

# Detection Qualification and Types of Detectors in HPLC

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# Detectors

## Detection Qualification and Types of Detectors in HPLC

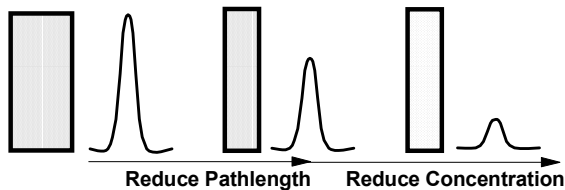
Dr. Shulamit Levin  
Medtechnica

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

## Beer's Law

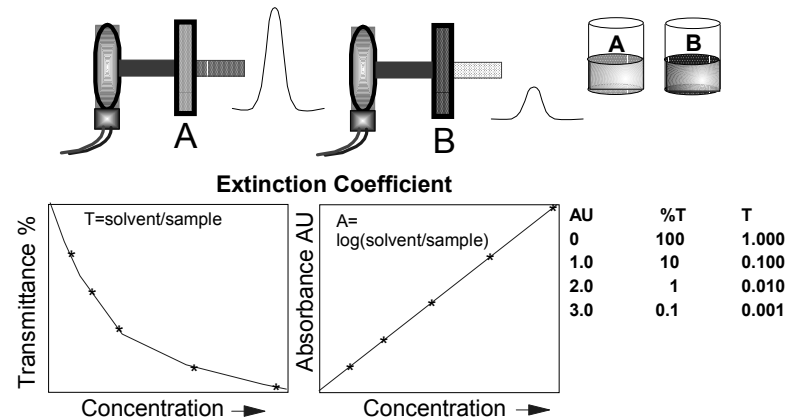
$$\text{Absorbance} = \text{Extinction Coefficient} \times \text{Pathlength} \times \text{Concentration}$$

Only for monochromatic light

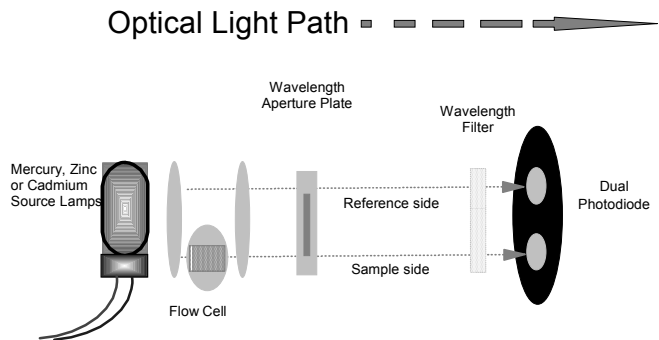


## Beer's Law

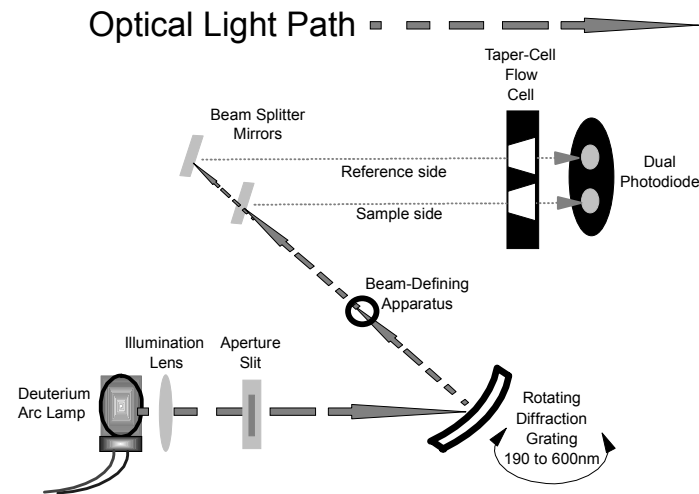
$$\text{Absorbance} = \text{Extinction Coefficient} \times \text{Pathlength} \times \text{Concentration}$$



# Single Wavelength UV Detector

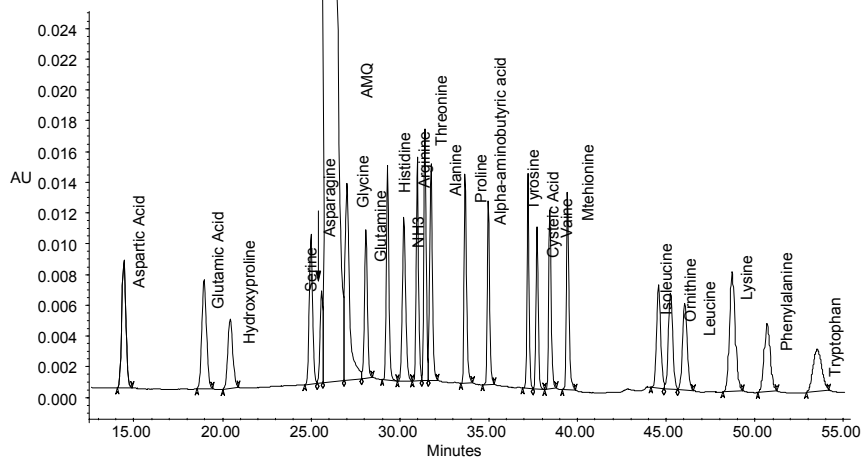


# 486 Detector Optical Bench

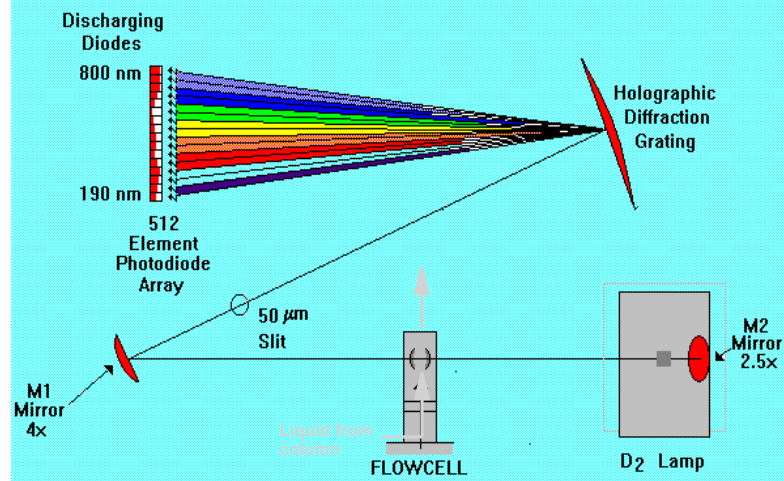


# UV Detection of AccQ-Tag Amino Acid Derivatives

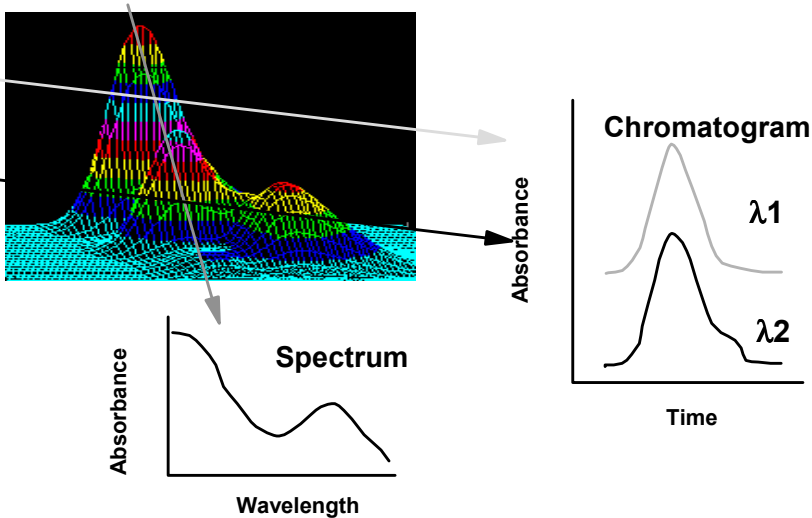
SampleName: Cult Std Vial: 1 Inj: 1 Ch: 486 Type: Standard



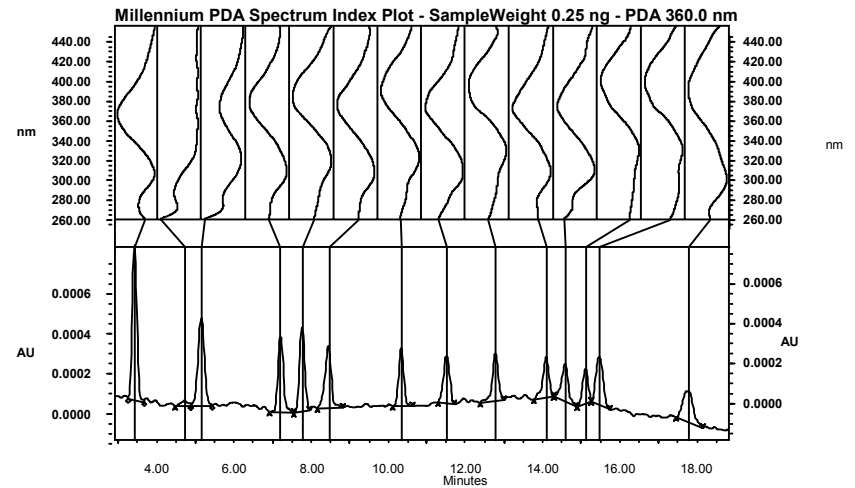
# Principle of Measurement



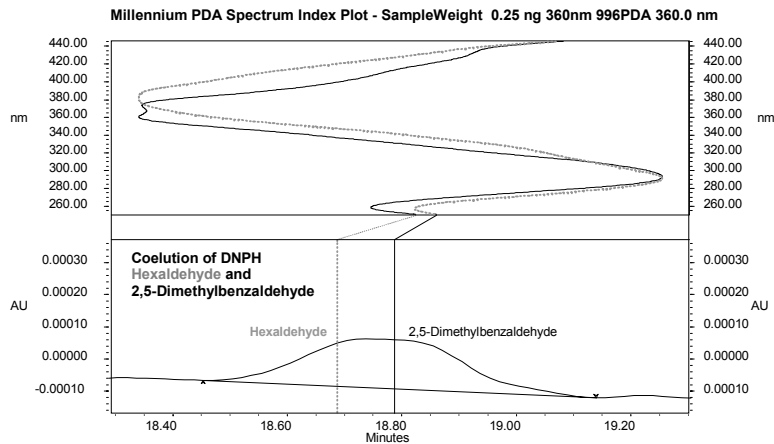
### Extraction of 3D Data



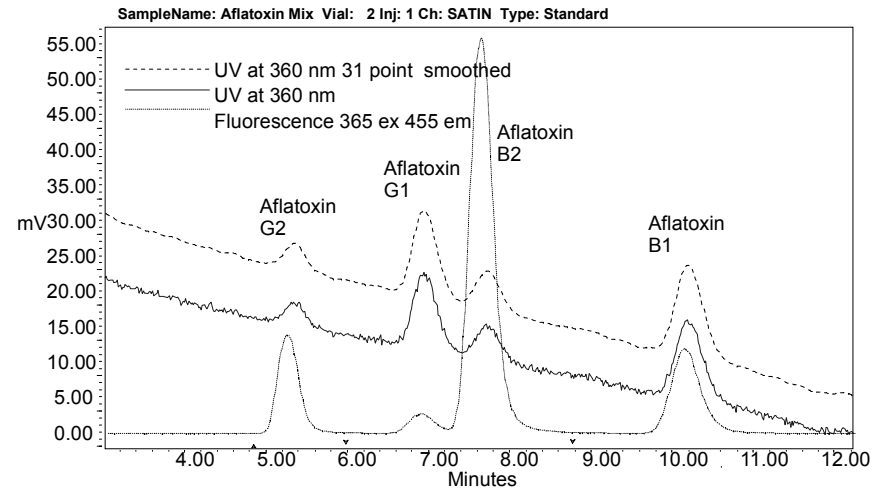
### 996 PDA Spectrum Index Plot DNPH Derivatives 0.25 ng Each Peak



### 996 Spectrum Index Plot Maximum Impurity Detection

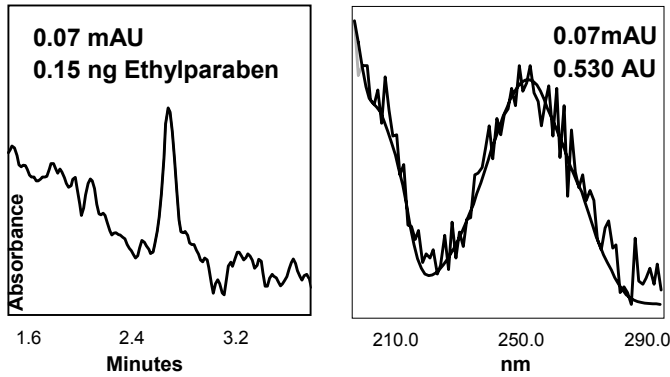


### PDA and fluorescent Detector Comparisons for Aflatoxin Analysis



# Photo-diode array

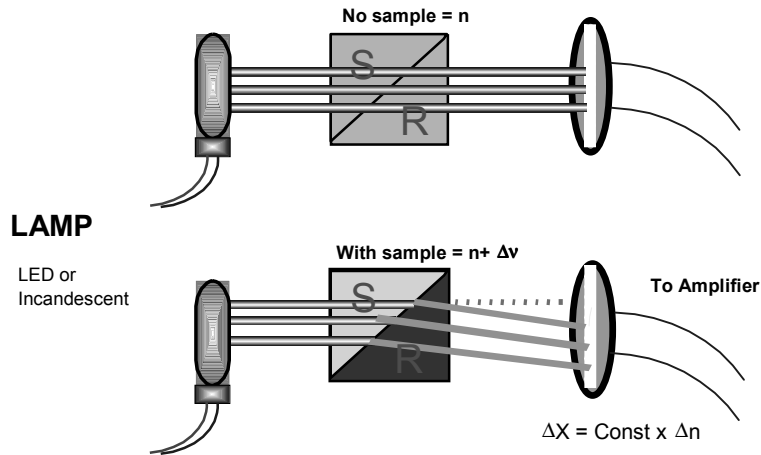
## Chromatographic and Spectral Sensitivity



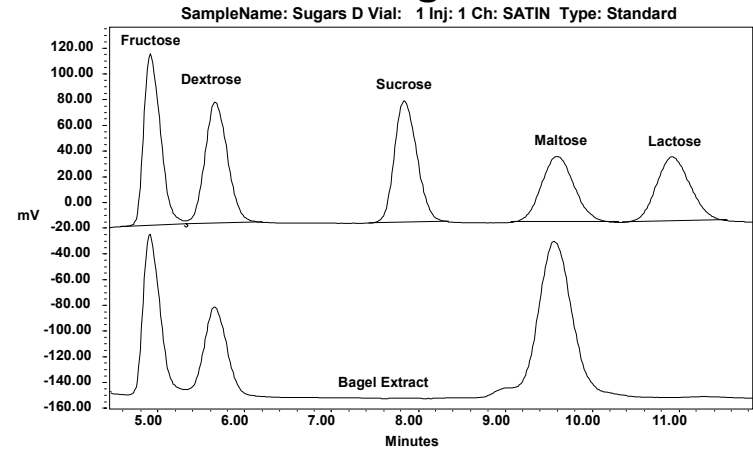
# Detectors

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

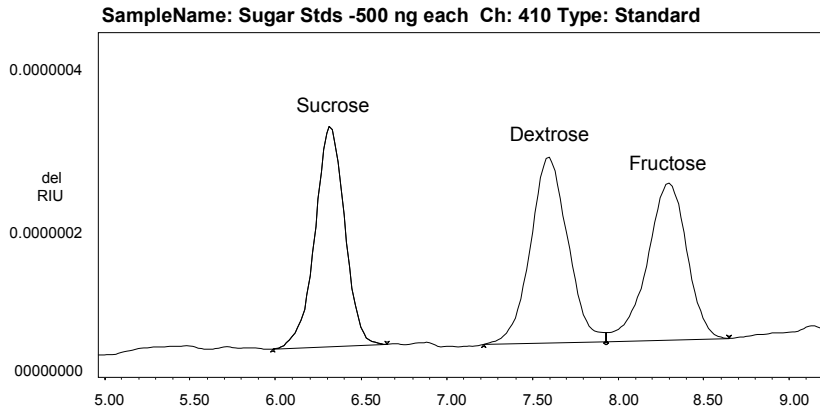
## Differential Refractive Index Detector



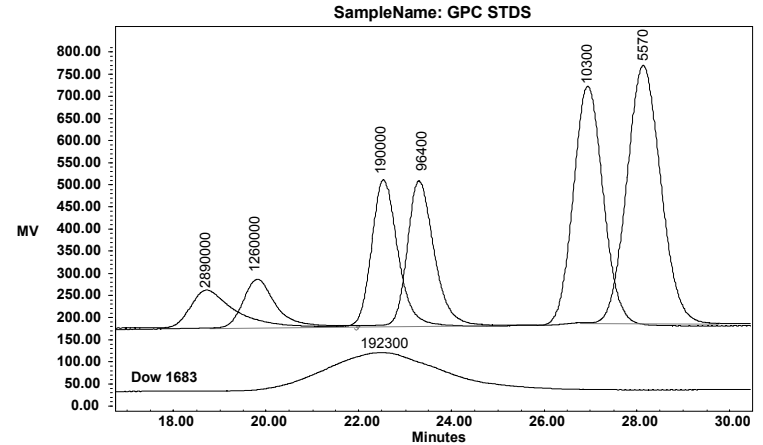
## Refractive Index Detection with Differential RI - Sugars



# Refractive Index Detection with Differential RI - Sugars

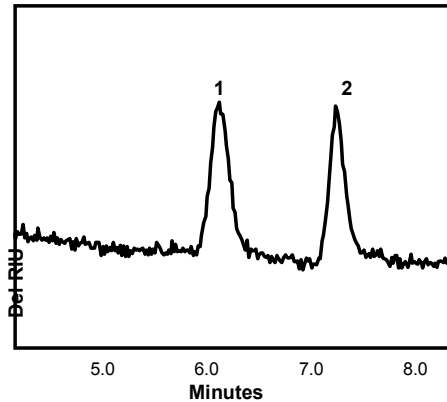


# Refractive Index Detection with Differential RI - Polymers



## Sensitivity

### Waters 410 Refractive Index Detector

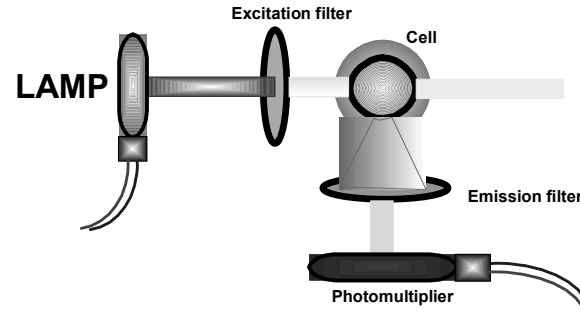


- ▶ 250 ng on column  
1=Tristearin  
2=Myristic acid
- ▶ Styragel HR 0.5,  
4.6 x 300 mm,  
35°C, 0.35 mL/min
- ▶ dRI sensitivity =  
32X, 32°C

# Detectors

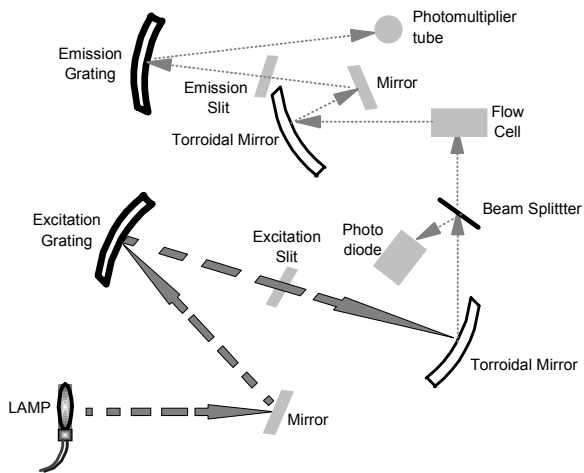
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# Fluorescence Detectors



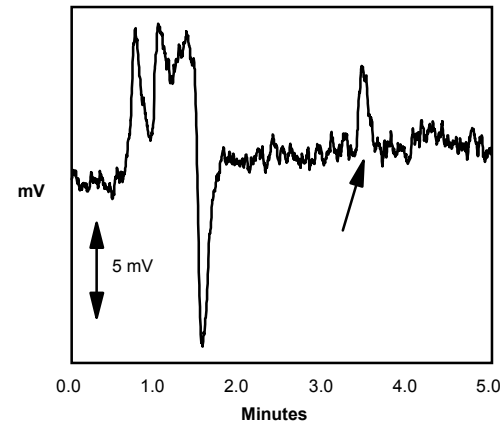
Short pass - transmits all wavelengths below a specified cutoff  
 Long pass - transmits all wavelengths above a specified cutoff  
 Band pass - blocks all wavelengths outside a specified band

# 474 Detector Optical Bench



# Sensitivity

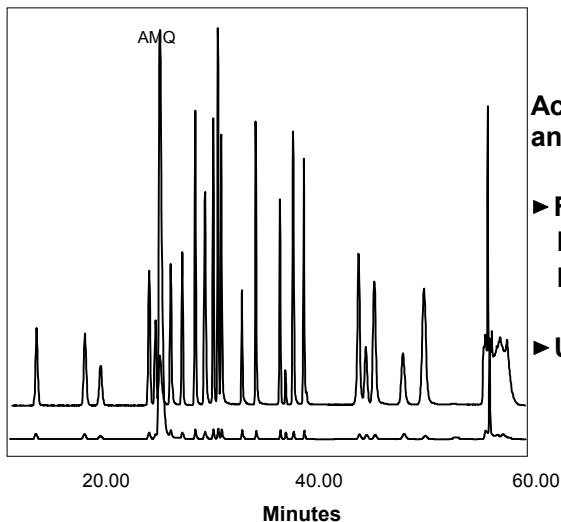
## Waters 474 Fluorescence Detector



0.1 pg Anthracene

Excitation = 251 nm  
 Emission = 406 nm

# Fluorescence vs. UV Detection



AccQ-Tag amino acid analysis

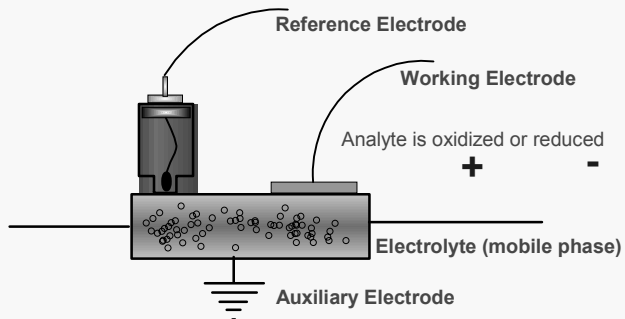
► Fluorescence  
Excitation=250 nm  
Emission=395 nm

► UV 254 nm

## Detectors

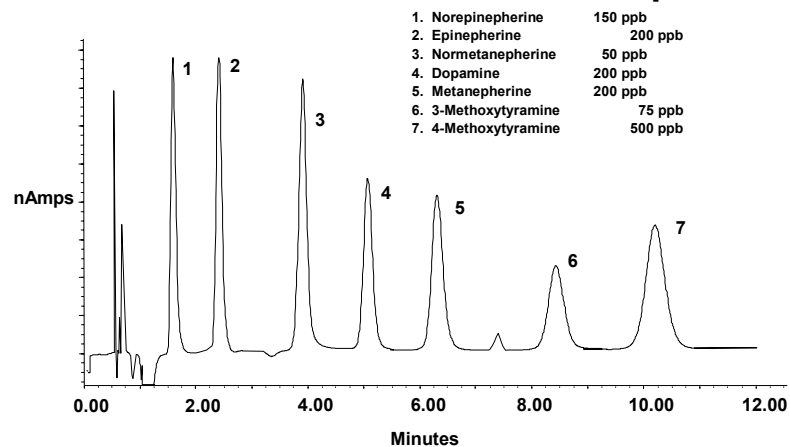
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## Electrochemical Detector

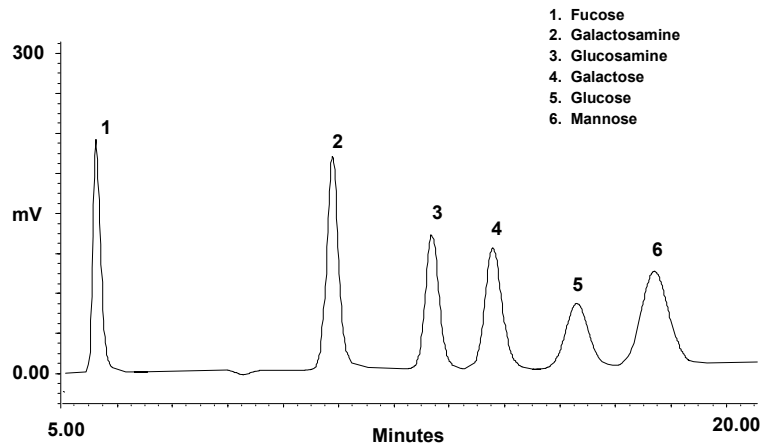


As compounds are oxidized or reduced, a current proportional to concentration is produced.

## 464 Electrochemical Detection of Catecholamines & Related Compounds



# 464 Pulsed Amperometric Detection of Monosaccharides

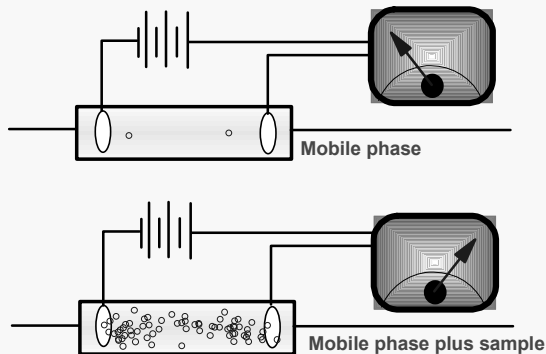


# Detectors

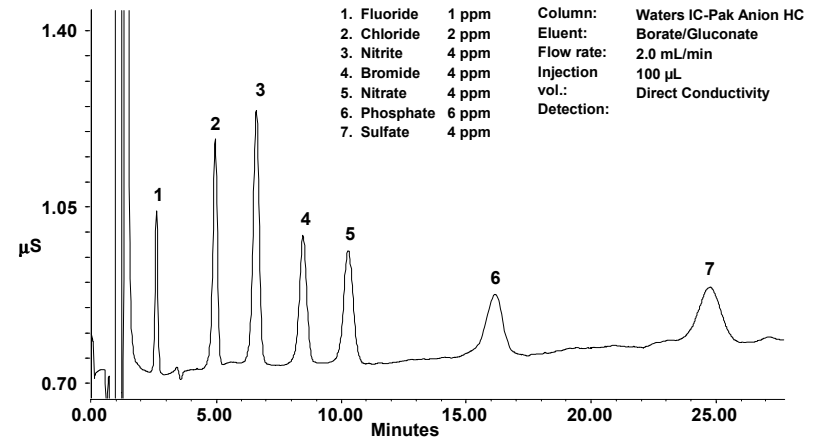
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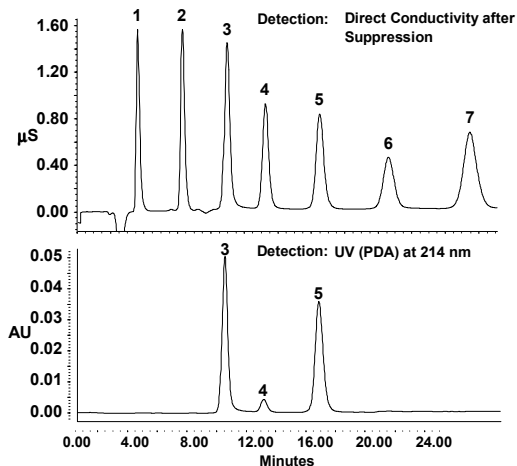
## Conductivity Detector



# 432 Conductivity Detection of Seven Anion Standard



# 432 Conductivity and 996 PDA Detectors in Series



- 1. Fluoride 1 ppm
- 2. Chloride 2 ppm
- 3. Nitrite 4 ppm
- 4. Bromide 4 ppm
- 5. Nitrate 4 ppm
- 6. Phosphate 6 ppm
- 7. Sulfate 4 ppm

Column: Waters IC-Pak Anion HR  
 Eluent: 1.2 mM Sodium Carbonate/  
 1.2 mM Sodium Bicarbonate  
 Flow rate: 1.0 mL/min  
 Injection vol.: 50 µL

## 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

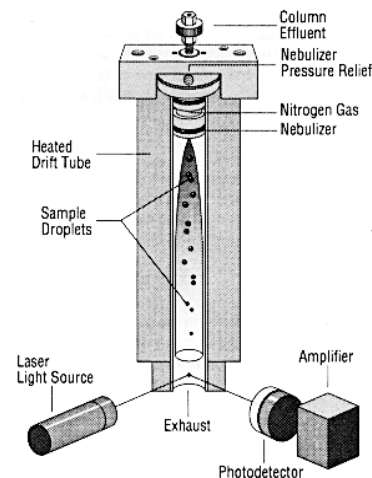
No normal phase separations

## Detectors

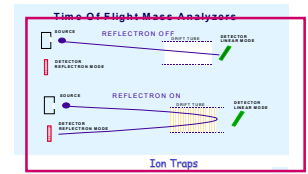
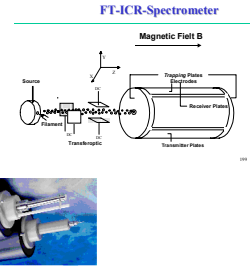
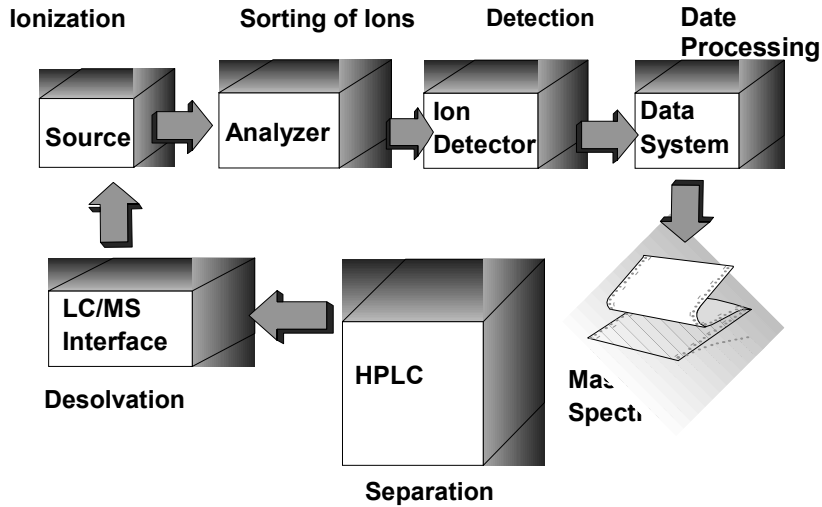
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## EVAPORATIVE LIGHT SCATTERING

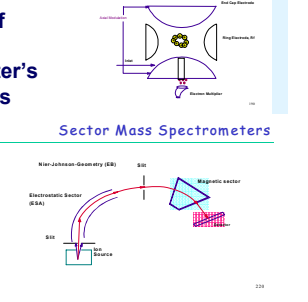
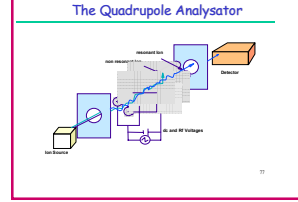
- The scattered light is detected by a silicone photodiode located at a 90° angle from the laser. The photodiode produces a signal which is sent to the analog outputs for collection. A light trap is located 180° from the laser to collect any light not scattered by particles in the aerosol stream.



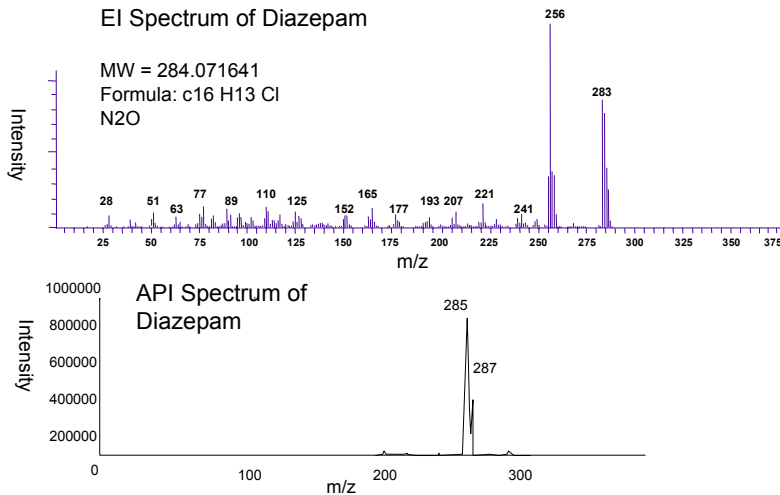
# How LC-MS Works



## Types of Mass Spectrometer's Analyzers

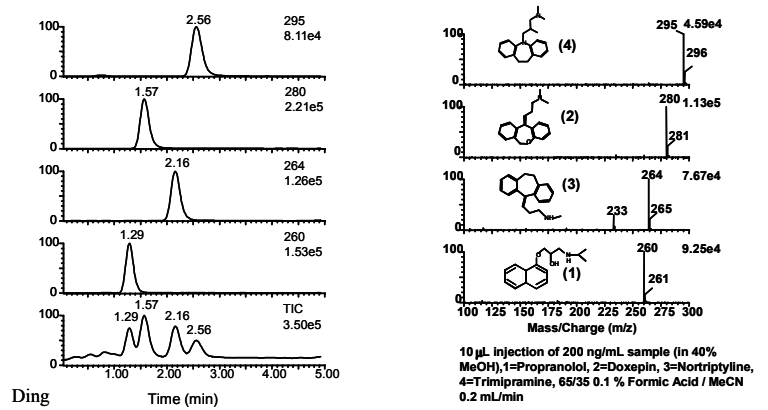


## EI vs. API Mass Spectrum

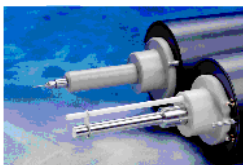


## Fast LC-MS Analysis

XTerra™ MS C18, 2.1 x 50 mm ( 5 μm)

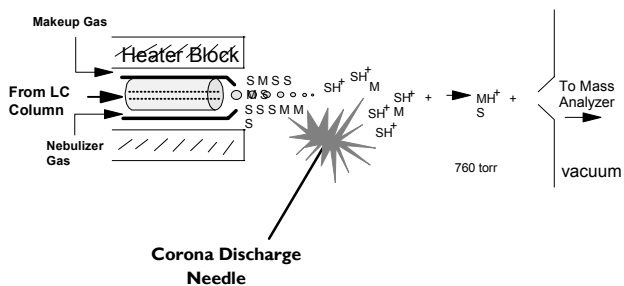


## HARDWARE - ES/APCI Ion Source



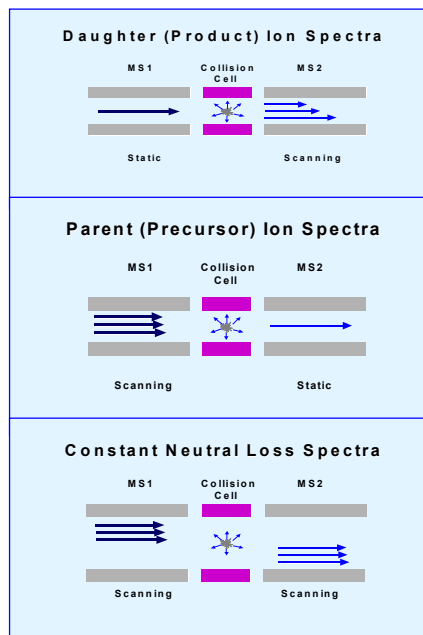
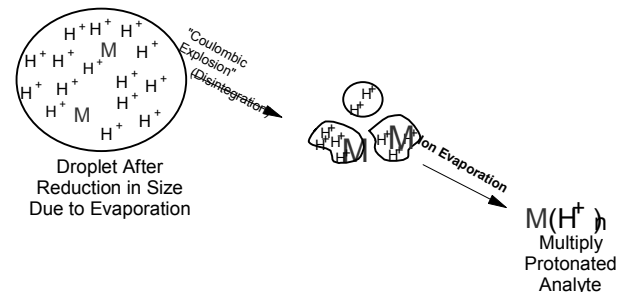
- ESI and APCI are easily interchangeable in seconds without venting the system
- ESI and APCI use a unique counter electrode to optimize sampling from the liquid spray and to aid sample desolvation
- Automatic Probe recognition

## APCI Probe Equipped With a Heated Nebulizer

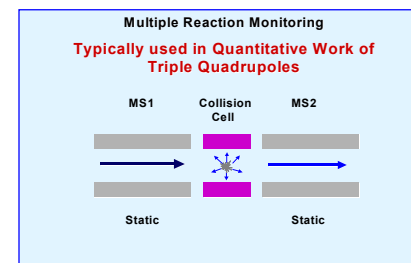


- Generates molecular weight and structural information
- APCI flow rate: 0.2 to 2mL/minute
- Option: Crossflow interface

## ESI-MS Ion Formation



## Triple Quadrupoles MS-MS - Modes of Operation

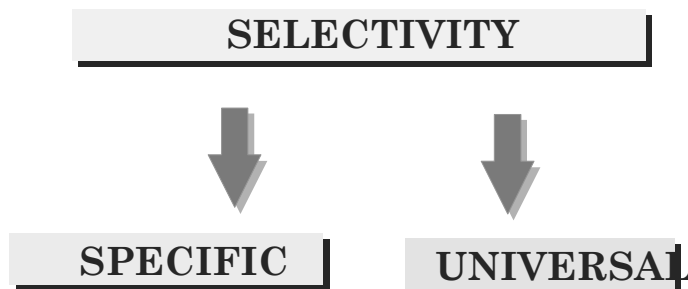


# BASIC DETECTOR REQUIREMENTS

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.

## PROPERTIES OF DETECTORS

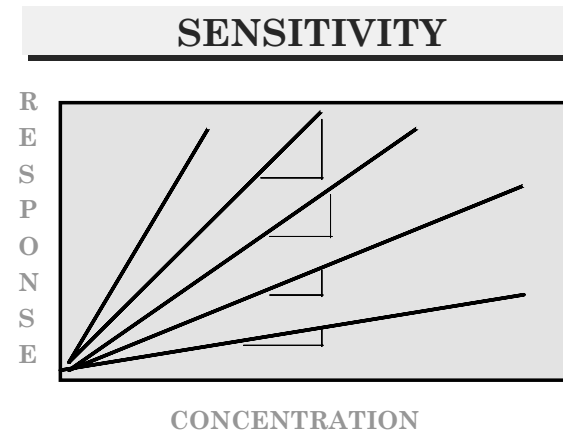


- A selective detector allows one to see only components of interest despite of their co-elution with any others.

## Detector Criteria

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

## PROPERTIES OF DETECTORS

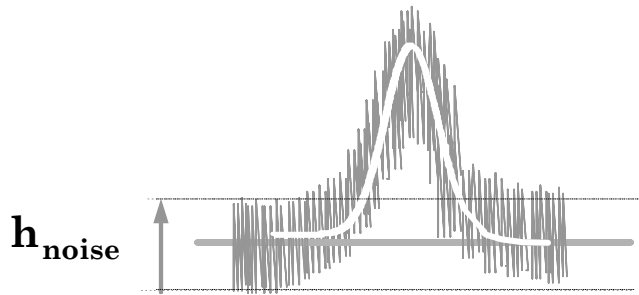


Sensitivity of a detector is the minimum amount that can be detected.

# PROPERTIES OF DETECTORS

## DETECTION LIMIT

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

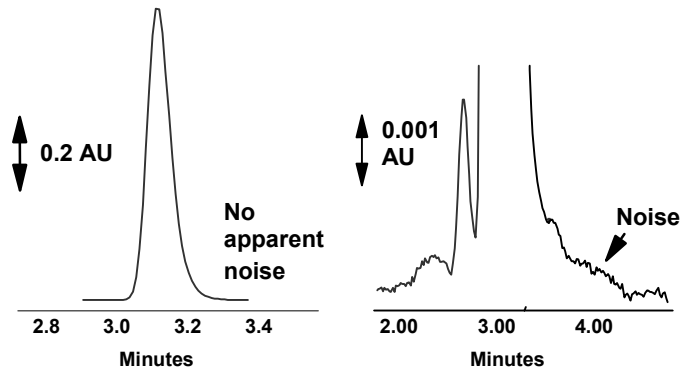


## Detector Sensitivity

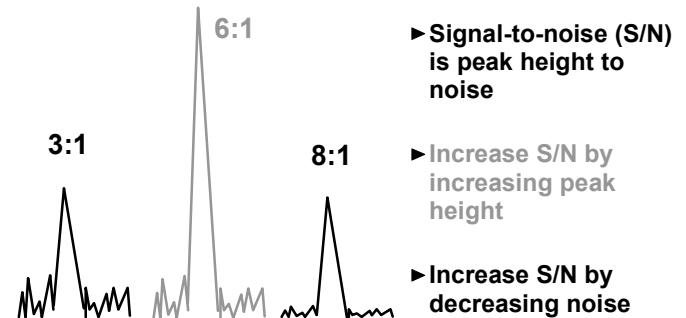
- **Limit of detection**
  - Lowest concentration that can be detected
  - Signal-to-noise ratio of 2:1 or 3:1
- **Limit of quantitation**
  - Lowest concentration that can be determined with acceptable precision
  - Signal-to-noise ratio of 10:1

## Chromatographic Sensitivity

### Signal-to-Noise Ratio



## Increase Signal-to-Noise Ratio

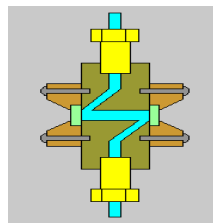


## Factors Increasing Signal

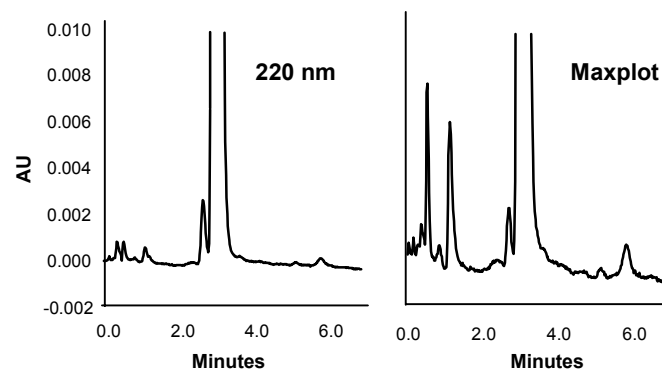
- ▶ Increase sample concentration
- ▶ Increase injection volume
- ▶ Choice of wavelength (s)
- ▶ Low volume flow cell
- ▶ Flow cell pathlength

## Factors Affecting Noise in HPLC Detectors

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

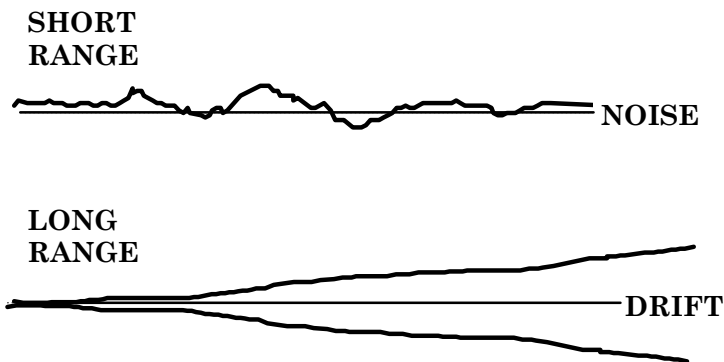


## Chromatographic Sensitivity Single Wavelength vs Maxplot



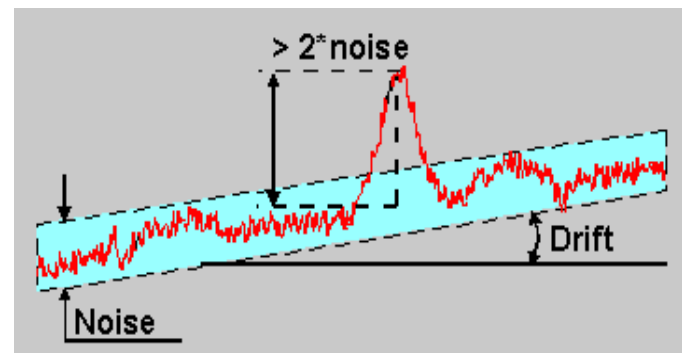
## PROPERTIES OF DETECTORS

### BASELINE STABILITY



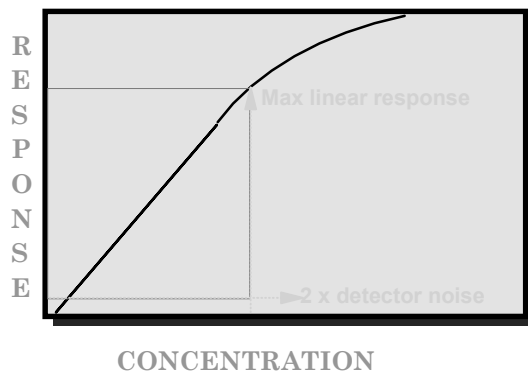
## Noise and drift

- Noise, drift, and smallest detectable peak.



# PROPERTIES OF DETECTORS

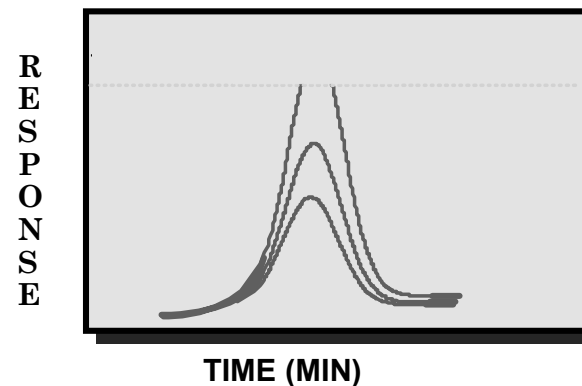
## LINEAR RANGE



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

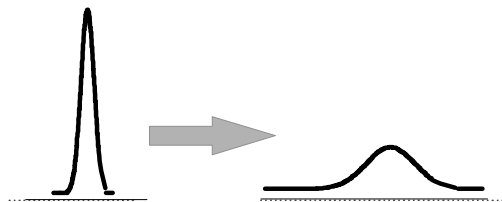
## CONTRIBUTION TO BAND BROADENING



RESPONSE TIME



FLOW-CELL VOLUME



# PROPERTIES OF DETECTORS

## REPEATABILITY OF RESPONSE



TEMPERATURE, FLOW RATE, ELECTRONICS