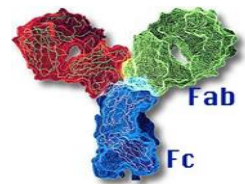# Establishment of Specifications for Monoclonal Antibodies

Chuanfei Yu
PhD

中国食品药品检定研究院

National Institutes for Food and Drug Control

# Disclaimer:

**This report represents only personal views and does not reflect the opinions of the regulatory authorities or the National Institutes for Food and Drug Control.**

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# Report Outline

**1** **Overview**

**2** **Compendial and regulatory requirements and experience with similar products**

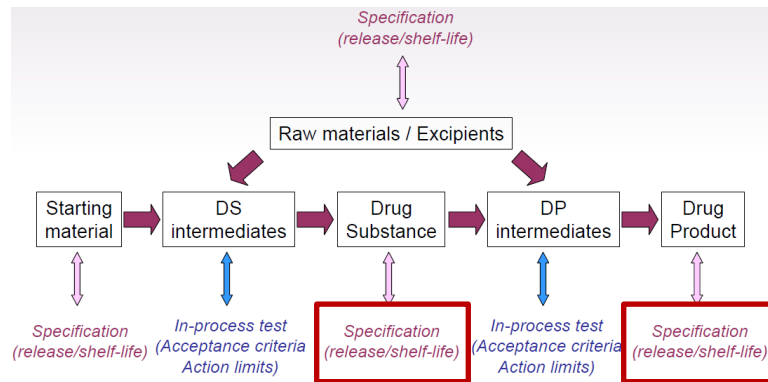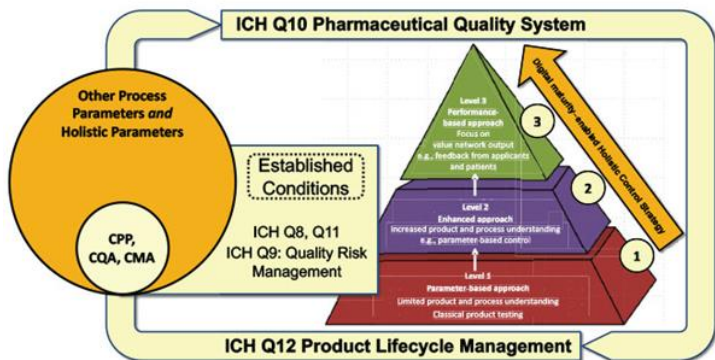**3** **Examples of specification setting for purity and potency**

**4** **Summary**

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Specifications are one part of a total control strategy designed to ensure product quality and consistency. Other parts of this strategy include thorough product characterization during development, upon which many of the specifications are based, adherence to Good Manufacturing Practices, a validated manufacturing process, raw materials testing, in-process testing, stability testing, etc.

# ICH Q6B

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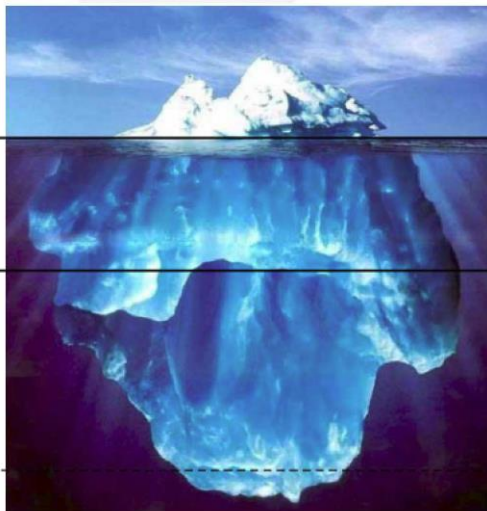**Comprehensive method validation**



Release Tests (Specifications)

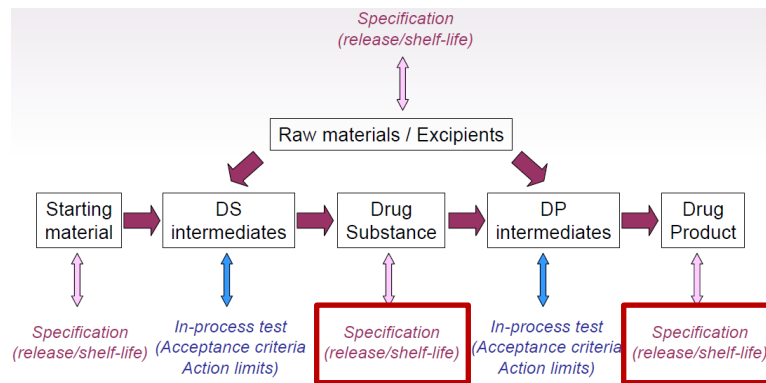Extended Characterization (Process & Product)

Process Control
- Procedures
- Materials
- In-process testing
- Monitoring
- Validation

Unknown
Learned over time – update control strategy

from: Koszlowski, S. & Swann, P. (2006) Adv. Drug Delivery Revs.



Specifications are one part of a total control strategy designed to ensure product quality and consistency. Other parts of this strategy include thorough product characterization during development, upon which many of the specifications are based, adherence to Good Manufacturing Practices, a validated manufacturing process, raw materials testing, in-process testing, stability testing, etc.

# ICH Q6B

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# Considerations for Specification Item Setting

**Using the QbD approach, lot release & stability testing should be risk-based and address highly critical or less well-controlled attributes**

A specification is defined as a list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described.
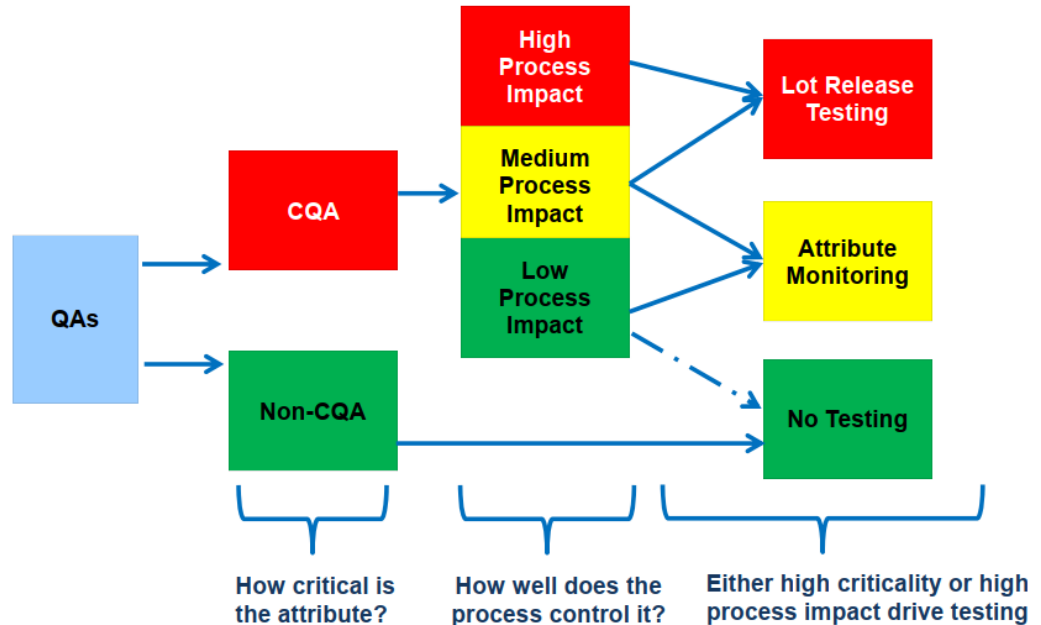
ICH Q6B

# Typical Specification for Monoclonal Antibodies

| Category | Quality Attribute | Release Specification[a] | Stability Specification[b] | Additional Consideration |
|---|---|---|---|---|
| Appearance and Description | Clarity | DP | DP | Appearance and description include specific tests for color, clarity and particles which are typically expected as tests performed on DP. More limited appearance and description testing is typically performed on DS. For lyophilized DP, an assessment of appearance is performed prior to and following reconstitution. Visible Particles may be categorized under Particulate Matter. |
| | Coloration | DP | DP | |
| | Visual appearance | DP | DP | |
| | Visible Particles | DP | DP | |
| Particulate Matter in Injections | Subvisible Particles | DP | DP | |
| Identity | Identity | DS, DP | | |
| Quantity or Strength | Protein Concentration | DS, DP | DS, DP | Although not a stability-indicating assay, protein concentration is frequently included in the stability program. |
| | Content Uniformity | DP | | In-line fill weight checks may be performed in lieu of release testing |
| Potency | Potency (Biological Activity) | DS, DP | DS, DP | |
| General Characteristics | pH | DS, DP | | The decision to include pH testing on stability should be driven by development data |
| | Osmolality | DP | | Often tested for DS. In process testing may be performed, particularly for DS, in lieu of release testing if justified |
| | Reconstitution Time | DP | DP | Performed for lyophilized drug products |
| | Moisture Content | DP | DP | Performed for lyophilized drug products |
| | Extractable Volume | DP | | |
| Excipients[c] | Polysorbate Concentration | DP | | Polysorbate testing for DS is not required but may be considered to mitigate business risk, or in place of DP testing if no formulation change at DP and no change on stability. In process testing for DP could be considered in lieu of release testing. |
| Purity and Product Variants | Size, Charge, Glycans, product specific PQAs | DS, DP | Attribute specific, based on a data-driven assessment | See Table 3 |
| Process-Related Impurities | Residual Host Cell Protein | | | The final control strategy for process-related impurities are determined based on process understanding, and novel control strategies may be considered. Process related impurities may be part of the drug substance release specification, an in-process control, or not required if suitable clearance is demonstrated during process characterization and validation. |
| | Residual Protein A | | | |
| | Residual Host Cell DNA | | | |
| Microbiological Attributes[d] | Endotoxin | DS, DP | | |
| | Bioburden | DS | | |
| | Sterility | DP | DP EOSL | Container closure integrity may be performed in lieu of Sterility on stability |
| | Container Closure Integrity | | DP EOSL | |

# ICH-Q6B

- **Linked to Manufacturing Process**
  - Proposed specification should be based on data from manufacturing process with demonstrated manufacturing consistency.
  - A lack of manufacturing consistency is not a good justification for a broad specification.
- **Account for drug substance and drug product stability**
  - May need serval stability indicating assays to address the inherent product complexity.
- **Linked to preclinical and clinical studies**
  - The quality of commercial scale batches should be representative of the batches used in preclinical and clinical studies.
- **Linked to analytical procedures**
  - Analytical technology often involves in parallel with product development. It is important to confirm that data generated during product development correlate with data generated at the time of marketing authorization application.

# Considerations for Specification Limit Setting

**Compendial requirements**

- ChP
- USP

**Product understanding**

- Literature knowledge
- Experience with similar products
- Characterization studies
- Structure-function relationship
- Product stability

**Process understanding**

- Process development/ characterization
- PPQ

**Method variability**

- Accuracy and precision
- Long-term operating trends

**Data statistical analysis**

- Clinical/process validation batches
- Release & long-term stability data

# Report Outline

**1**   Overview

**2**   Compendial and regulatory requirements and experience with similar products

**3**   Examples of specification setting for purity and potency

**4**   Summary

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# Compendial Requirements

**Appearance:**

**Physical state**

**Clarity**

**Color**

**Test for visible particles**

| Reference suspension No. | 0.5 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| Opalescence reference standard solution (mL) | 2.50 | 5.0 | 10.0 | 30.0 | 50.0 |
| Water (mL) | 97.50 | 95.0 | 90.0 | 70.0 | 50.0 |

| Formazin suspensions | Opalescent values (NTU) |
|---|---|
| Reference suspension I | 3 |
| Reference suspension II | 6 |
| Reference suspension III | 18 |
| Reference suspension IV | 30 |
| Standard of opalescence | 60 |
| Primary opalescent suspension | 4000 |

**ChP:**

**Clear**: The solution which is termed "clear" means that the clarity of the solution of the substance being examined is the same as that of the solvent being used for the preparation of the solution, or its opalescence is not more pronounced than that of reference suspension **No. 0.5**.

**Almost clear**: The term of "almost clear" for a solution means that the opalescence of the solution of the substance being examined is as pronounced as that of the reference suspension between **No. 0.5 and No. 1.**

**Ph. Eur.:**

**Clear**: A liquid is considered clear if its clarity is the same as that of water R or of the solvent, or if its opalescence is not more pronounced than that of reference **suspension I** (theoretical value is 3 NTU)..

# Compendial Requirements

## Appearance:

**Physical state**
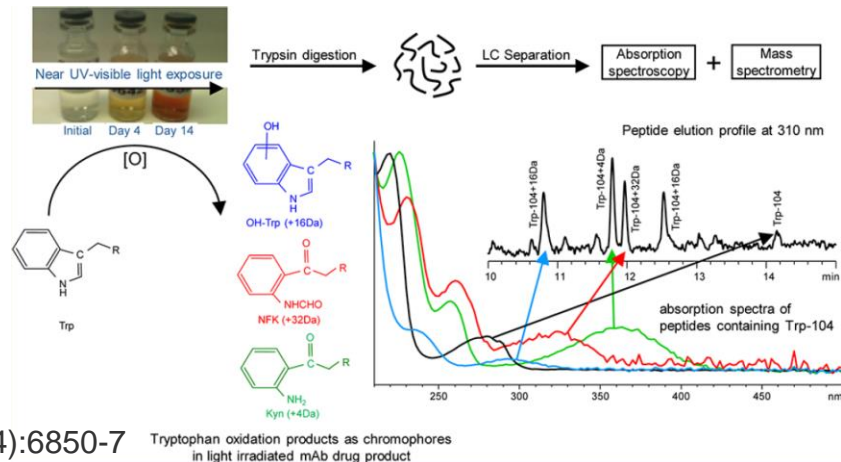
**Clarity**

**Color**

**Test for visible particles**

**Definition:**

- They provide a floor standard for batch-to-batch consistency of drug quality.
- Consistent with the product label description, they provide an expectation standard for healthcare professionals and patients.

**Other points of attention:**

Specifications should be reasonably established for release, stability, accelerated, and stressed samples.





ANAL CHEM. 2014-07-15;86(14):6850-7

# Compendial Requirements

The obviously visible particles such as broken bits of metal or glass, fibers with a length of more than 2 mm, blocks with a size dimension of more than 2 mm, the visible smoky precipitate composed by particles formed by gently inverting the vial after a period of stationary standing, the cluster of particles which are difficult to count, the precipitate not dispersed on shaking, and the protein flocculus which are difficult to count within the specified time should not be found.

If tiny visible particles such as dots and short fibers or blocks with a length or a size dimension of less than 2 mm are detected in the samples, and if the translucent protein flocculus or protein particles with length of less than 1 mm are detected in biochemical medicine or biologics, they should comply with the requirements listed in the following tables, unless otherwise specified.

## Appearance:

**Physical state**

**Clarity**

**Color**

**Test for visible particles**

**Instance:**

**Should comply with the requirements, except possibly containing a trace amount of translucent to white amorphous protein particles in the solution.**

| Category | Limits of tiny visible particles | |
|---|---|---|
| | 20 vials in the primary test | 40 vials in the primary and repeat tests |
| Injection | For an extractable volume of ≤ 50 mL, the number of tiny visible particles in each vial should be ≤ 3; for a fill volume of > 50 mL, the number of tiny visible particles in each vial should be ≤ 5 | If 2 or more vials are beyond the test limits, the test item does not meet the requirements |

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# Compendial requirements

## Chinese Pharmacopoeia requirements

**Sub-visible particles**

pH

**Osmolality**

| | Particulate matter ≥ 10 μm | Particulate matter ≥ 25 μm |
|---|---|---|
| Product ≥ 100 mL | ≤ 25 particles/mL | ≤ 3 particles/mL |
| Product < 100 mL | ≤ 6000 particles per vial | ≤ 600 particles per vial |

USP <787> Subvisible Particulate Matter in Therapeutic Protein Injections
USP <1787> Measurement of Subvisible Particulate Matter in Therapeutic Protein Injections

Particles below 10 μm are potentially immunogenic and should be collected for characterization

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# Compendial requirements

## Chinese Pharmacopoeia requirements

**Sub-visible particles**

**pH**

**Osmolality**

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|---|---|---|
| Product ≥ 100 mL | ≤ 25 particles/mL | ≤ 3 particles/mL |
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USP <787> Subvisible Particulate Matter in Therapeutic Protein Injections
USP <1787> Measurement of Subvisible Particulate Matter in Therapeutic Protein Injections
Particles below 10 µm are potentially immunogenic and should be collected for characterization

1. Based on process development
   Target $\pm$ 0.X pH units or target $\pm$ XX mOsmol
2. The acceptance criterion is based on currently available release and stability data ranging from pH 5.1 to 5.4 with the addition of $\pm$0.2 pH units over the range obtained from the limited number of production batches and assay variability, i.e., 4.9 to 5.6

Meaning
- Correct recipe
- Patient tolerance requirements
- Product stability

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# Compendial requirements and experience with similar products

## Compendial requirements

- **Sterility: no growth**
- **Microbial limit: pay attention to packaging when submitting for inspection**
- **Endotoxin:** K = 5 EU/(kg.h)
- **Residual DNA: 10 ng/dose**

## Experience with similar products

- **Protein content: target $\pm$ 10%**
- **Polysorbate 80: target $\pm$ 50%**
- **HCP: ≤ 100 ng/mg (ppm)**
- **Protein A: ≤ 10–100 (ppm)**

# Report Outline

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# Statistical Methods Used and Batches Used

**(Min, Max)**

**Minimum and maximum values observed in all batches**

**The tolerance interval is the 95% confidence interval of the 99% population data within which the values of the product characteristic lie. Tolerance intervals are used to predict changing trends in long-term data.**

**Prediction Interval**

**Used for changing trends of stability**

**Reference Interval**

**Mean +/- k•SD (k is usually between 2–4)**

**Tolerance Interval**

**The tolerance interval contains at least the population at the specified proportion. It is a confidence interval that specifies the overall proportion (not the mean or standard deviation). The tolerance calculation interval results in a 95% confidence interval containing 99% of the future results.**

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# Statistical Methods Used and Batches Used

**(Min, Max)**
**Minimum and maximum values observed in all batches**

**Prediction Interval**
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**Tolerance Interval**
**The tolerance interval contains at least the population at the specified proportion. It is a confidence interval that specifies the overall proportion (not the mean or standard deviation). The tolerance calculation interval results in a 95% confidence interval containing 99% of the future results.**

➤ **Batches representative of the commercial process;**

➤ **Batches for clinical studies (partial end-of-shelf-life batches);**

➤ **Release & long-term stability data**

**Batches produced before major process changes should not be included**

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# Statistical Methods Used and Batches Used

**(Min, Max)**
**Minimum and maximum values observed in all batches**

**Prediction Interval**
**Used for changing trends of stability**

**Reference Interval**
**Mean +/- k•SD (k is usually between 2–4)**

**Tolerance Interval**
**The tolerance interval contains at least the population at the specified proportion. It is a confidence interval that specifies the overall proportion (not the mean or standard deviation). The tolerance calculation interval results in a 95% confidence interval containing 99% of the future results.**

**ICH Q5C:**
- **At least 3 representative final process batches.**
  - **1) Representative of clinical study batch quality**
  - **2) Representative of commercial process and storage conditions**
  - **3) Identical packaging materials**
- **Shelf-life ≤ 1 year: 0, 1, 2, 3, 6, 9, and 12 months**
  **Shelf-life > 1 year: 0, 3, 6, 9, 12, 18, 24, 36, 48......**
- **Tests:**
  **Potency, purity, appearance, visible particles, pH, osmolality, water content, etc.**

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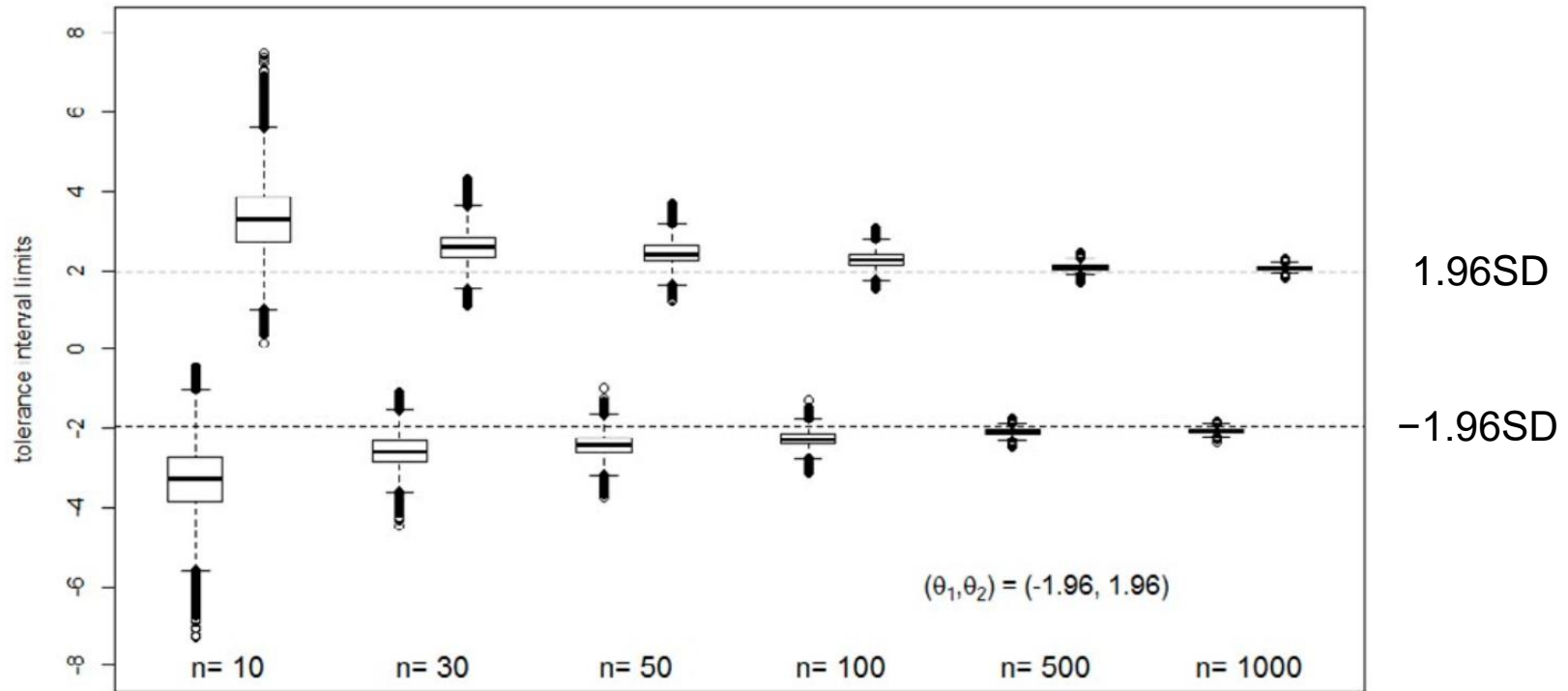
**Figure 4** Boxplot of the lower and upper bounds of specification using (97.5%, 95%) one-sided tolerance interval from $10^5$ Monte Carlo simulations on the standard normal distribution with a targeted interval of (−1.96, 1.96).
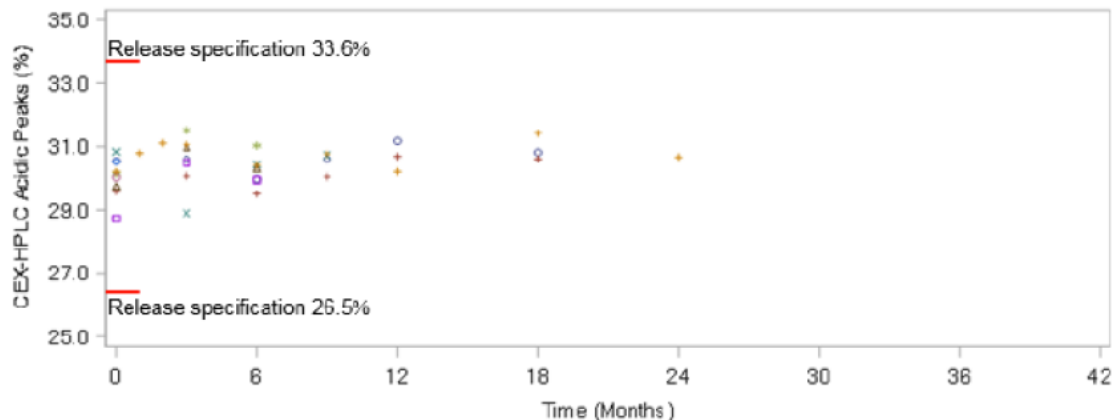
Batch release data of drug substance

| Main peak (%) | Acidic peak (%) | Basic peak (%) |
| --- | --- | --- |
| 57.3 | 30.2 | 12.5 |
| 57.0 | 30.5 | 12.5 |
| 57.6 | 29.6 | 12.9 |
| 57.8 | 30.8 | 11.4 |
| 58.6 | 29.7 | 11.7 |
| 58.2 | 28.7 | 13.1 |
| 58.1 | 30.1 | 11.8 |
| 57.6 | 30.5 | 11.9 |
| 56.6 | 32.0 | 11.3 |



Min-Max (N = 9): 29.6–32.0%

1. 95%/99 TI (N = 9): 27.1%–33.0%

Intermediate precision: 0.6%

2. Release specification: 26.5–33.6
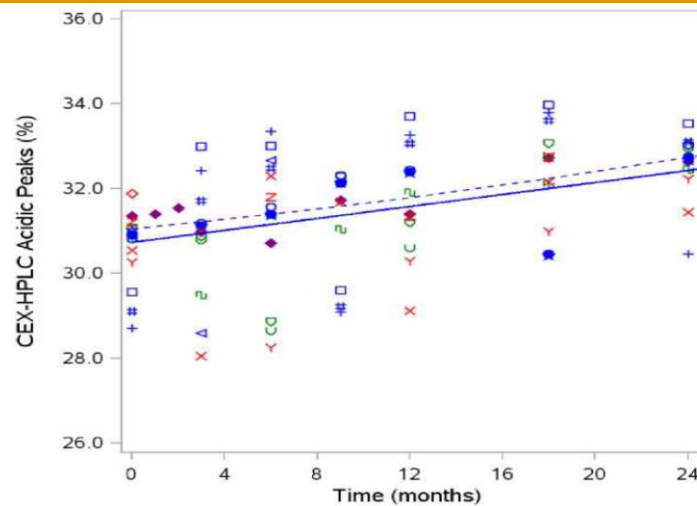
Stability specifications = release specifications

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## Calculation 2:

Stability variation: 0.0889%/month (mean)

Stability specification: ≤ 35.7

(33.6 + 2.1 = 35.7%)

## Calculations 3:

Stability variation: 0.1132%/month

(95%/99 TI upper limit)

Stability specification: ≤ 36.3

(33.6 + 2.7 = 36.3%)

## Calculation 1:

Release specification: 26.5%–33.6% (same as drug substance)

(1-1) 95%/99 TI at 2-year time point (N = 9): 30.5%–34.7%

Intermediate precision: 0.6%

(1-2) Stability specification: ≤ 35.3%

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# Establishment of active substance specifications

### Batch release data

Relative potency (%)

| 102 |
| 87 |
| 87 |
| 99 |
| 124 |
| 92 |
| 96 |
| 101 |
| 101 |

| Sample | Results obtained by analyst (CV%) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 40% simulated potency | 8 | 5 | 16 | 9 | 15 | 11 |
| 60% simulated potency | 11 | 7 | 12 | 7 | 8 | 9 |
| 80% simulated potency | 5 | 9 | 12 | 6 | 8 | 15 |
| 100% simulated potency | 3 | 8 | 6 | 11 | 9 | 11 |
| 130% simulated potency | 9 | 1 | 7 | 6 | 11 | 10 |
| 160% simulated potency | 10 | 3 | 8 | 7 | 13 | 11 |
| DS | 8 | 9 | 10 | 12 | 13 | 17 |
| Acceptance criterion | CV% ≤ 20% | | | | | |

## 95%/99 TI: 47%–151%

## Unacceptable!

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# Establishment of active substance specifications
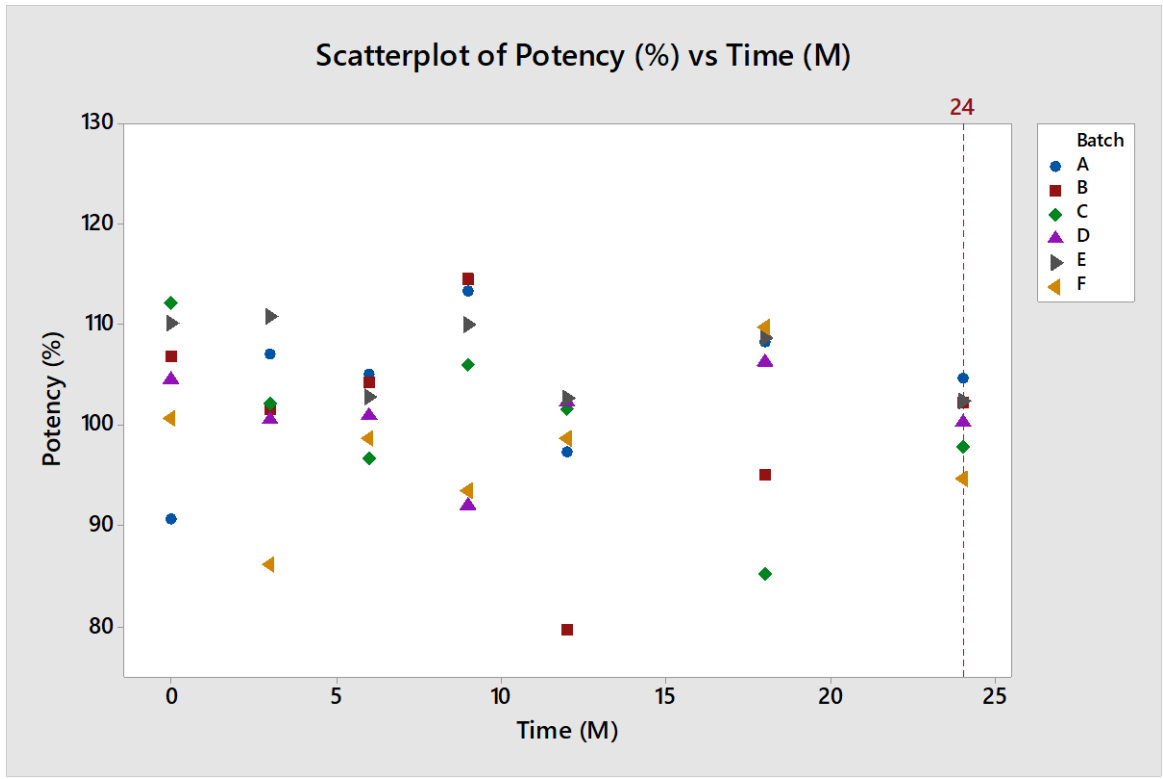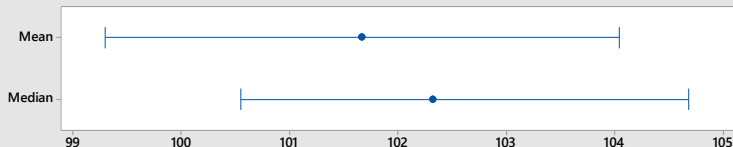
# Establishment of active substance specifications



Summary Report for Potency (%)
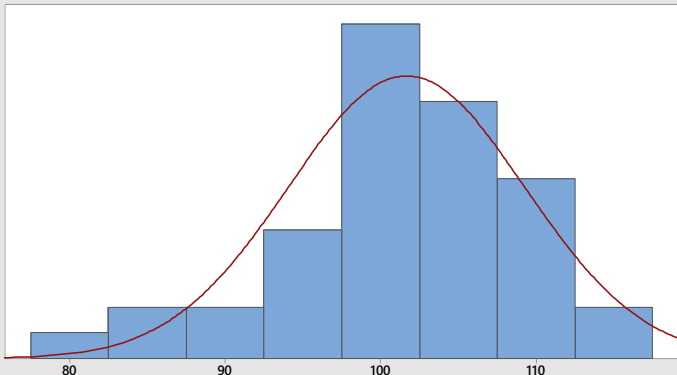
Anderson-Darling Normality Test

| | |
|---|---|
| A-Squared | 0.49 |
| P-Value | 0.208 |
| Mean | 101.67 |
| StDev | 7.62 |
| Variance | 58.03 |
| Skewness | -0.796881 |
| Kurtosis | 0.822304 |
| N | 42 |
| Minimum | 79.68 |
| 1st Quartile | 97.74 |
| Median | 102.33 |
| 3rd Quartile | 106.94 |
| Maximum | 114.55 |

95% Confidence Interval for Mean
99.30    104.05

95% Confidence Interval for Median
100.55    104.69

95% Confidence Interval for StDev
6.27    9.71

95% Confidence Intervals

- **Lower limit = Mean − 3SD = 101.67 − 3 $\times$ 7.62 = 78.81 (%)**

- **Upper limit = Mean + 3SD = 101.67 + 3 $\times$ 7.62 = 124.53 (%)**

- **Proposed specifications: 75%–125%**

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# Introduction of Process Capability Concepts

$$C_{pk} = \min\left(\frac{URL - \bar{x}}{3s}, \frac{\bar{x} - LRL}{3s}\right)$$

Cpk = $\dfrac{\text{Distance from Mean to Nearest Spec Limit}}{\text{Distance from Mean to Process Edge}}$

Mean to Process Edge (Spread)

Mean to Nearest Spec Limit (Location)

Mean

LSL          USL
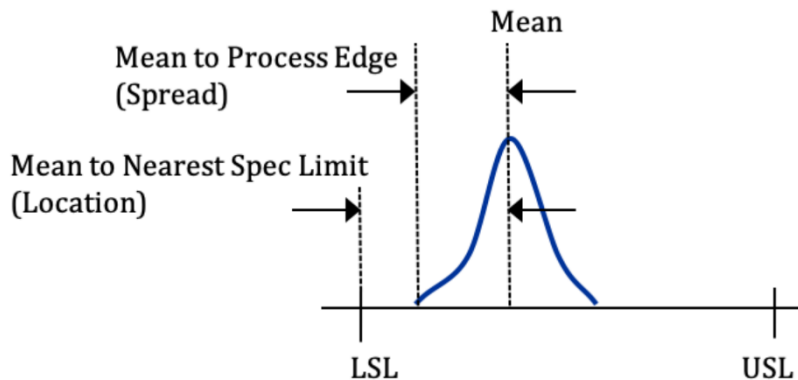
**Cpk** accounts for both the spread and location of the process.

$C_{pk} = 1$

LRL          URL

$C_{pk} < 1$

LRL          URL

$C_{pk} < 1$

LRL          URL

$C_{pk} > 1$

LRL          URL

**SPEC**

Cpk < 1.0
Not Capable

Cpk > 1.33
Capable

**Process**

N
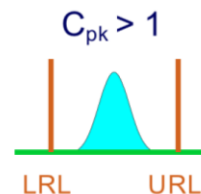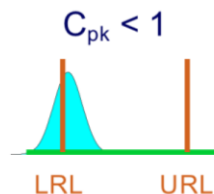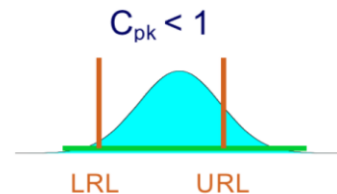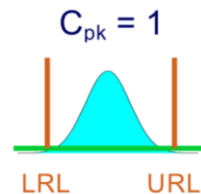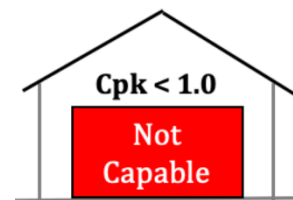
# Introduction of Process Capability Concepts



Process Capability Report for Potency (%)

| Process Data | |
|---|---|
| LSL | 75 |
| Target | * |
| USL | 125 |
| Sample Mean | 100 |
| Sample N | 42 |
| StDev(Overall) | 7.61773 |
| StDev(Within) | 7.53146 |

| Overall Capability | |
|---|---|
| Pp | 1.09 |
| PPL | 1.09 |
| PPU | 1.09 |
| Ppk | 1.09 |
| Cpm | * |

| Potential (Within) Capability | |
|---|---|
| Cp | 1.11 |
| CPL | 1.11 |
| CPU | 1.11 |
| Cpk | 1.11 |

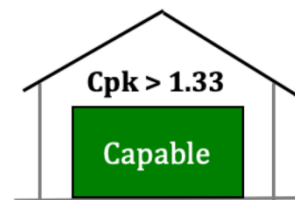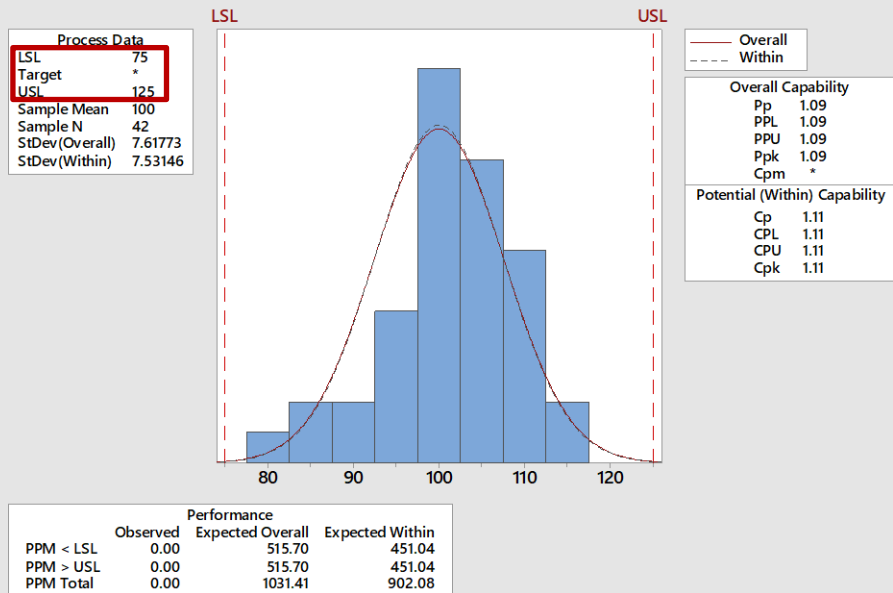| Performance | Observed | Expected Overall | Expected Within |
|---|---|---|---|
| PPM < LSL | 0.00 | 515.70 | 451.04 |
| PPM > USL | 0.00 | 515.70 | 451.04 |
| PPM Total | 0.00 | 1031.41 | 902.08 |

Failure rate: 0.1%

Process Capability Report for Potency (%)

| Process Data | |
|---|---|
| LSL | 70 |
| Target | * |
| USL | 135 |
| Sample Mean | 101.672 |
| Sample N | 42 |
| StDev(Overall) | 7.61773 |
| StDev(Within) | 7.53146 |

| Overall Capability | |
|---|---|
| Pp | 1.42 |
| PPL | 1.39 |
| PPU | 1.46 |
| Ppk | 1.39 |
| Cpm | * |

| Potential (Within) Capability | |
|---|---|
| Cp | 1.44 |
| CPL | 1.40 |
| CPU | 1.48 |
| Cpk | 1.40 |

| Performance | Observed | Expected Overall | Expected Within |
|---|---|---|---|
| PPM < LSL | 0.00 | 16.08 | 13.04 |
| PPM > USL | 0.00 | 6.07 | 4.82 |
| PPM Total | 0.00 | 22.15 | 17.86 |
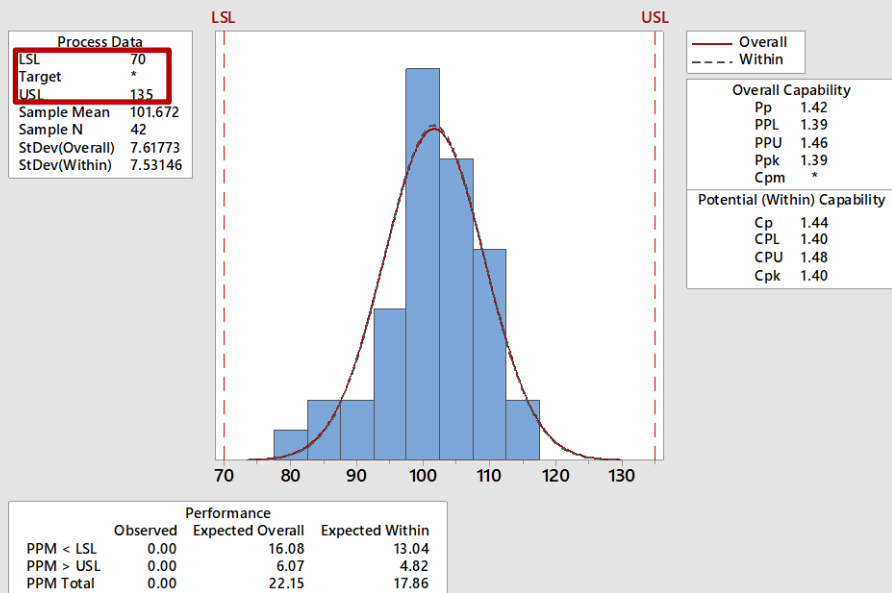
Failure rate: 0.002%

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Generally, not recognized by regulatory authorities

## White Paper: FDA Pharmaceutical Quality Oversight

drug products fit for the intended use. Clinically relevant specifications (CRSs) identify and reject drug product batches that are likely to perform inadequately in the indicated patient populations. Important goals include the establishment of acceptance criteria (e.g., for impurities) and dissolution parameters based on clinical relevance, instead of process capability or manufacturing process control.



**E**stablished clinical range and/or justified by *in-vitro* studies

**A**cceptance criteria wider than the limits calculated statistically is justified when there is a low risk of impact to patient safety and efficacy
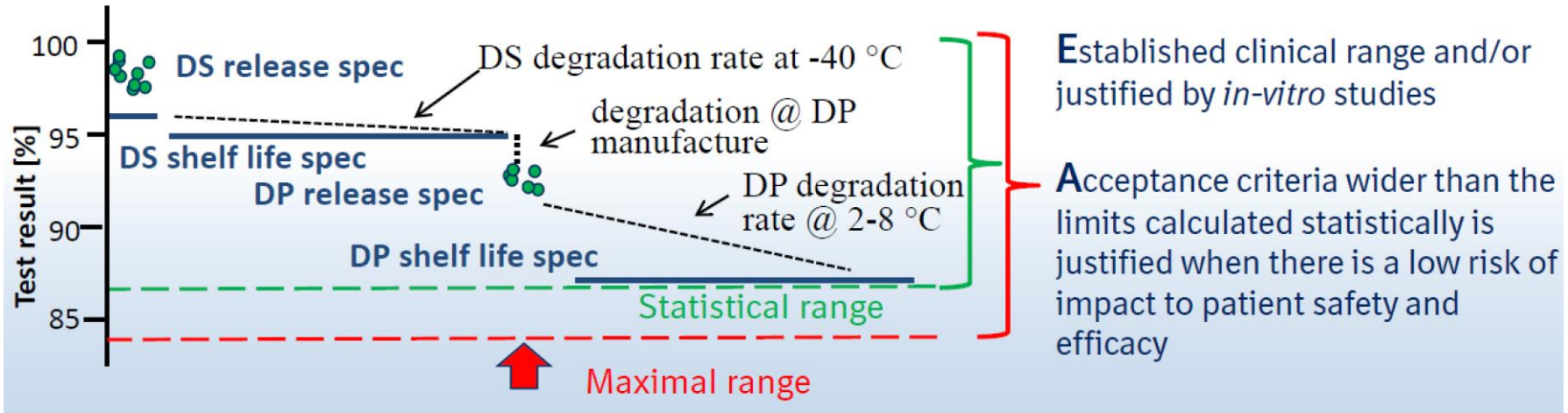
Advantage: Patient-centric specifications enable appropriate control over higher risk CQAs to ensure product quality for the patient, and flexibility for lower risk PQAs for a sustainable supply chain.

# Patient-Centered Specification Setting

## ICH Q6B

- **Linked to Manufacturing Process**
- **Account for drug substance and drug product stability**
- **Linked to preclinical and clinical studies**
- **Linked to analytical procedures**

> **Clinical range**
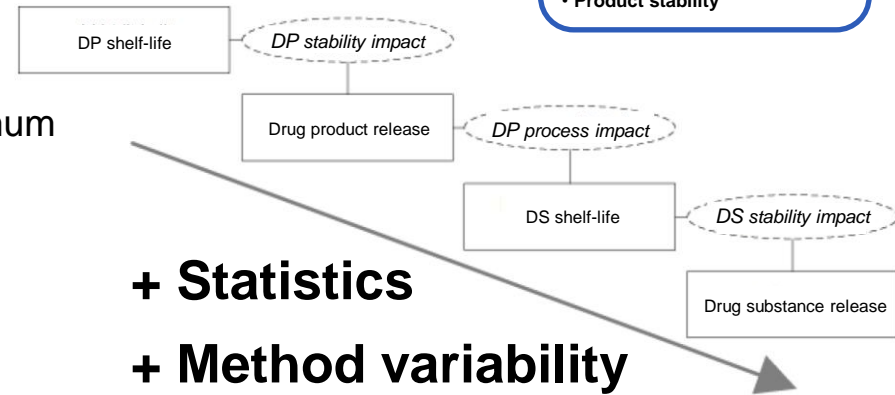>
> **Manufacturing range**
>
> **Specification range**

- Safety: toxicology + clinical dose correlation and maximum exposure
- Immunogenicity: clinical data + clinical dose correlation and maximum exposure
- Pharmacokinetics: FcRn binding
- Efficacy: Structure-function relationship

**Product understanding**

- Literature knowledge
- Experience with similar products
- Characterization studies
- Structure-function relationship
- Product stability

DP shelf-life — *DP stability impact*

Drug product release — *DP process impact*

DS shelf-life — *DS stability impact*

Drug substance release

**+ Statistics**

**+ Method variability**

# Report Outline

**1** Overview

**2** Compendial and regulatory requirements and experience with similar products

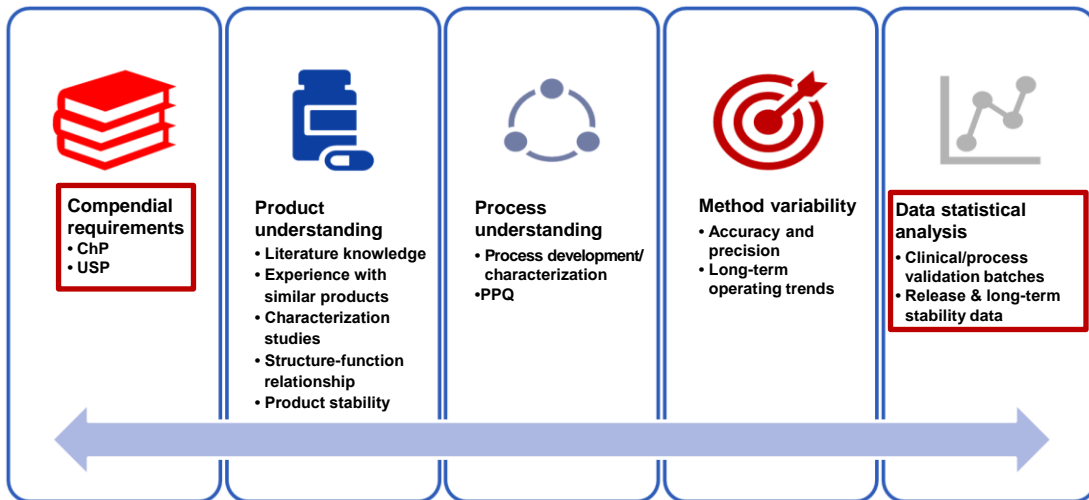**3** Examples of specification setting for purity and potency

**4** Summary

National Institutes for Food and Drug Control

# Summary

**Compendial requirements**
• ChP
• USP

**Product understanding**
• Literature knowledge
• Experience with similar products
• Characterization studies
• Structure-function relationship
• Product stability

**Process understanding**
• Process development/ characterization
• PPQ

**Method variability**
• Accuracy and precision
• Long-term operating trends

**Data statistical analysis**
• Clinical/process validation batches
• Release & long-term stability data

- **Strategies vary from company to company but should be scientific and logical.**
- **Competition and negotiattion between the sponsor and regulatory authorities**

## Patient-Centered Specification Setting