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

SELECTIVITY

SPECIFIC

UNIVERSAL

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

Sensitivity

CONCENTRATION

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%.

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Signal to Noise

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

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

Importance of Sensitivity: Impurity Profile

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**Increase Signal-to-Noise Ratio**

- Signal-to-noise (S/N) is peak height to noise
- Increase S/N by increasing peak height
- Increase S/N by decreasing noise

**Detector Noise**

Detector Noise Comparison

**Flow Cell Comparison - Noise**

Flow Cell Comparison - Noise

**HPLC System 1**

HPLC System 1

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Baseline Stability

SHORT RANGE

LONG RANGE

NOISE

DRIFT

Noise and Drift

Noise, drift, and smallest detectable peak.

Baseline Noise

Analytical High Sensitivity

Linearity-Linear Dynamic Range

Max linear response

2 x detector noise

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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

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Dynamic Range
Detector Response
Paclitaxel at 7.00 mg/mL

Waters 2487
Waters 486

Conditions:
Column: Symmetry® C8 (3.0 x 150 mm)
Buffer: 2mM NH₄Ac (pH 5.0) / ACN : MeOH 50:40:10
Flow Rate: 1 mL/min Temperature: 35°C
Inj. Volume: 5 µL Wavelength: 227 nm

Contribution to Band Broadening

RESPONSE TIME → FLOW-CELL VOLUME

Flow Cell Comparison - Sensitivity

Time constant

Effect of Filter Time Constant

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**Maintenance**

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

**Wavelength Accuracy Test**

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