MICROSOLV

Validating HPLC and LC-MS Methods for Regulated Labs - Primer

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Analytical method validation is a critical process in regulated environments such as pharmaceutical, environmental, and forensic laboratories. It ensures that your HPLC or LC-MS method is reliable, reproducible, and suitable for its intended purpose under SOP, GMP, GLP, or other regulatory frameworks.

Below is a simplified overview of the key validation parameters. Always consult your Regulatory Affairs team for specific requirements.

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1. Accuracy

- Measures how close your results are to the true value.
- Demonstrated through:
  - Comparison to a certified reference standard
  - Percent recovery studies
  - Standard addition experiments

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2. Precision

Defined by the **International Council for Harmonisation (ICH)** in three levels:

- **Repeatability**: Same analyst, same instrument, short time frame.
- **Intermediate Precision**: Different days, analysts, instruments.
- **Reproducibility**: Across different laboratories (often during method transfer).

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3. Linearity

- Assesses how well the detector response correlates with analyte concentration.
- Typically evaluated using:
  - A calibration curve
  - Correlation coefficient (R^2)
  - Y-intercept and slope

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4. Range

- The span between the **lowest and highest concentrations** where the method is accurate, precise, and linear.
- Must cover expected sample concentrations.

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5. Limit of Detection (LOD) & Limit of Quantitation (LOQ)

- **LOD**: Lowest concentration that can be reliably detected (S/N ≈ 3).
- **LOQ**: Lowest concentration that can be reliably quantified (S/N ≈ 10).
- Can also be calculated using:
    - $LOD = 3.3\ S0/b$
    - $LOQ = 10\ S0/b$
        - where (S_0) is the standard deviation of the response and (b) is the slope of the calibration curve.

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6. Specificity

- The method's ability to distinguish the analyte from other components in the matrix.
- Demonstrated through:
    - Chromatographic separation
    - Forced degradation studies
    - Orthogonal methods
    - Selective detection (e.g., MS or immunoassays)

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7. Ruggedness

- Evaluates method consistency under **variable conditions**, such as:
    - Different analysts
    - Instrument models
    - Column lots
    - Solvent suppliers

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8. Robustness

- Assesses how small, deliberate changes in method parameters affect results.
- Examples include:
    - Flow rate
    - Column temperature
    - Mobile phase pH
    - Gradient slope
- Results are compared to system suitability criteria (e.g., resolution, retention time).

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9. Octanol-Water Partition Coefficient (Log P)

- Describes a compound's **hydrophobicity**.
- Calculated as the ratio of concentrations in octanol vs. water at equilibrium.
- Useful for predicting:
    - Retention behavior in reversed-phase HPLC
    - Solubility and extraction efficiency

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10. Recovery

- Measures how much of the analyte is recovered from the sample matrix after processing.

- Important for methods involving extraction or sample prep.