Quality Assurance and Calibration Methods

Chapter 5

"If your experiment needs statistics, you ought to have done a better experiment." Ernest Rutherford, British chemist/physicist, 1908 Nobel Prize in Chemistry

Quality Assurance - procedure followed to obtain the correct answer for the desired purpose

- **use objectives** - states purpose for which results will be used
- **specifications** - sampling protocol, accuracy and precision, selectivity, sensitivity, detection limit, blanks, QC or blind samples
  - false positive / false negative
  - blanks - method, reagent, field
  - matrix
  - spiked sample
- **assessment** - data collected, procedures documented to verify that use objectives are met

Method Validation

- **specificity** - can distinguish analyte, no interference
- **linearity** of calibration curves - from LINEST and calculators

\[ R^2 = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})^2}{\sum(x_i - \bar{x})^2(y_i - \bar{y})^2} \]

we will use frequently

- **accuracy** - some ways to assess
  - reference material - Case 1 of Chapter 4
  - compare different analytical methods - Cases 2 and 3 of Chapter 4
  - analyze blank spiked with known concentration of analyte in same matrix
  - use standard addition to account for matrix effects - Chapter 5.3

- **precision** - determine reproducibility
  - instrument
  - intra-assay - same person, day, instrument
  - ruggedness - different people, day, instrument but same lab
  - interlaboratory - different people in another lab

- **range** - concentration range over which desired accuracy, precision, and linearity is obtained

Limits of Detection and Quantitation

- **limit of detection** (99% confidence)

  definition: signal detection limit

  \[ y_{dl} < y_{blank} + 3s \]

  \[ s \text{ from } N \geq 7 \text{ low concentration samples, } m \text{ from a calibration curve:} \]

  \[ y_{sample} - < y_{blank} > = mc_{sample} \]

  detection limit: substitute \( y_{dl} \) for \( y_{sample} \):

  detection limit = \( 3s/m \)

- **limit of quantitation**

  lower limit of quantitation = \( 10s/m \)
To determine the signal detection limit

1. measure the signal from ≥ 7 blanks and obtain the mean reading, \(< y_{\text{blank}} >\)
2. perform ≥ 7 trials that measure the signal from a sample containing a concentration of the analyte near the detection limit; obtain the mean reading, \(< y_{\text{sample}} >\), and its standard deviation, \(s_{\text{sample}}\)
3. define a detection limit that gives ~ 99% confidence that a signal above the detection limit arises from a sample that contains analyte by

\[
y_{\text{dl}} = < y_{\text{blank}} > + 3s_{\text{sample}}
\]

signal detection limit

Note that the one-sided Student’s \(t\) value for six degrees of freedom at the 99% confidence limit is 3.143.

The steps to continue to the determination of the concentration detection limit begins with a known linear relationship between the signal of a sample and the concentration of analyte (at concentrations higher than the detection limit). This relationship yields a linear calibration curve whose slope is \(m\).

4. the corrected signal is proportional to sample concentration

\[
< y_{\text{sample}} > - < y_{\text{blank}} > = mc_{\text{sample}}
\]

5. the concentration detection limit (minimum detectable concentration) is found by substituting for the sample concentration in step 4 the detection limit of step 3

\[
\text{minimum detectable concentration} = \frac{3s_{\text{sample}}}{m}
\]

concentration detection limit

EX. Concentrations of EDTA near the detection limit gave readings of 175, 104, 164, 193, 131, 189, 155, 133, 151, and 176. Ten blanks gave an average reading of 45.0 The slope of the calibration curve is 1.75 x 10^9 M^-1. Estimate the signal and concentration detection limits and the lower limit of quantitation.

The Tragedy of Thalidomide

Frances Kelsey, a pharmacologist and physician working at the FDA who, despite pressure from pharmaceutical companies, refused to approve thalidomide for marketing in the U.S.
7 Method Validation Reports

A method validation report, suitable for placing in the public docket, should be prepared. The report should address the method validation topics outlined in this guidance document (summarized in Table 1), and (a) background information on method development, (b) a description of the actual method validation techniques, (c) any changes made to the method as a result of the validation studies, and (d) any recommendations for future work.

Table 1
Method Validation Report Topic Areas

<table>
<thead>
<tr>
<th>Topic</th>
<th>Explanation</th>
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</thead>
<tbody>
<tr>
<td>Purpose</td>
<td>The purpose of the method is a clear and concise description of the measurement objectives and the intended use of the data.</td>
</tr>
<tr>
<td>Scope and Applicability</td>
<td>The method scope and applicability serve to define the range of method performance studies. The scope and applicability includes (a) the measurement process components validated, (b) the nature of the analytes and matrices studied, (c) the range of analyte levels for which the method is claimed to be suitable, (d) a description of any known limitations and any assumptions upon which a method is based, and (e) a description of how the method and analytical parameters chosen meet the data quality objectives for the specific application.</td>
</tr>
<tr>
<td>STANDARDS ADDITION</td>
<td>Selectivity is a performance characteristic that demonstrates the ability of the method to yield useful data for the analytes, analyte levels, and matrices defined within the scope of the method. Selectivity is especially important at action levels of concern, and must be demonstrated in the presence of species known or predicted to present analytical challenges to the method. Typically, selectivity is demonstrated by providing information that substantiates the identity of the analytes in the presence of expected matrix constituents.</td>
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<tr>
<td>Instrument Calibration</td>
<td>This performance characteristic is sometimes referred to as “instrument linearity.” In a broader sense, it addresses instrument calibration and the nature of the resulting calibration model. During method validation, sufficient data should be generated to demonstrate and justify the method-recommended calibration approach.</td>
</tr>
<tr>
<td>Precision</td>
<td>Precision is a performance characteristic that reflects sources of random error in a measurement process. There are several different types of precision studies (i.e., repeatability and reproducibility studies). Methods designed for demonstrating compliance with regulatory requirements should be evaluated for both repeatability (within-lab) and reproducibility (among labs).</td>
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<tr>
<td>Quantitation Range and Limits</td>
<td>A quantitation range corresponds to the range of analyte concentrations (or other quantity) characterized for measurement accuracy (trueness and precision) during method validation. Analytical measurement uncertainty should be well characterized over a quantitation range.</td>
</tr>
<tr>
<td>DETECTION LIMIT</td>
<td>This general term describes the lowest level of analyte that can be identified with confidence. A method validation study typically involves a specific detection limit definition, and includes an explanation of how the defined detection limit was estimated. Detection limit estimates derived from mathematical definitions or statistics must be verified by analyzing samples containing analyte at the claimed detection level. This performance characteristic may not be important for all analytical applications, and its relevance depends on the method scope and applicability.</td>
</tr>
<tr>
<td>Ruggedness</td>
<td>Ruggedness refers to the capacity of an analytical method to remain unaffected by small variations in operating conditions. Ruggedness testing involves experimental designs for examining method performance when small changes are made in operating or environmental conditions. The changes should reflect expected, reasonable variations that are likely to be encountered in different laboratories.</td>
</tr>
<tr>
<td>Interlaboratory Study</td>
<td>Interlaboratory studies determine whether an analytical method can be transferred for use in other laboratories and used for regulatory testing. The design and results of the interlaboratory study should be included in the method validation summary report. Data from the interlaboratory study should be reported in tabular form. There should also be a discussion included describing the details of, and rationale for, any changes made to the method resulting from the interlaboratory study.</td>
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