

# Detection Qualification and Types of Detectors in HPLC

Dr. Shulamit Levin

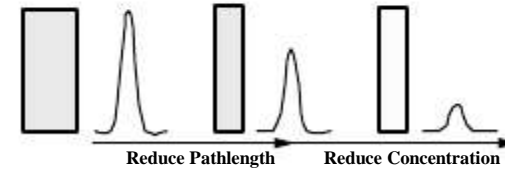
## Detectors

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

## Beer's Law

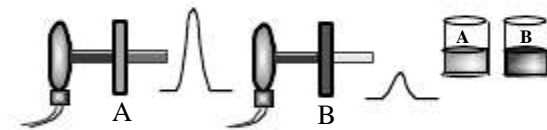
$$\text{Absorbance} = \text{Extinction Coefficient} \times \text{Pathlength} \times \text{Concentration}$$

Only for monochromatic light

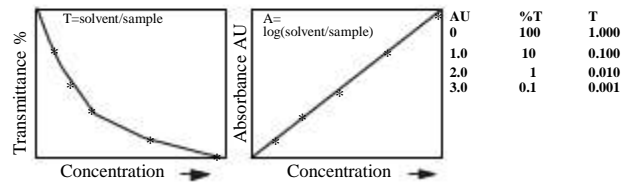


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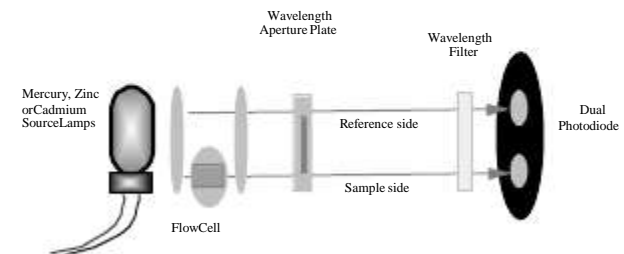


Extinction Coefficient

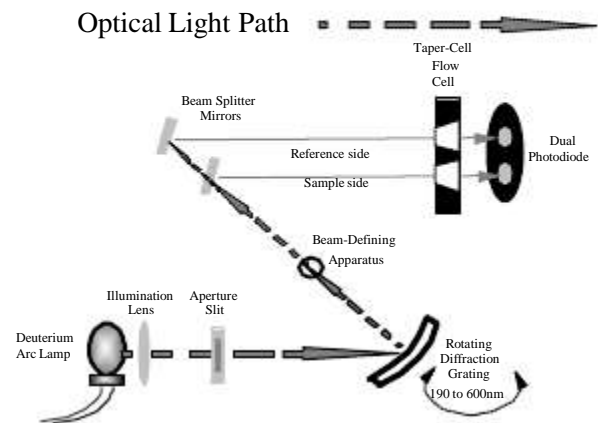


## Single Wavelength UV Detector

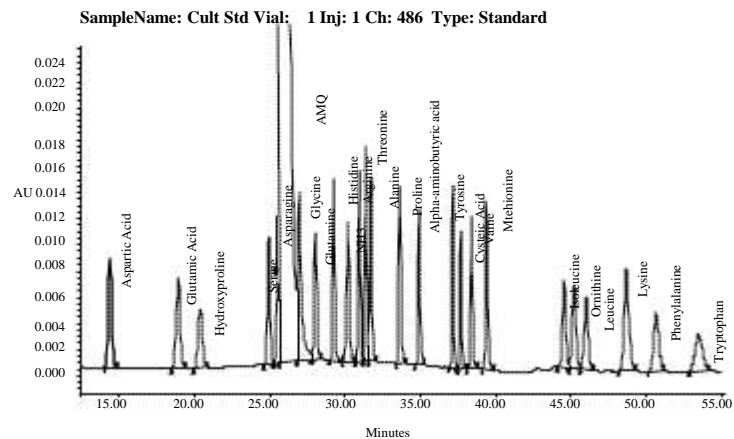
Optical Light Path



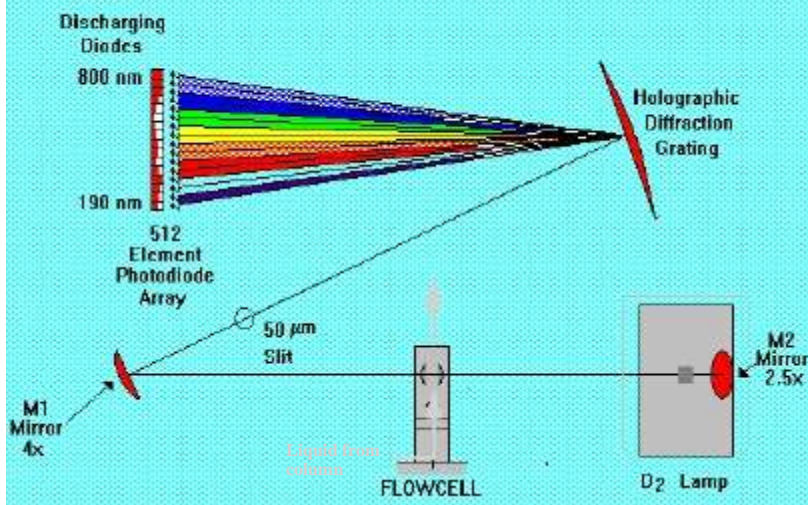
### UV-VIS Detector Optical Bench



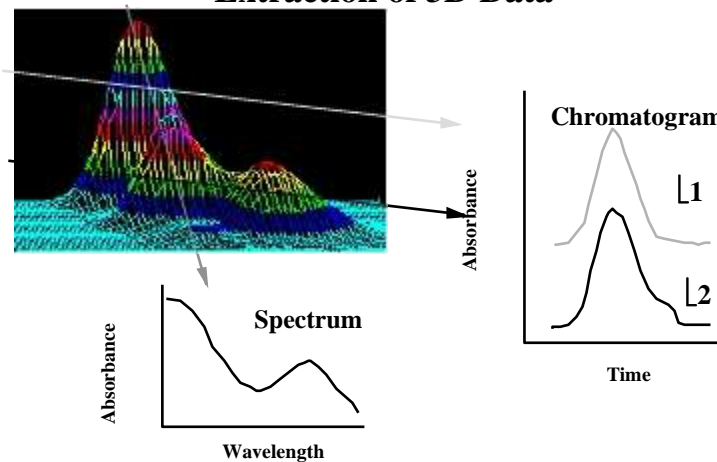
### UV Detection of AccQ-Tag Amino Acid Derivatives



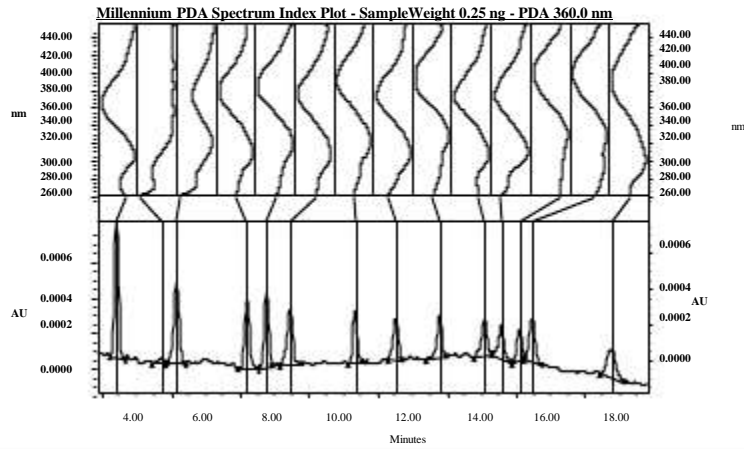
### Principle of Measurement



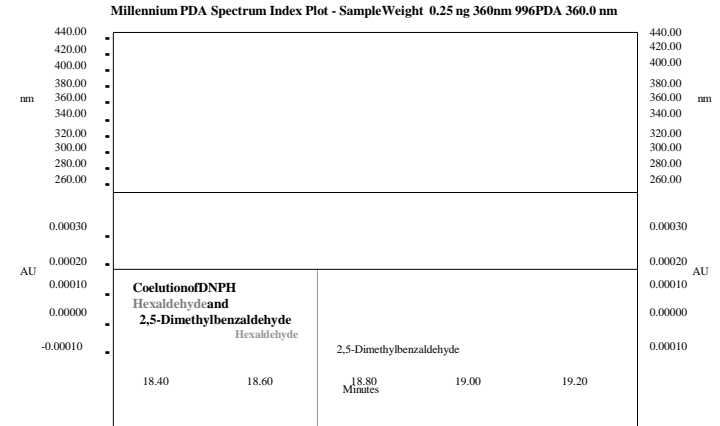
### Extraction of 3D Data



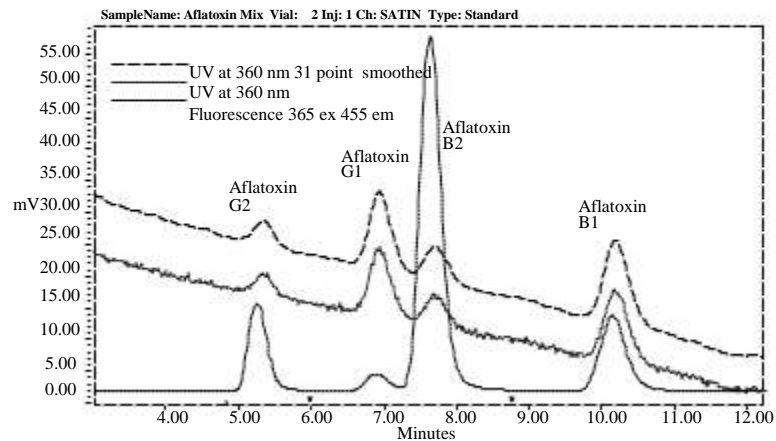
### PDA Spectrum Index Plot DNPH Derivatives 0.25 ng Each Peak



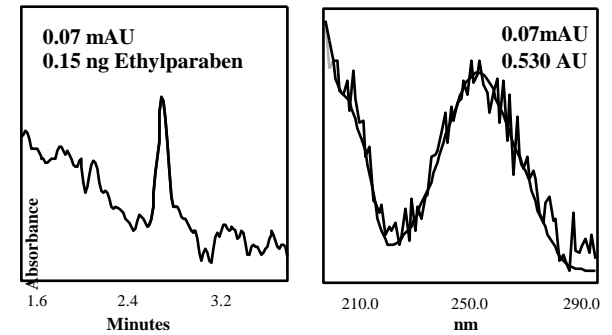
### Maximum Impurity Detection



### PDA and fluorescent Detector Comparisons for Aflatoxin Analysis



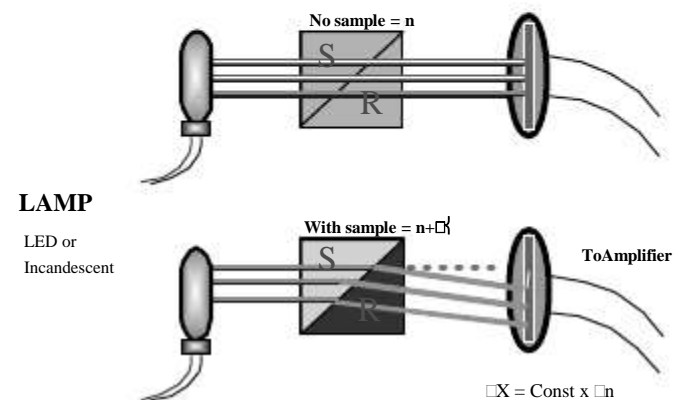
### Photo-diode array Chromatographic and Spectral Sensitivity



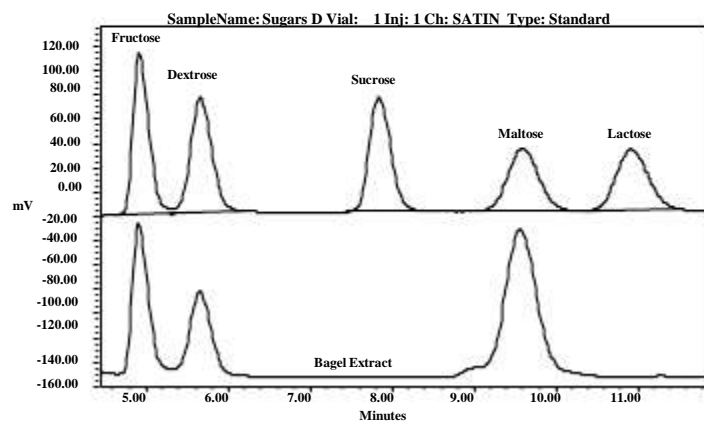
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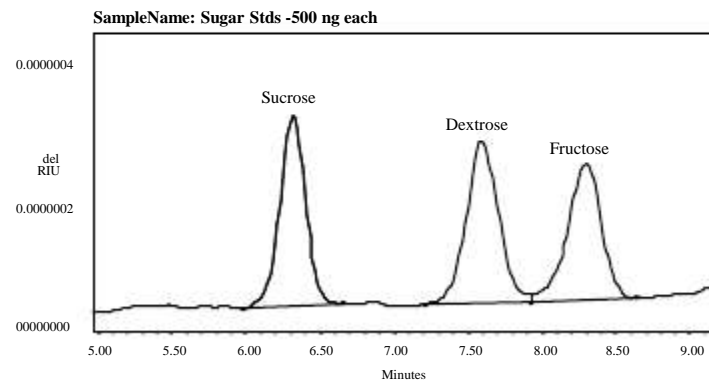
## Differential Refractive Index Detector



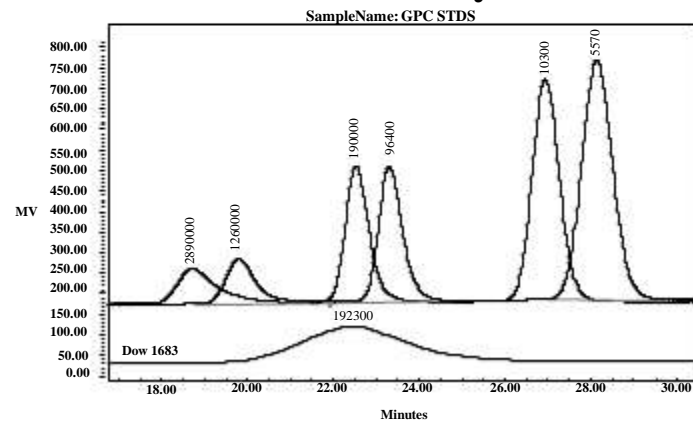
## Refractive Index Detection with Differential RI - Sugars



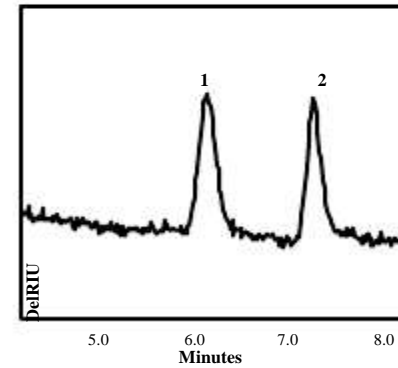
## Refractive Index Detection with Differential RI - Sugars



## Refractive Index Detection with Differential RI - Polymers



## Sensitivity Refractive Index Detector



- ▶ 250 ng on column
- 1=Tristearin
- 2=Myristic acid

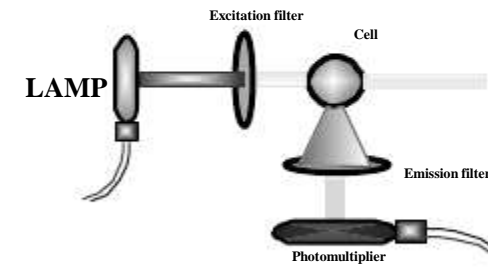
- ▶ Styragel HR 0.5,  
4.6 x 300 mm,  
35° C, 0.35 mL/min

- ▶ dRI sensitivity =  
32X, 32° C

## Detectors

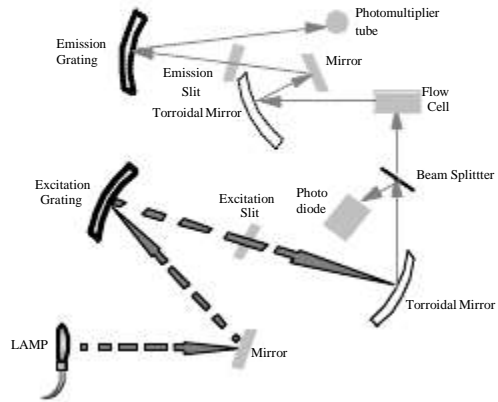
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## Fluorescence Detectors

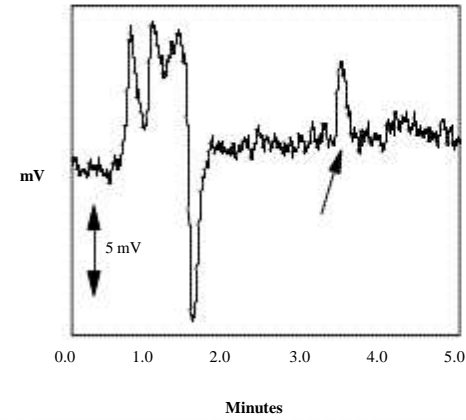


- Short pass - transmits all wavelengths below a specified cutoff
- Long pass - transmits all wavelengths above a specified cutoff
- Band pass - blocks all wavelengths outside a specified band

### Fluorescence Detector Optical Bench



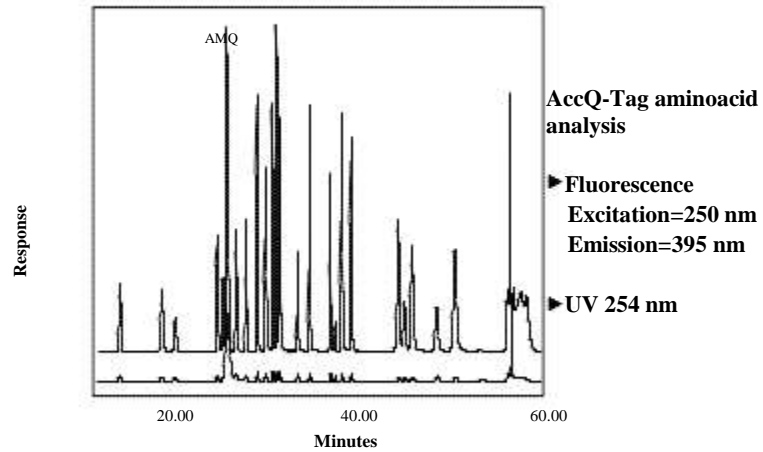
### Sensitivity Fluorescence Detector



0.1 pg Anthracene

Excitation = 251 nm  
Emission = 406 nm

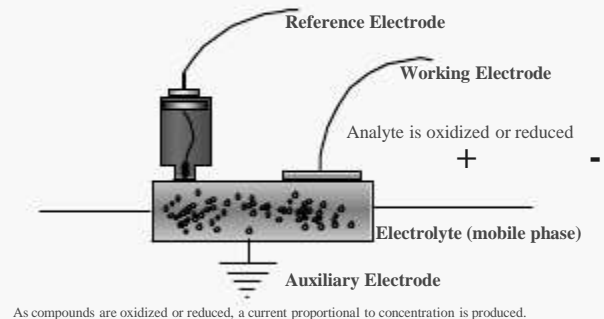
### Fluorescence vs. UV Detection



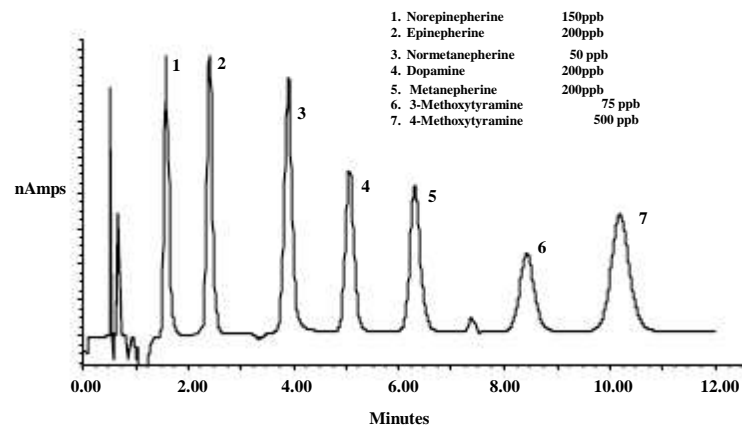
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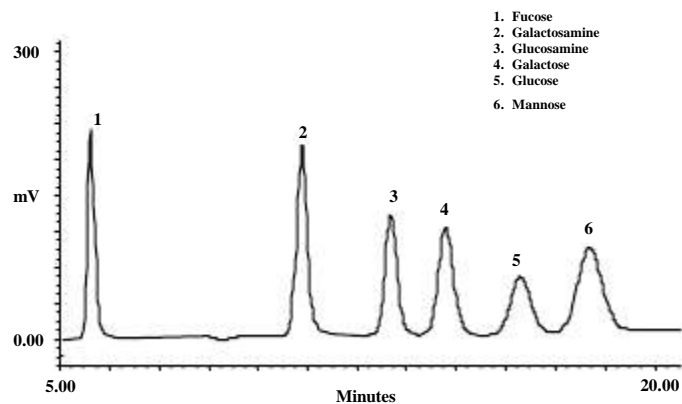
### Electrochemical Detector



### Electrochemical Detection of Catecholamines & Related Compounds



### Pulsed Amperometric Detection of Monosaccharides

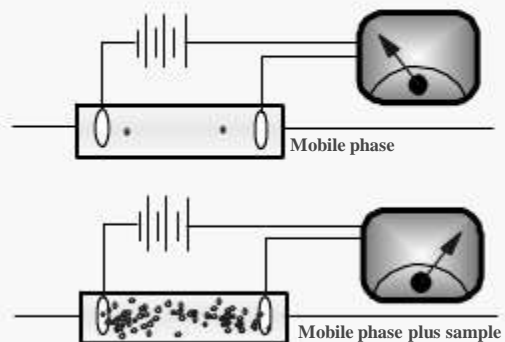


### Detectors

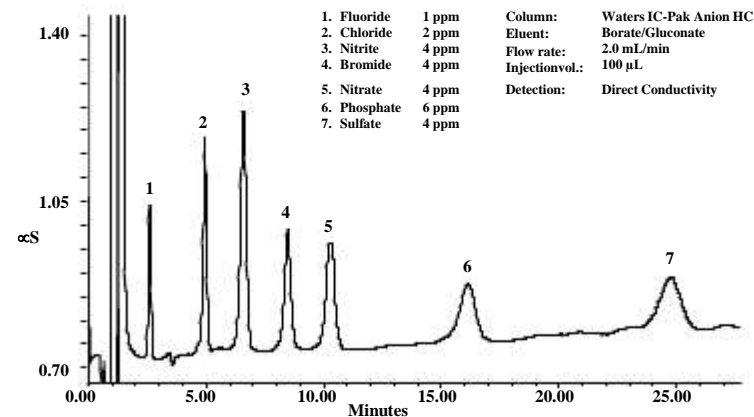
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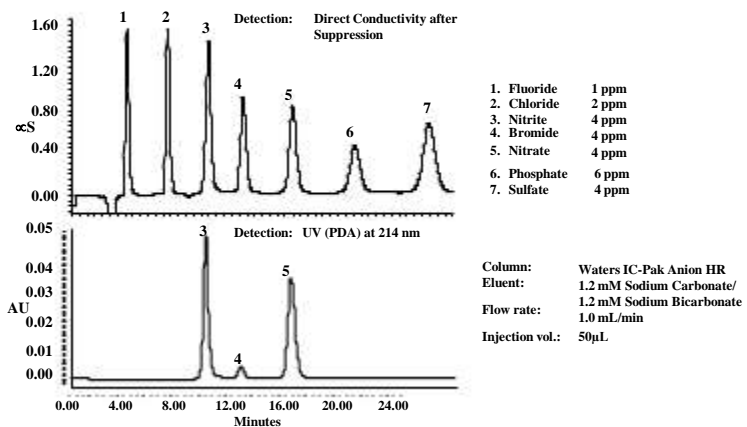
### Conductivity Detector



### 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**

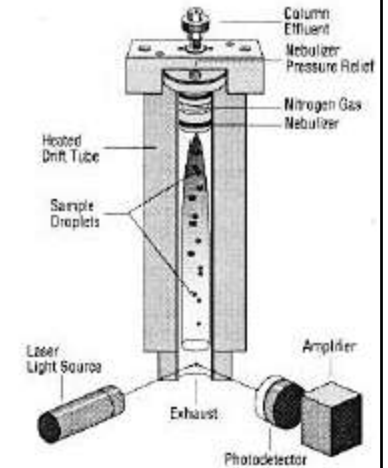
**No normal phase separations**

## Detectors

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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.

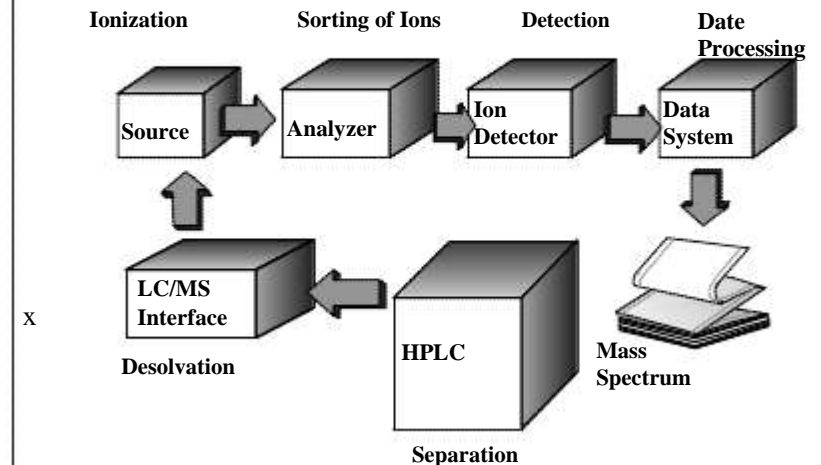


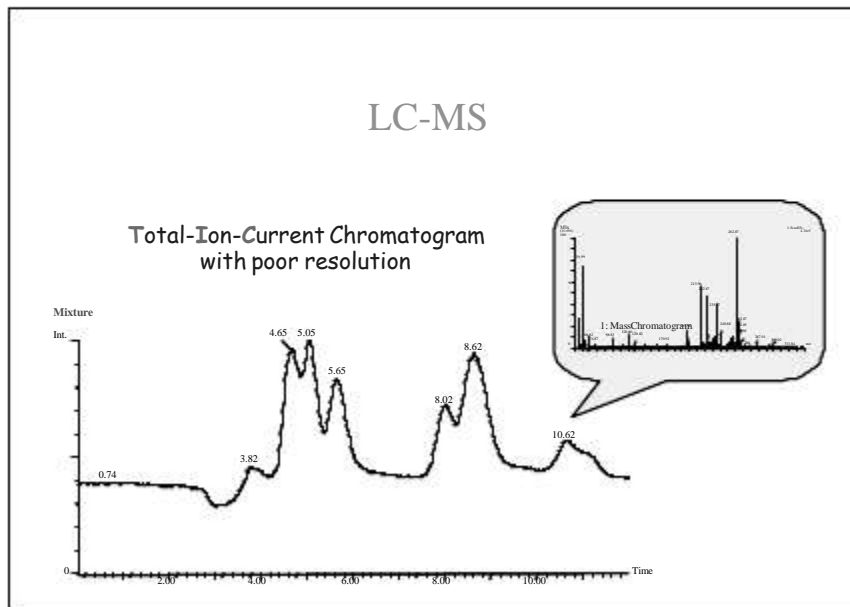
## Radioactive Detector

Primarily used for the measurement of  $^3\text{H}$ ,  $^{14}\text{C}$ , and  $^{32}\text{P}$ , 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

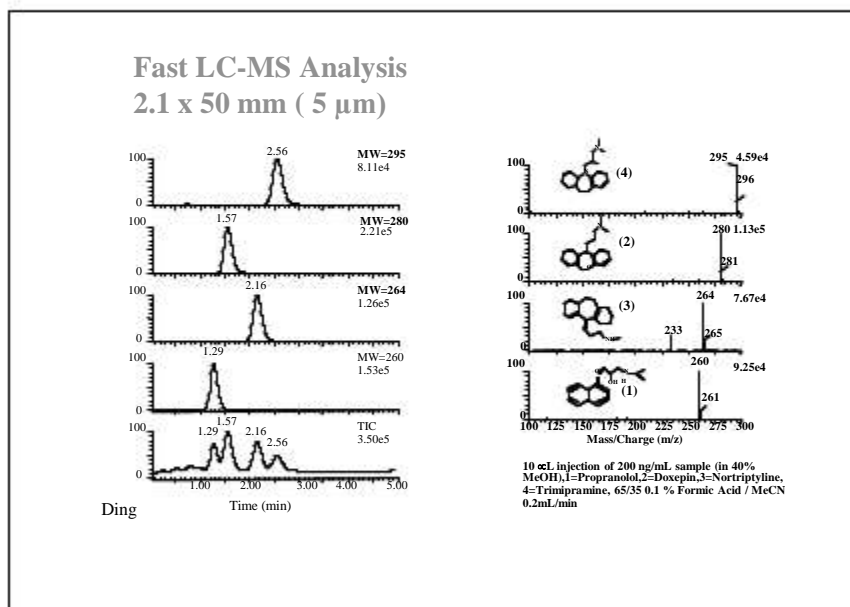
FT-ICR-Spectrometer

Magnetic Field B

Ion Traps

The Quadrupole Analyser

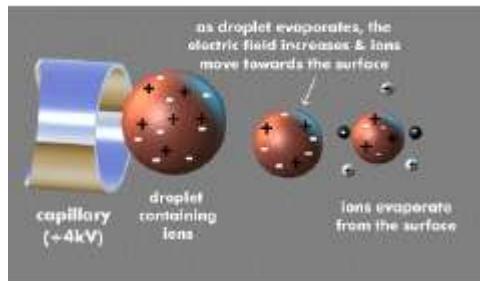
Sector Mass Spectrometers



### 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

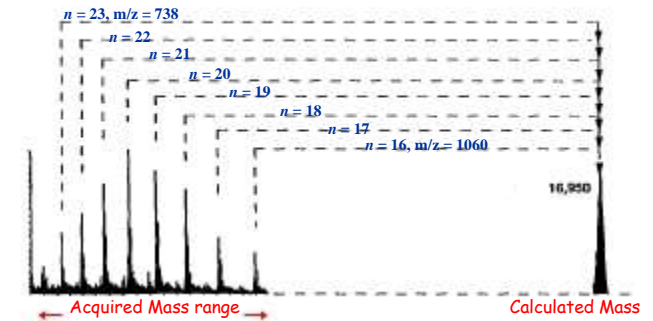
### ESI-MS Ion Formation



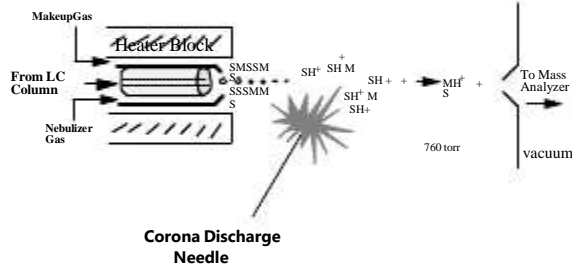
### Mass Range Multiply Charged Molecules

ANY ADJACENT PAIR OF IONS CAN BE USED TO CALCULATE THE NUMBER OF CHARGES AND THUS THE MOLECULAR WEIGHT

Horse Heart Myoglobin

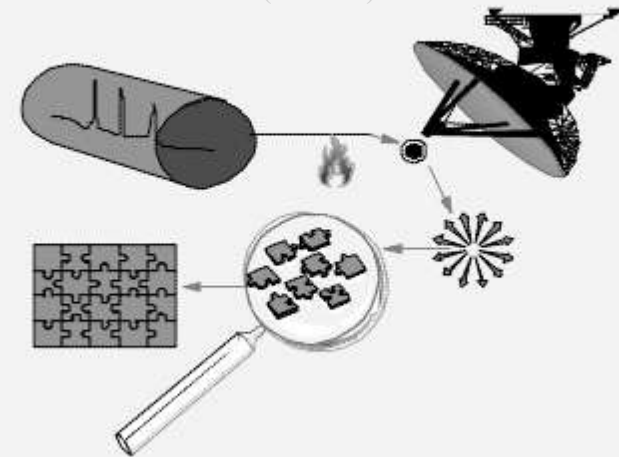


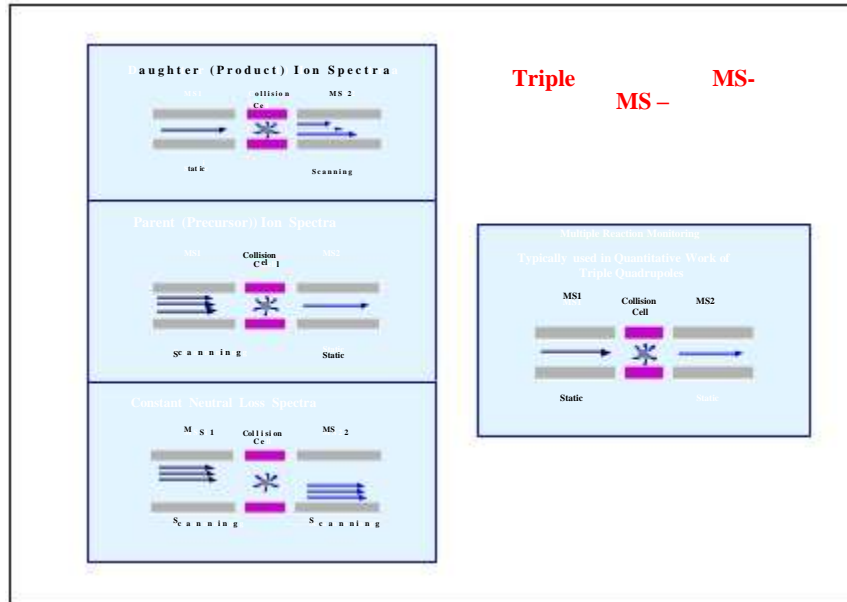
### APCI Probe Equipped With a Heated Nebulizer



- Generates molecular weight and structural information
- APCI flow rate: 0.2 to 2mL/minute
- Option: Crossflow interface

### Liquid Chromatography- Mass Spectrometry (LC-MS)





## 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 deadvolume (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

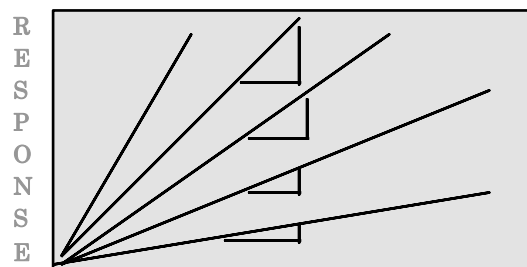


UNIVERSAL

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

## PROPERTIES OF DETECTORS

### SENSITIVITY



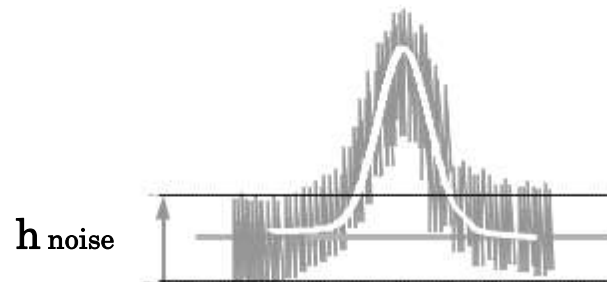
CONCENTRATION

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

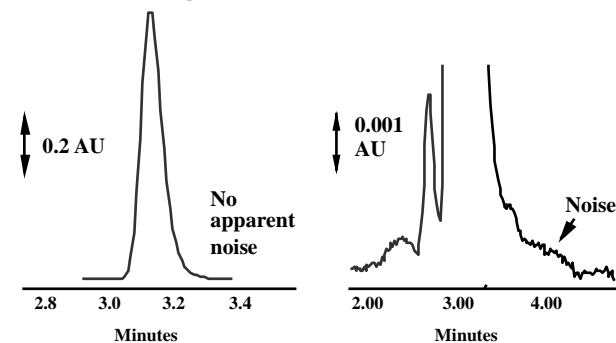
- Lowest concentration that can be detected
- Signal-to-noise ratio of 2:1 or 3:1

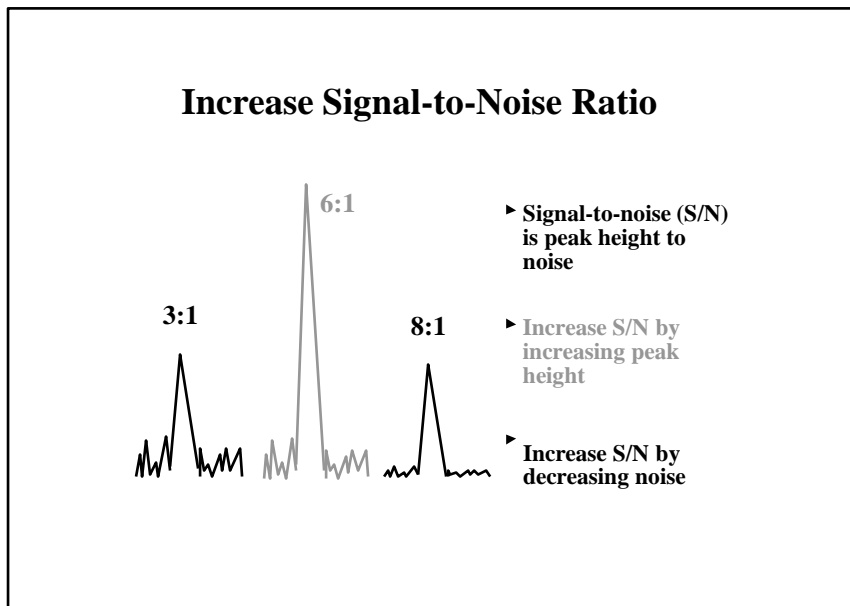
### ■ Limit of quantitation

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

## Chromatographic Sensitivity

### Signal-to-Noise Ratio



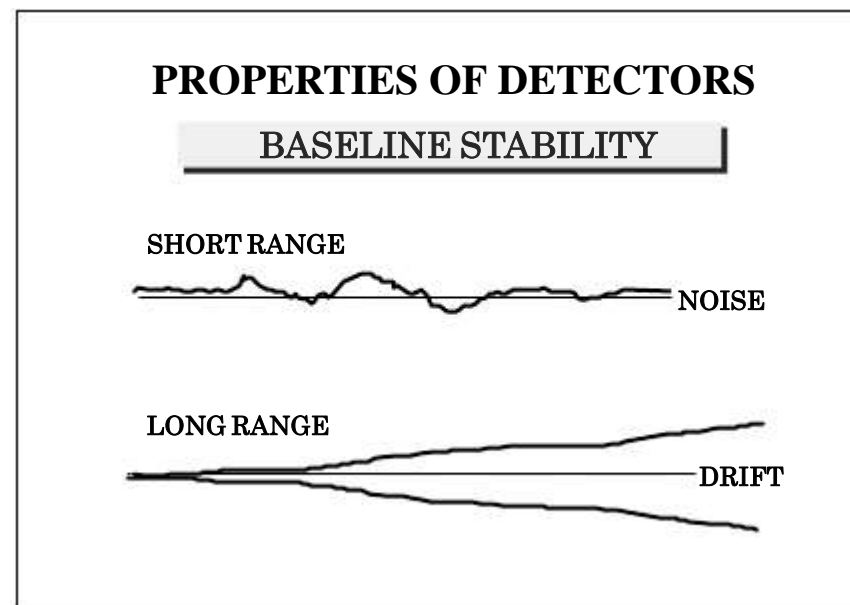
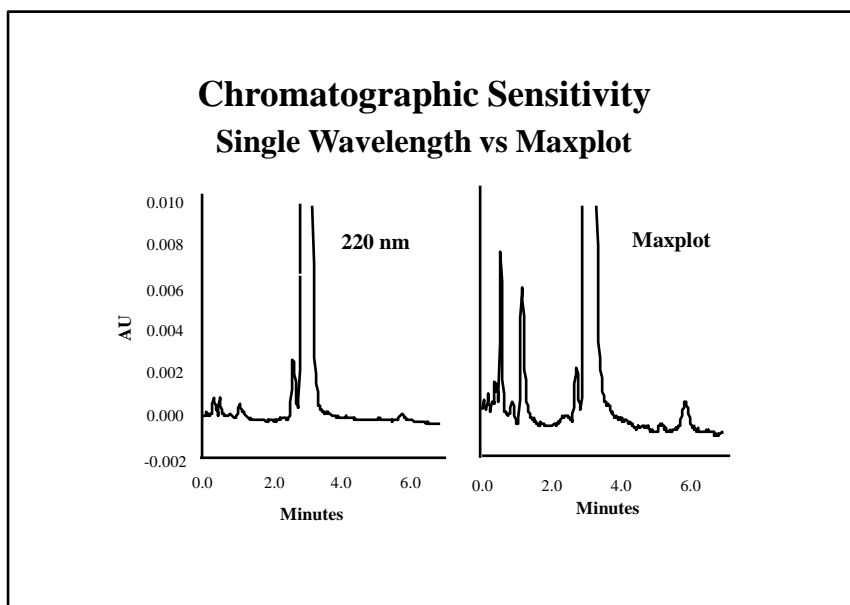


### Factors Increasing UV Signal

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

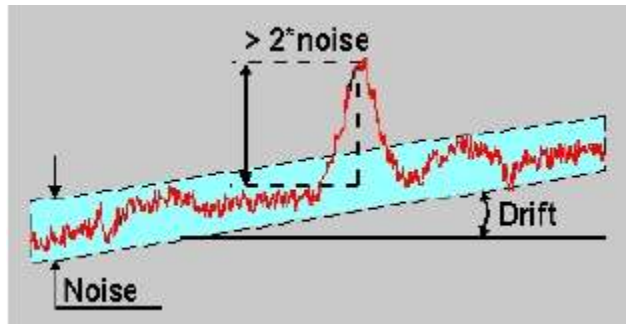
### Factors Affecting Noise in UV Detectors

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



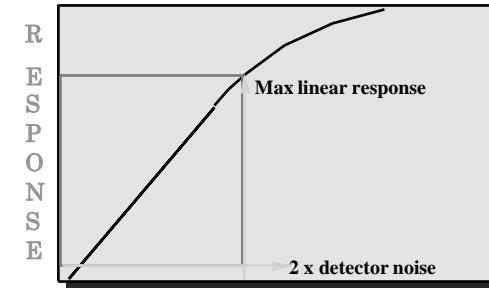
### Noise and drift

- Noise, drift, and smallest detectable peak.



### PROPERTIES OF DETECTORS

#### LINEAR RANGE

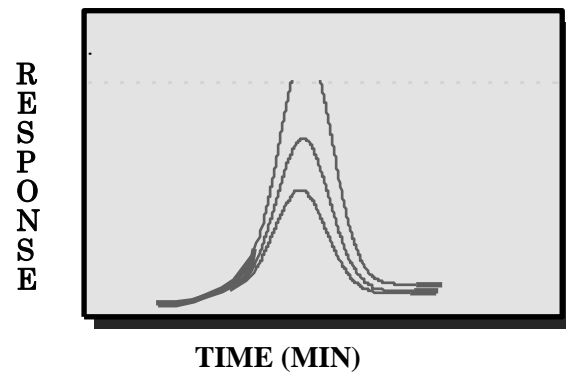


CONCENTRATION

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

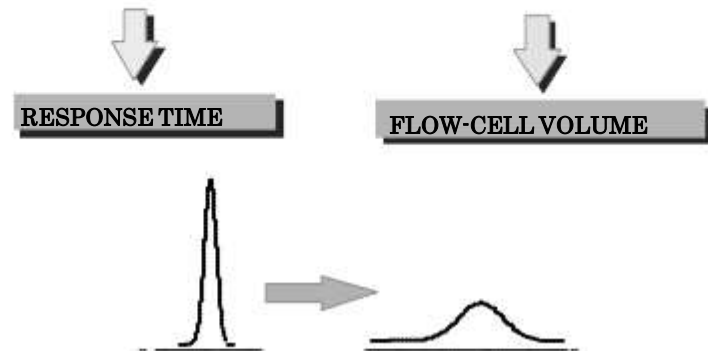
### PROPERTIES OF DETECTORS

#### 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**