Practical LC-MS method development and bioanalytical method validation
(Formerly “Introduction to GLP Regulations and Bioanalytical Method Validation by LC-MS/MS”)
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Selectivity ($\alpha$) impacts the separation most! unfortunately, it is the most difficult parameter to understand and predict.

If you fully understand this equation, you are a great chromatographer!
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Combination of LC with MS – a perfect marriage
Validation batch design for **A&P runs**

Prepare 3 runs in different days

<table>
<thead>
<tr>
<th>Calibration Standards Name</th>
<th>Replicate</th>
<th>Quality Control Samples Name</th>
<th>Replicate</th>
<th>Other Validation Samples Name</th>
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</table>

**QC1 ≤ 3 × LLOQ**
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Accuracy and precision expressions

**True = Actual = Theoretical = Nominal value**

\[
\text{Accuracy} = \frac{\text{Determined Value}}{\text{True value}} \quad \text{100 ± 15%}
\]

\[
\text{Accuracy} = \text{bias/error/deviation/difference}
\]

\[
\frac{\text{Determined Value} - \text{True Value}}{\text{True value}} \times 100 \quad \pm 15\%
\]

**Precision:** RSD = CV (coefficient of variation)
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Matrix effect and extraction recovery

\[ ME = \frac{\#2 \text{ PkAr}}{\#1 \text{ PkAr}} \]

\[ R = \frac{\#3 \text{ PkAr}}{\#2 \text{ PkAr}} \]

Stable isotopically labeled IS can compensate for extraction recovery and matrix effects
Case study (one run = Batch 1 + Batch 2)

Based on 2018 BMV acceptance
75% and a minimum of six standards (6/8 = 75%)
Both batches passed – each batch was processed individually
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Decision tree for reporting re-assay results

- **One value?**
  - Yes: **<LLOQ?**
    - Yes: Report BQL
    - No: Report value
  - No: Calculate median
    - Median within 100 ±15% of at least one valid result?
      - Yes: Report value
      - No: Sample left and stable?
        - Yes: Reassay
        - No: Report NR