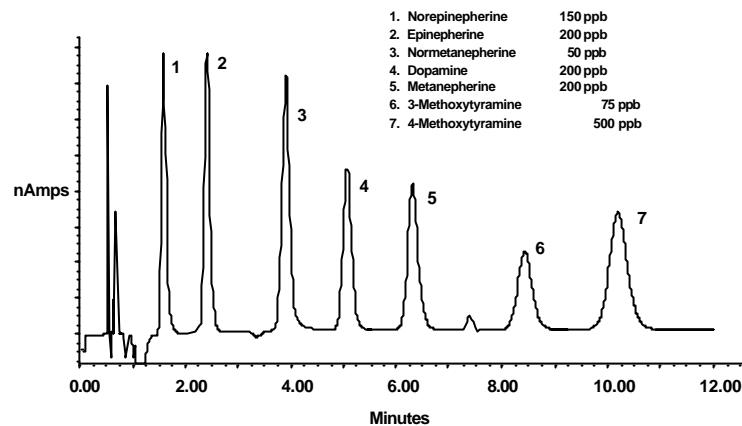
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Electrochemical Detector

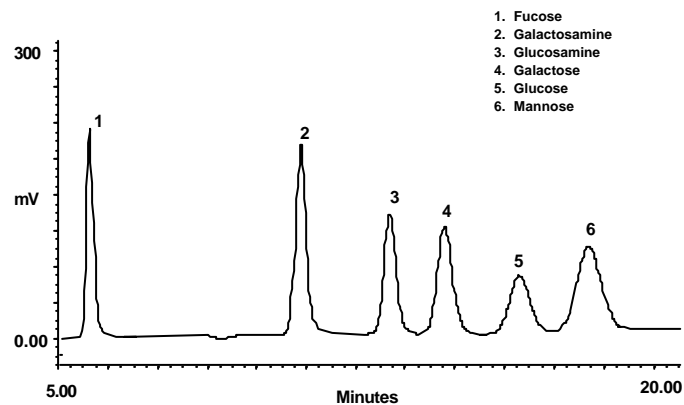


As compounds are oxidized or reduced, a current proportional to concentration is produced.

Electrochemical Detection of Catecholamines & Related Compounds



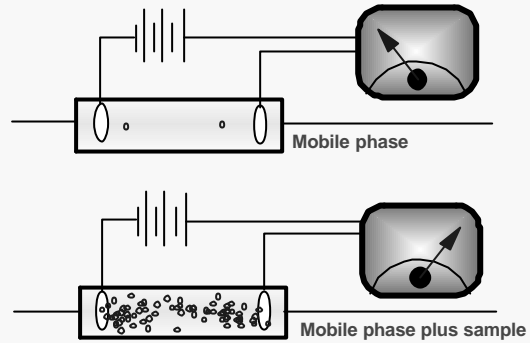
Pulsed Amperometric Detection of Monosaccharides



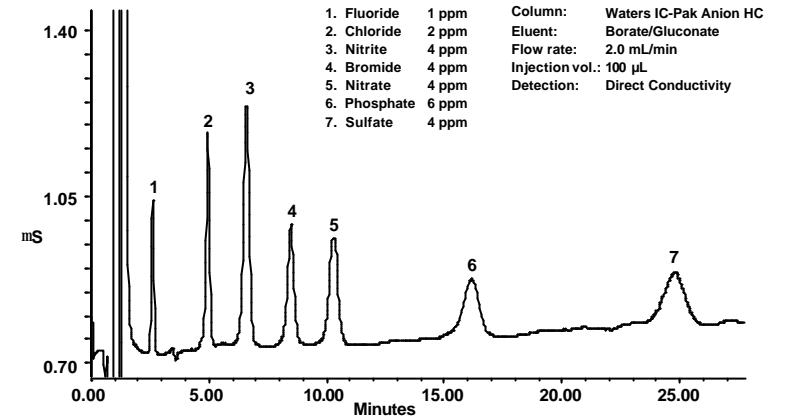
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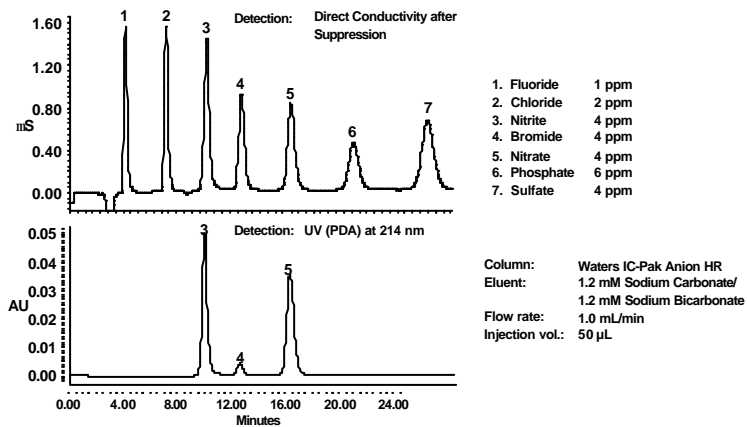
Conductivity Detector



Conductivity Detection of Seven Anion Standard



Conductivity and UV 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

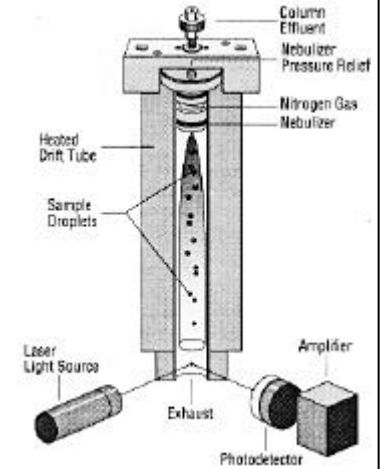
No normal phase separations

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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.

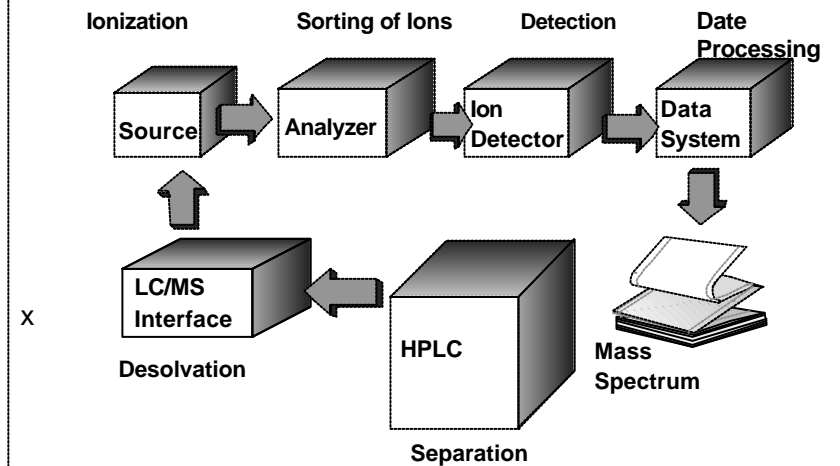


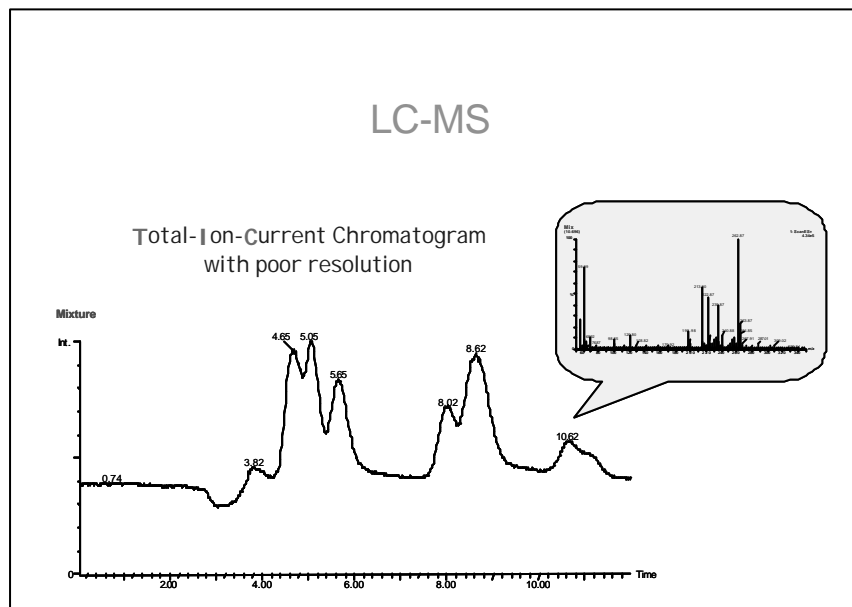
Radioactive Detector

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.



How LC-MS Works





Types of Mass Spectrometer's Analyzers

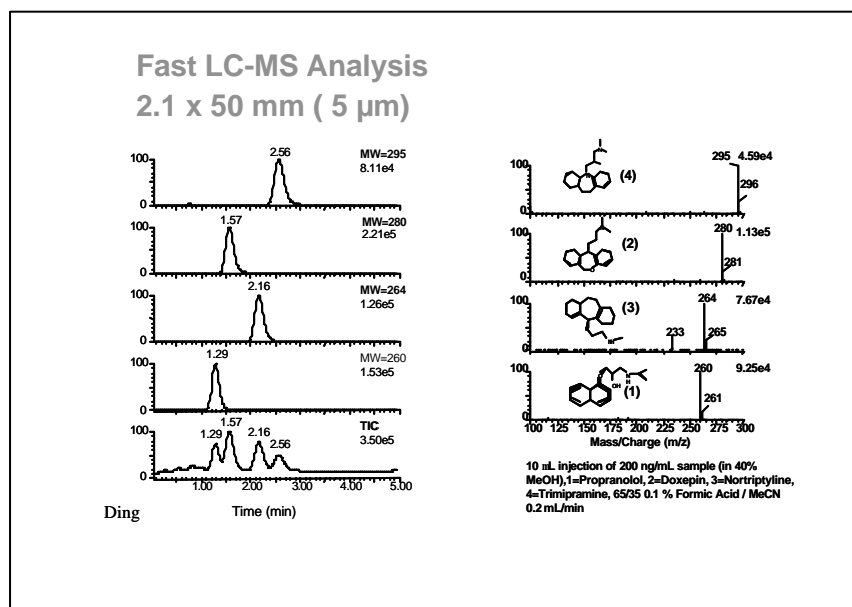
FT-ICR-Spectrometer

Time Of Flight Mass Analyzers

Ion Traps

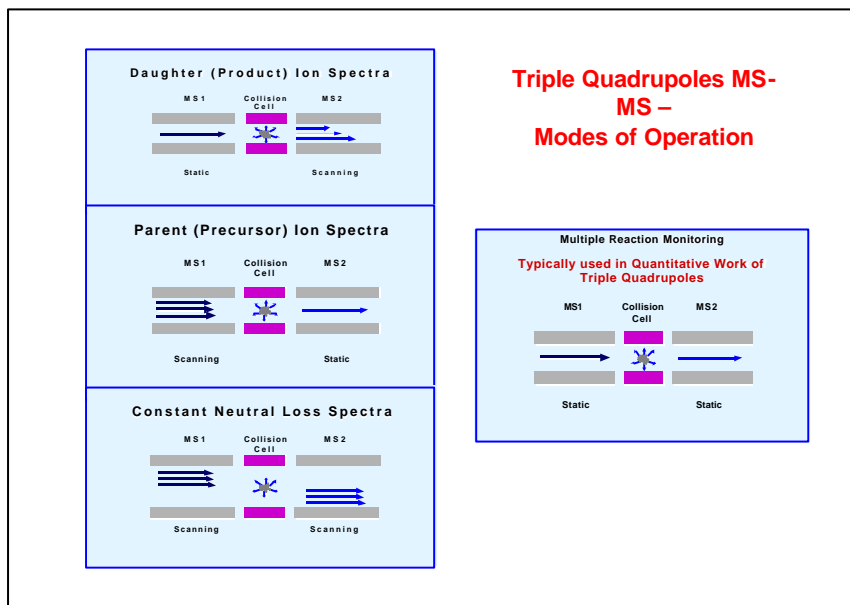
The Quadrupole Analyzator

Sector Mass Spectrometers:



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

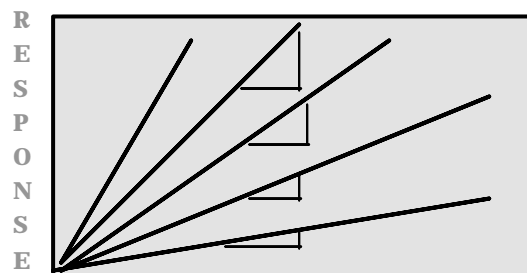


UNIVERSAL

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

PROPERTIES OF DETECTORS

SENSITIVITY



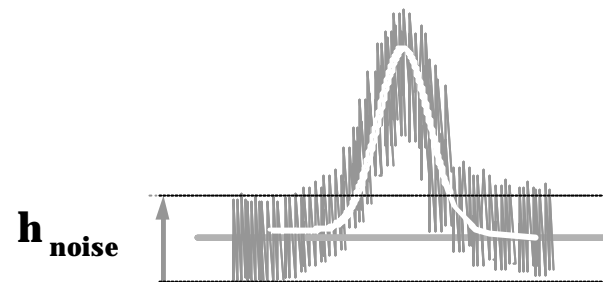
CONCENTRATION

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

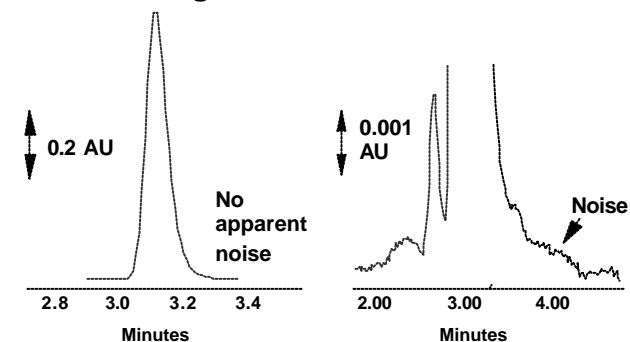
- Lowest concentration that can be detected
- Signal-to-noise ratio of 2:1 or 3:1

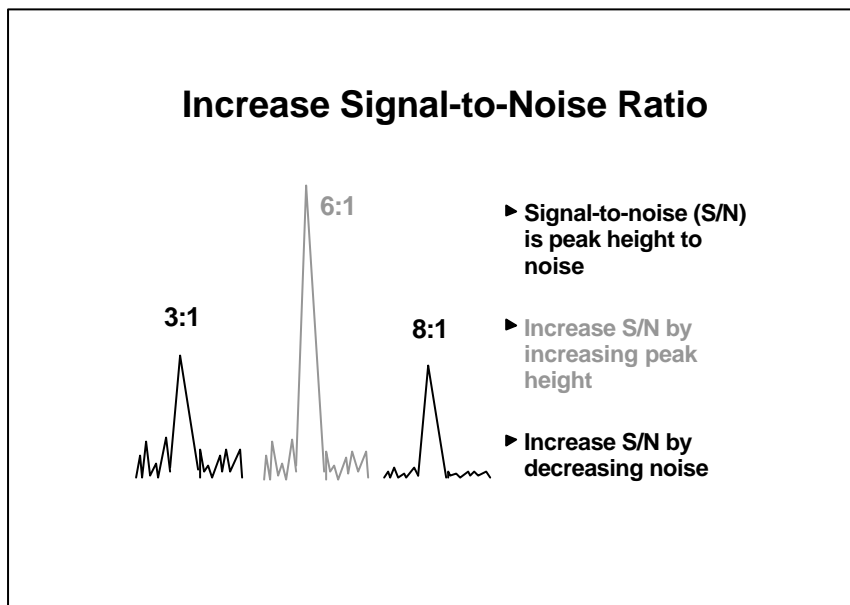
■ Limit of quantitation

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

Chromatographic Sensitivity

Signal-to-Noise Ratio



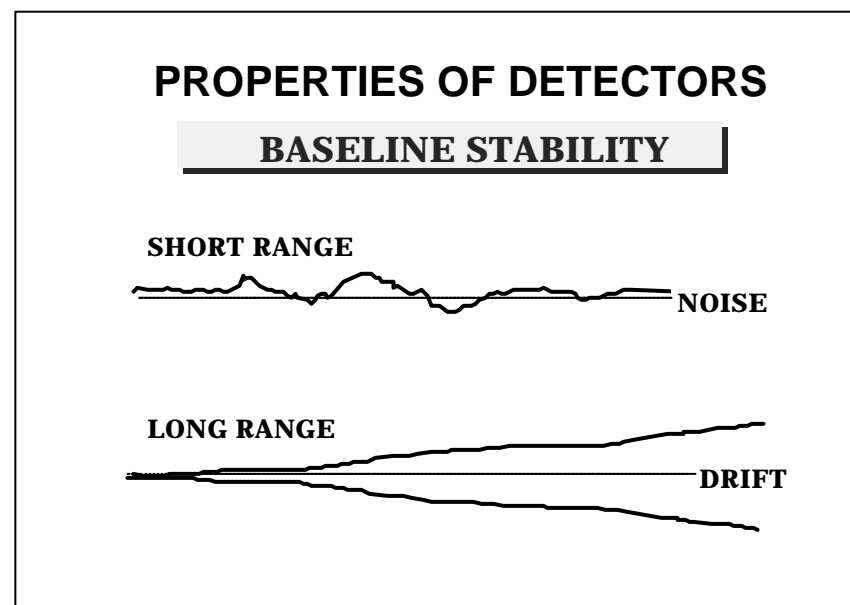
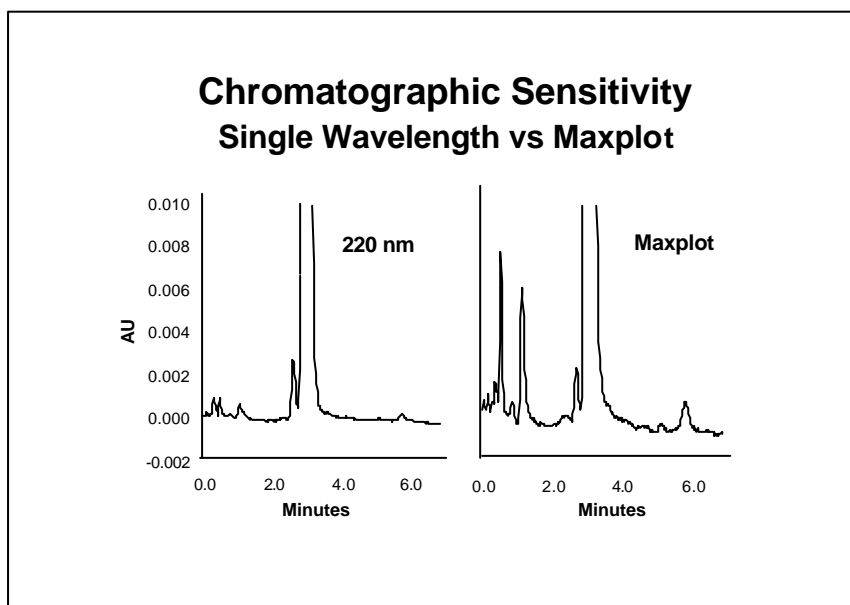


Factors Increasing UV Signal

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

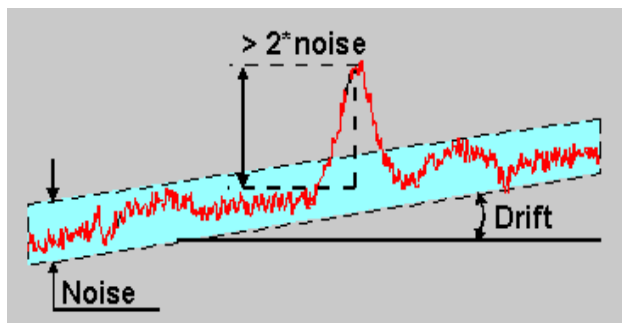
Factors Affecting Noise in UV Detectors

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



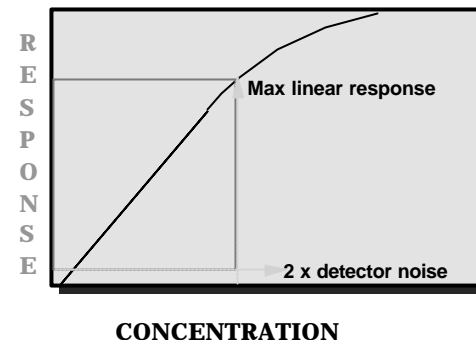
Noise and drift

- Noise, drift, and smallest detectable peak.



PROPERTIES OF DETECTORS

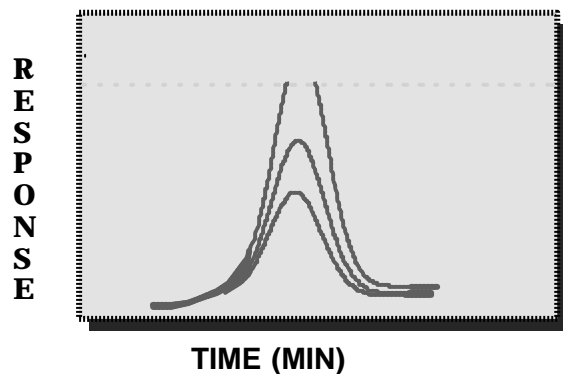
LINEAR RANGE



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

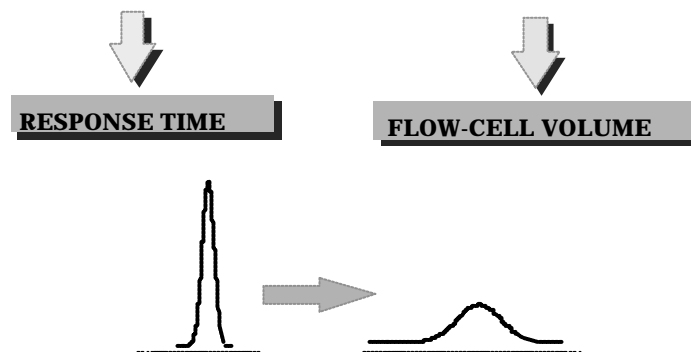
PROPERTIES OF DETECTORS

DYNAMIC RANGE



PROPERTIES OF DETECTORS

CONTRIBUTION TO BAND BROADENING



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