CMC Regulatory Experiences and Expectations for Peptides

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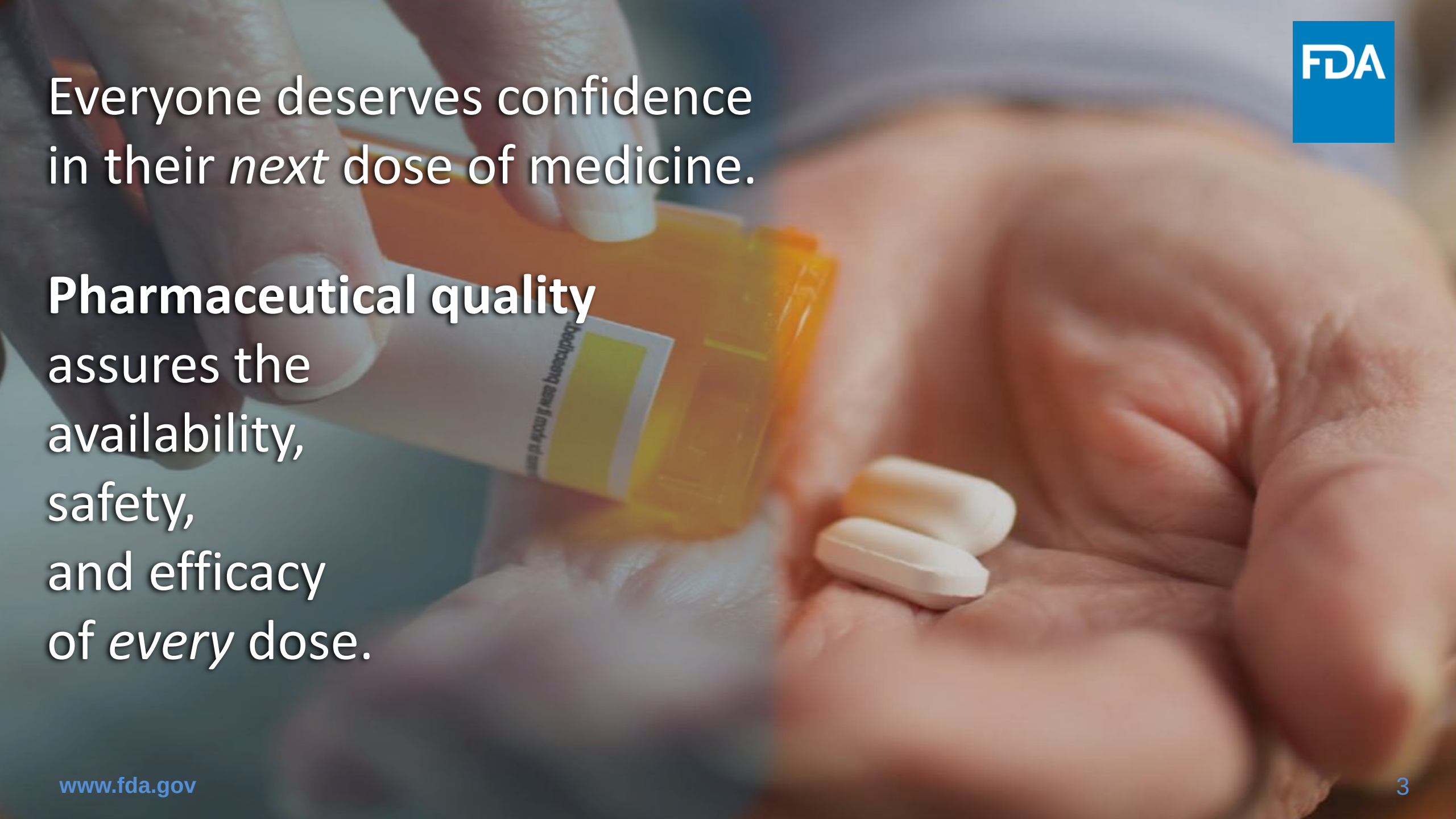
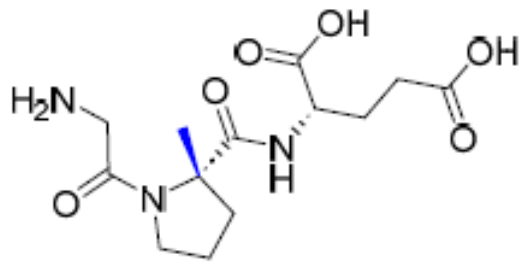


Everyone deserves confidence in their *next* dose of medicine.

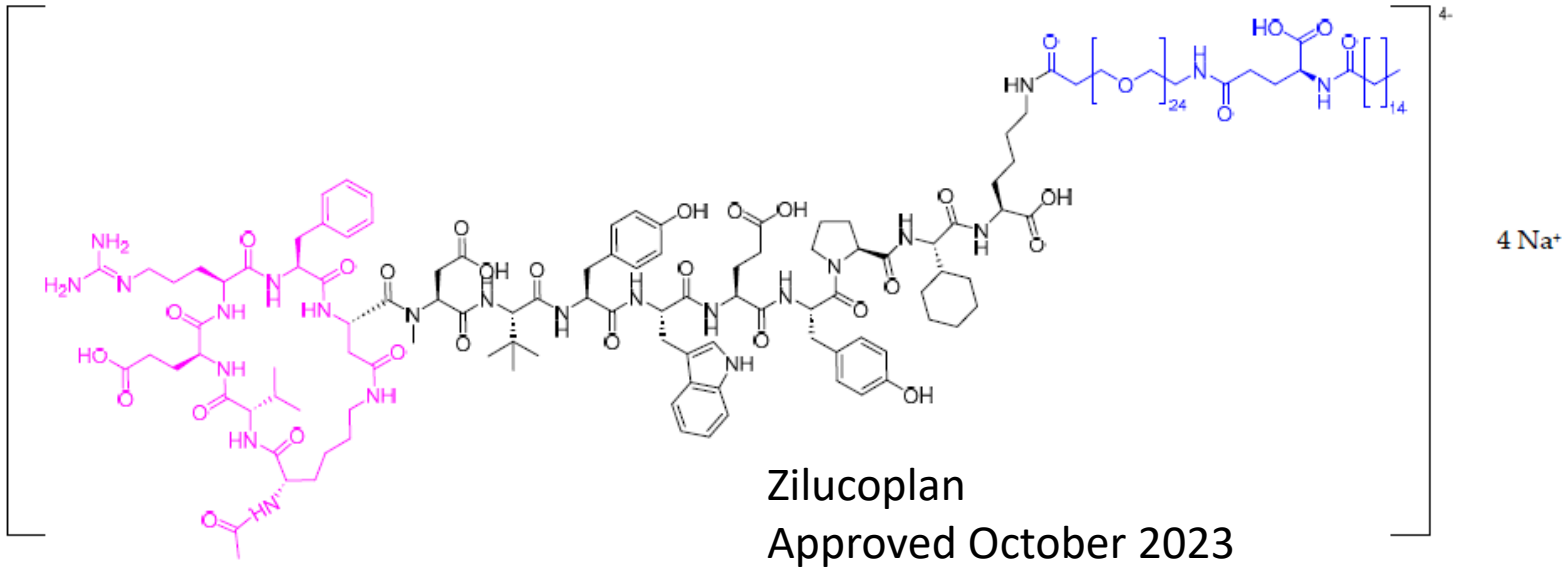
Pharmaceutical quality assures the availability, safety, and efficacy of *every* dose.

Peptide Therapeutics

- Peptides make up approximately 9% (31/370) of the new drugs approved by the FDA between 2016–2023[1]
- The global peptide therapeutics market was estimated at $43.45 billion in 2023[2]



Trofinetide
Approved March 2023
Administration: Oral



Zilucoplan
Approved October 2023
Administration: Subcutaneous

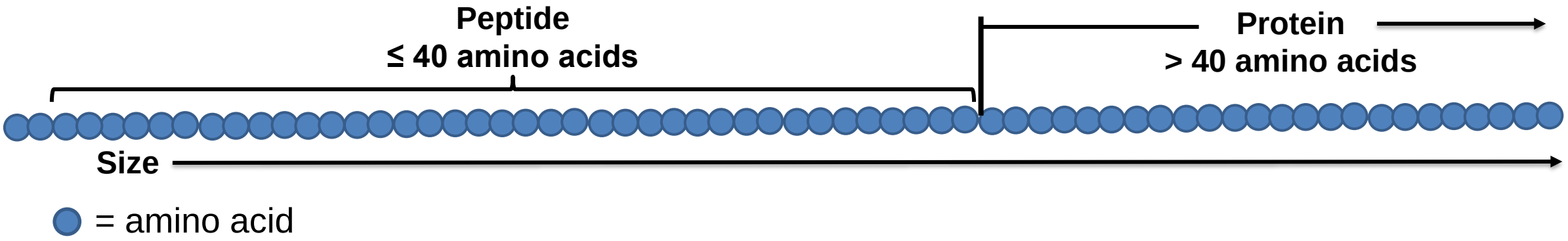
(1) "2023 FDA TIDES (Peptides and Oligonucleotides) Harvest" *Pharmaceuticals,* **2024:** doi: 10.3390/ph17020243
(2) Peptide Therapeutics Market Size and Share [2023 Report]. https://www.grandviewresearch.com/industryanalysis/peptide-therapeutics-market (accessed on 18 March 2024).

What is a Peptide?

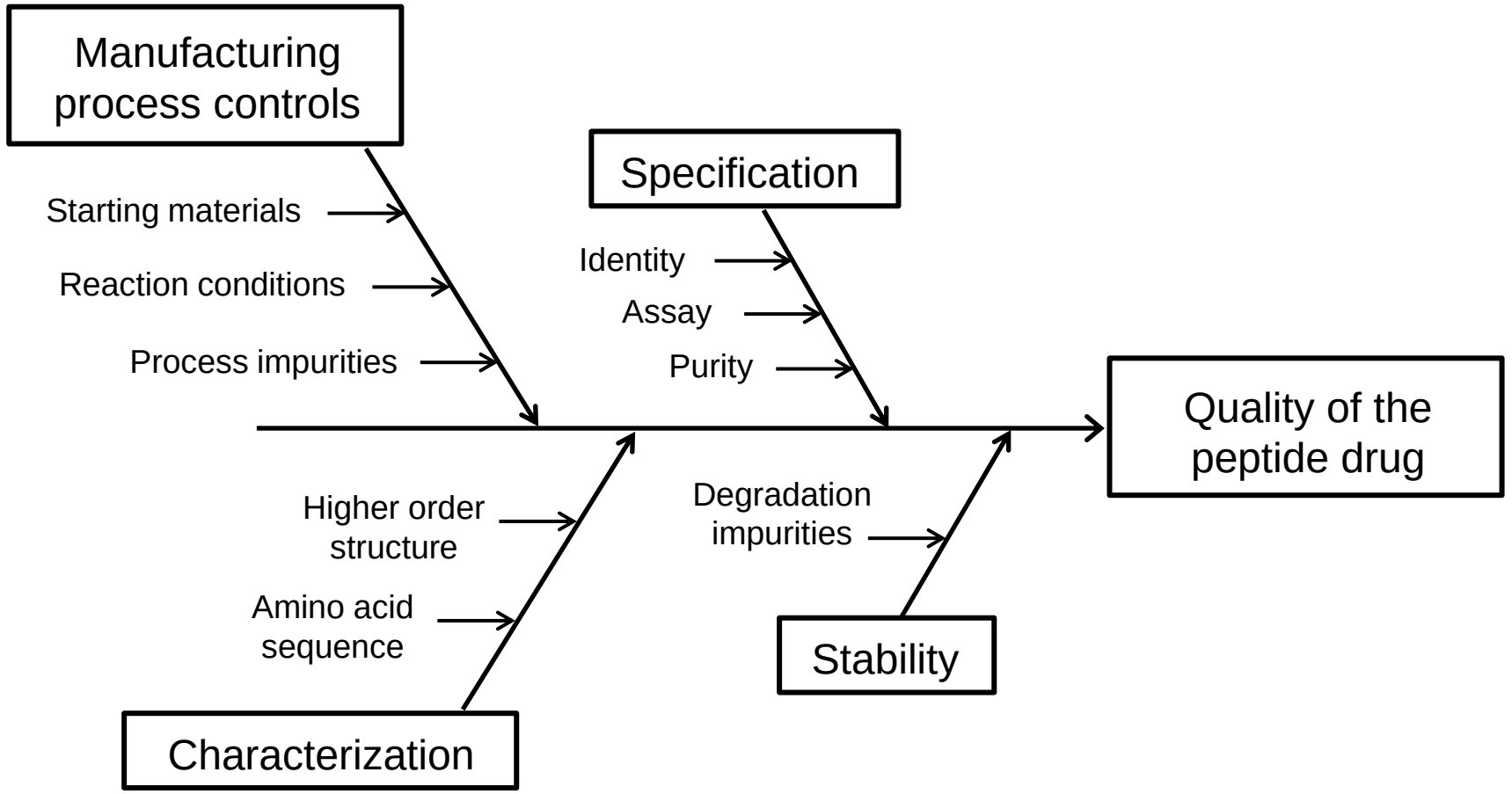
Protein defined in the FDA Final Rule "Definition of the Term 'Biological Product'" (85 FR 10057 March 23, 2020):

> *"the term **protein** would mean any alpha amino acid polymer with a specific defined sequence that is **greater than 40 amino acids in size**…"*

Peptide: any polymer composed of 40 or fewer amino acids



Elements of a Control Strategy



Adapted from Wu, L.C., et al. International Journal of Pharmaceutics 518.1-2 (2017): 320-334.

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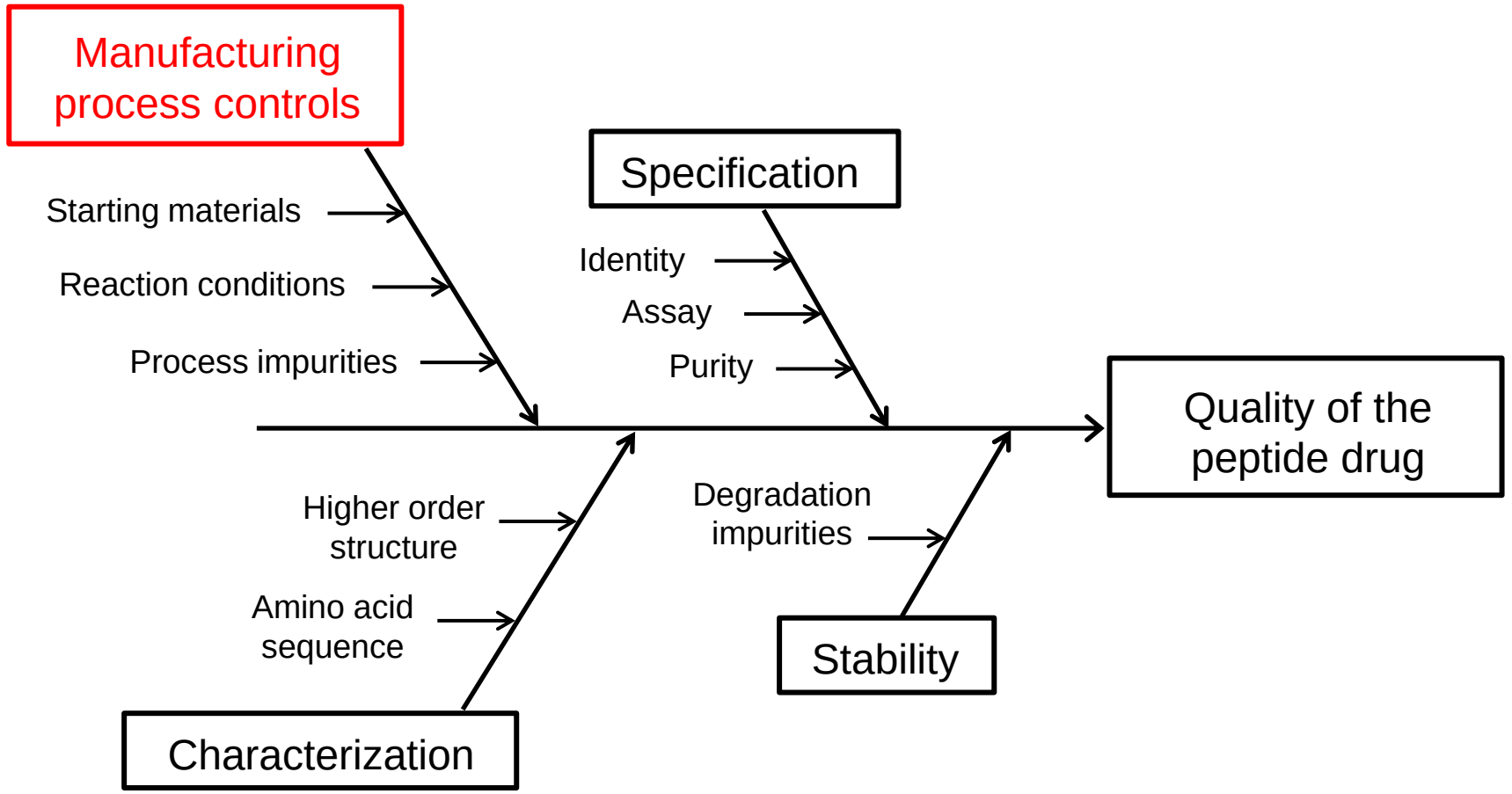

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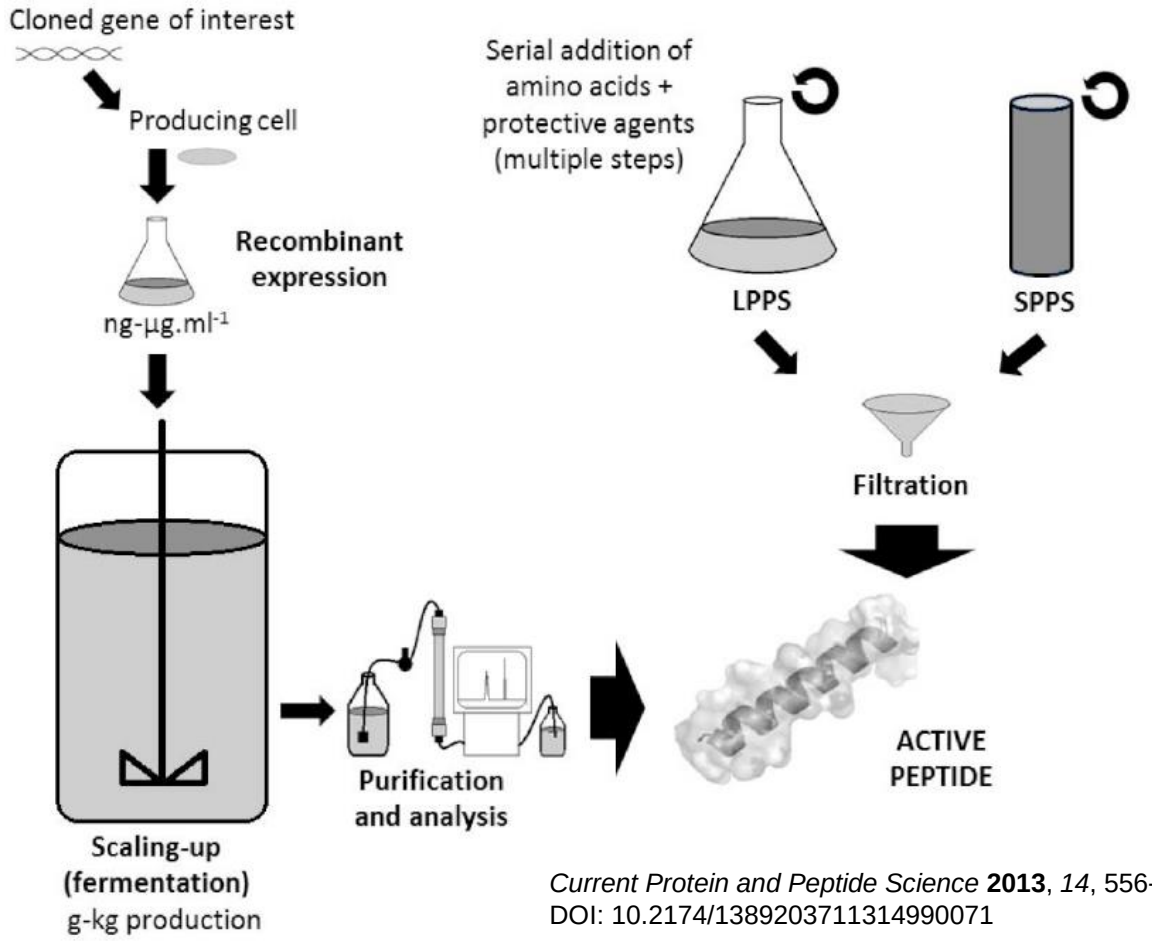
Peptide Manufacturing Approaches

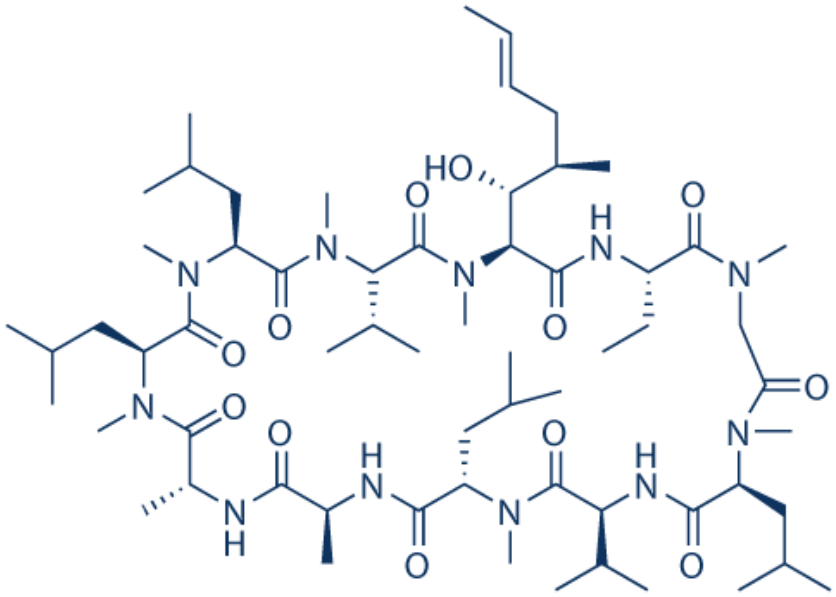
- Recombinant DNA technology
- Extraction from natural sources, including fermentation products
- Chemical synthesis



Current Protein and Peptide Science **2013**, *14*, 556-567.
DOI: 10.2174/1389203711314990071

Biologically-Derived Peptides

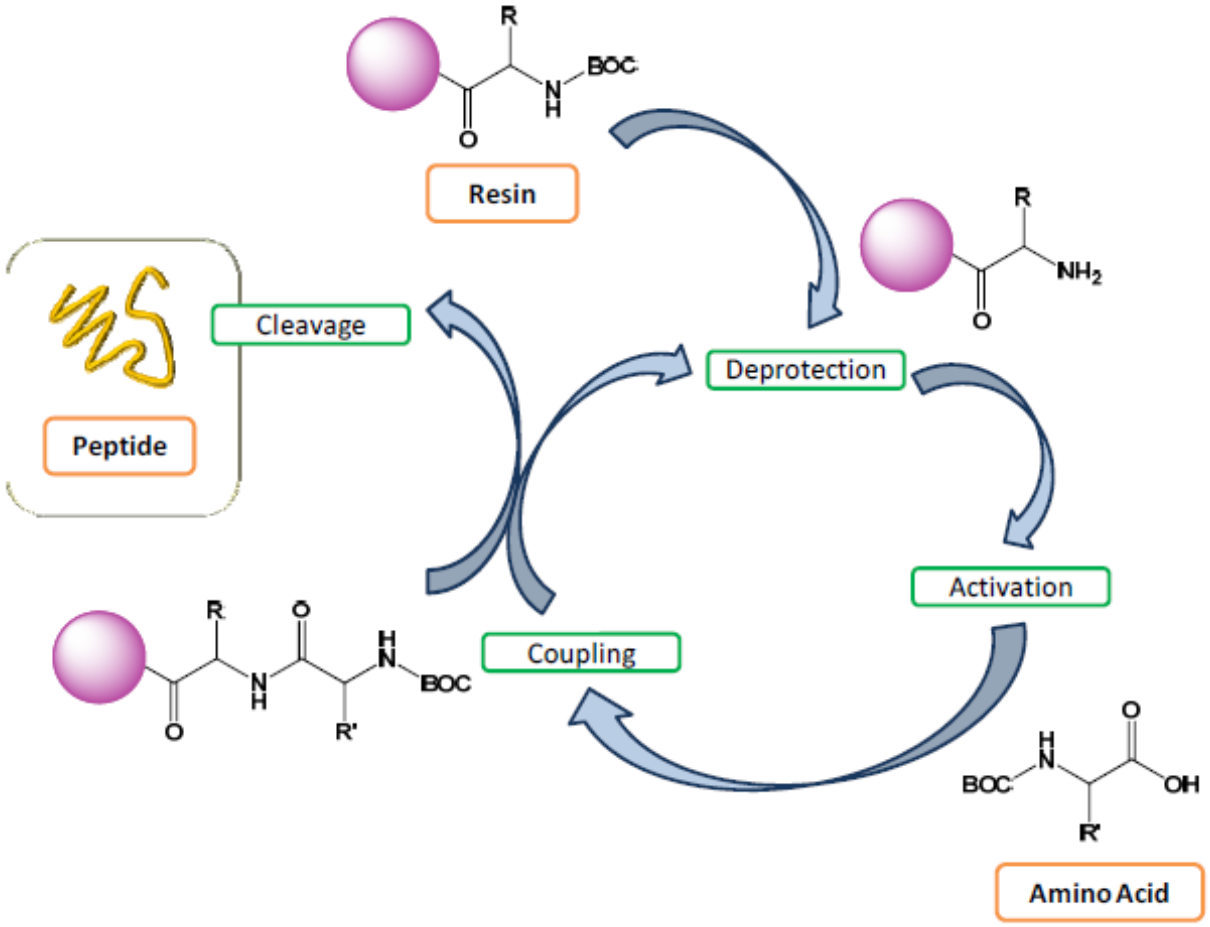
- Examples: Glucagon, calcitonin, cyclosporin A (fermentation product)
- Starting materials: Cell banks
  - Master cell banks
  - Working cell banks
- Process impurities can include host cell proteins, host cell DNA, media residue, processing reagents, etc.
- Refer to ICH Q5A, Q5B, and Q5D for regulatory considerations



Cyclosporin A: Fermentation product from Tolypocladium inflatum (aerobic fungi)

Synthetically-Derived Peptides: Solid-Phase Peptide Synthesis

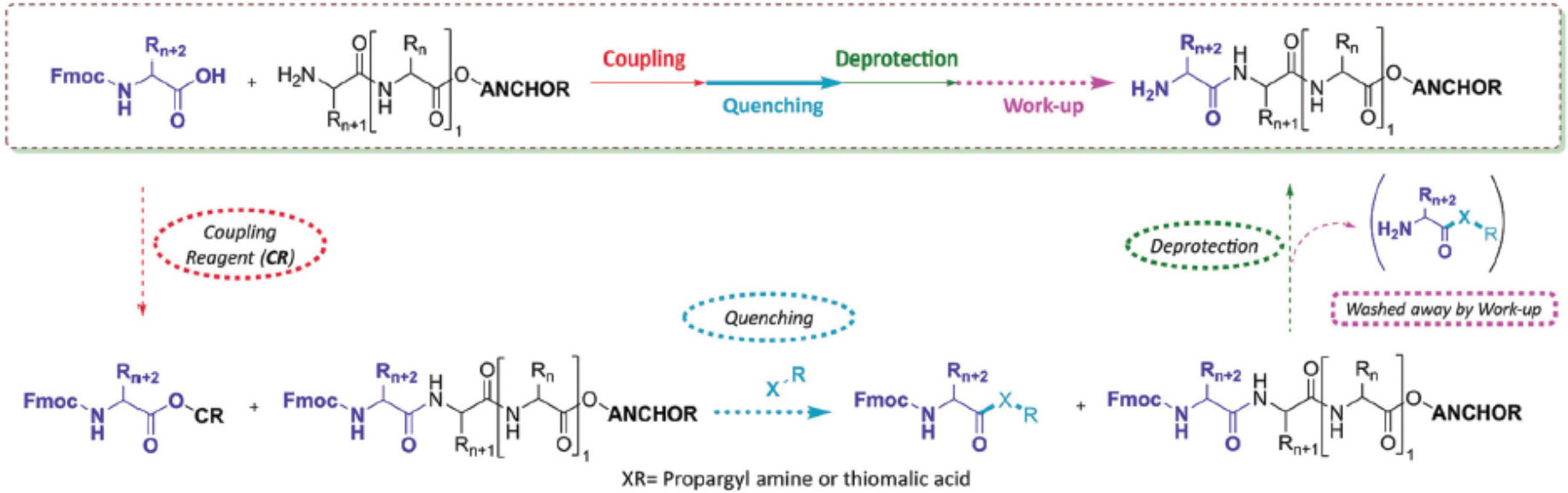
- Chemistry developed in the 1960s
- Most common approach
- Process-related impurities may include residual solvents, reagents, and elemental impurities



Polymers **2014**, *6*, 515-551. DOI: 10.3390/polym6020515

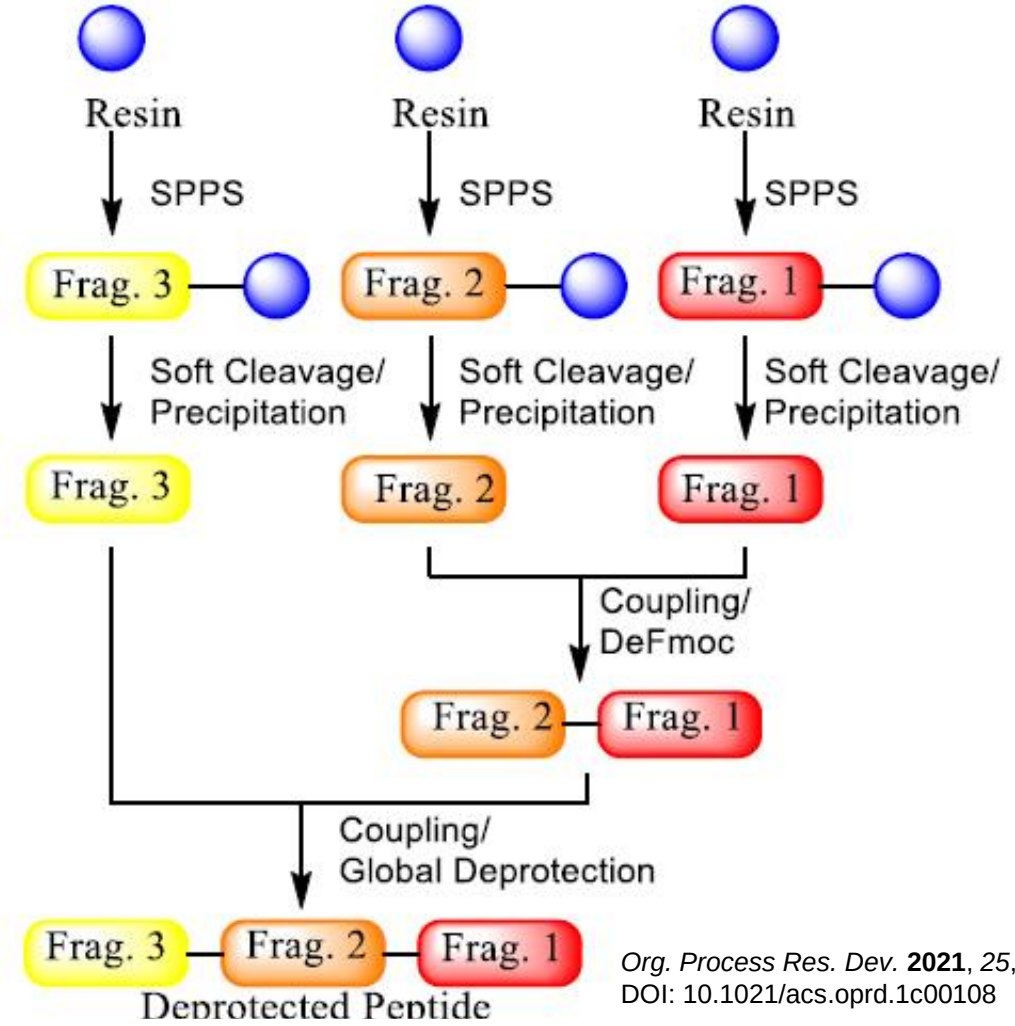
Synthetically-Derived Peptides: Liquid-Phase Peptide Synthesis

- Less common approach
- Works well for smaller peptides
- Typically slower than SPPS



Synthetically-Derived Peptides: Hybrid SPPS/LPPS Process

- Shorter peptide fragments manufactured through SPPS
- Fragments coupled in the liquid phase

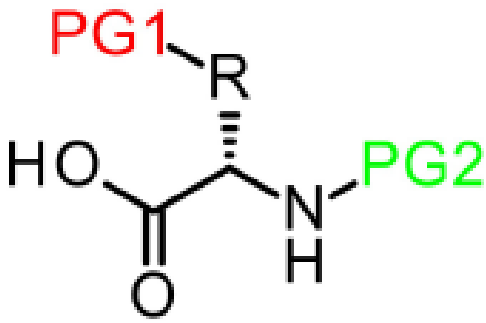


Org. Process Res. Dev. **2021**, *25*, 1628-1636. DOI: 10.1021/acs.oprd.1c00108

Enfuvirtide: Nat Rev Drug Discov. **2023** *2*, 587-593. DOI: 10.1038/nrd1133

Synthetically-Derived Peptides: Starting Materials

- Starting materials may include:
  - Protected amino acids
  - Solid supports (resins)
  - Any covalently linked moiety (e.g., lipid, fatty acid, polyethylene glycol)



AA derivative with orthogonal protection groups (PG)

Synthetically-Derived Peptides: Protected Amino Acid Starting Materials

- Protected amino acid specifications may include tests for
  - Identification
  - Purity
  - Related substance impurities (e.g., partially unprotected amino acids, β-alanine impurities, dipeptides)
  - Chiral purity (enantiomer content)
  - Assay

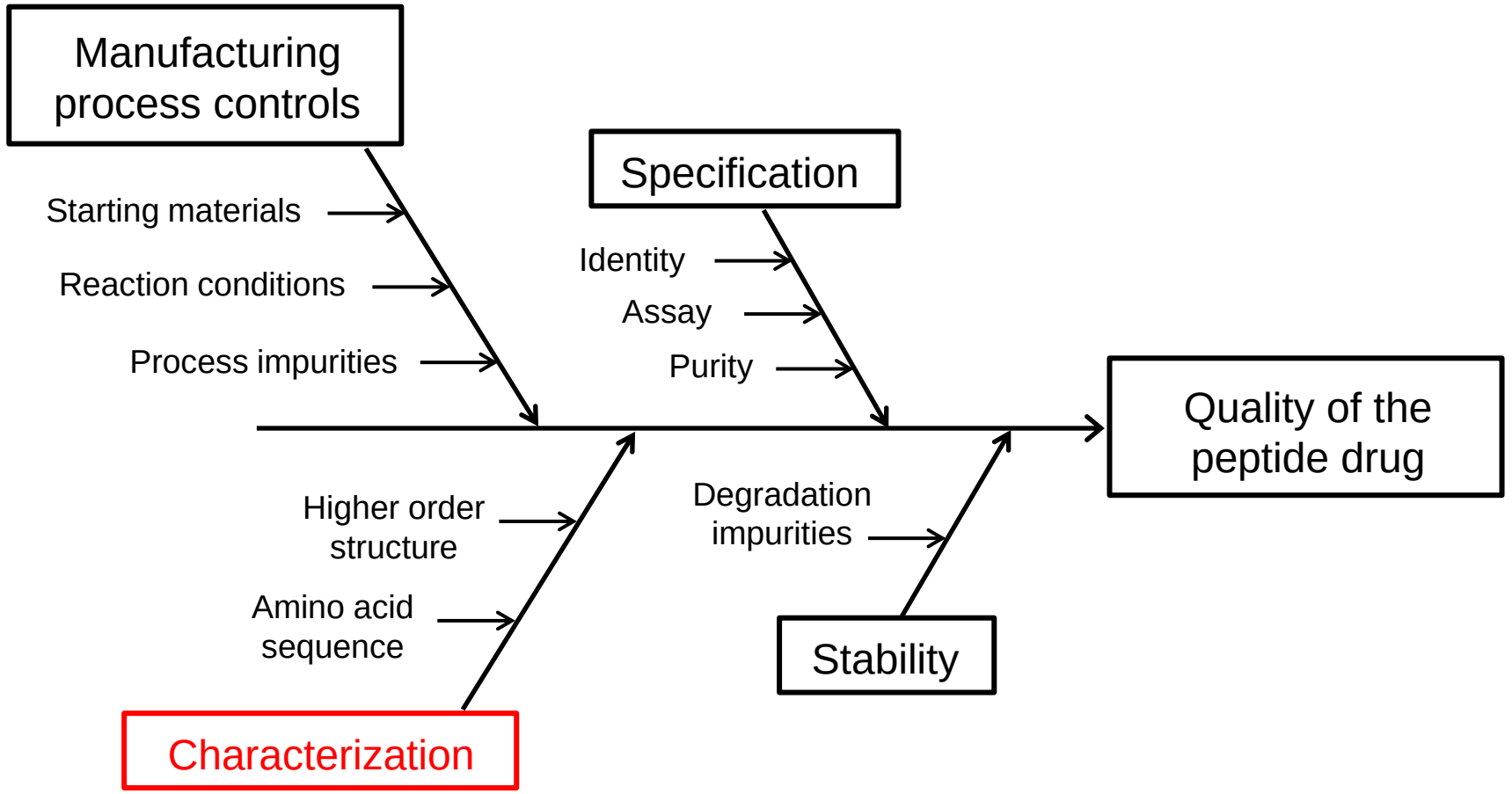
Synthetically-Derived Peptides: Starting Materials with Additional Considerations

- Additional information (e.g., synthesis information, characterization, chromatographic behavior) may be needed for:
  - D-amino acids
  - Non-proteinogenic amino acids
  - Custom-synthesized moieties for conjugation
- Refer to USP chapter <1504> Quality Attributes of Starting Materials for the Chemical Synthesis of Therapeutic Peptides

Manufacturing Process Considerations

- Follow ICH Q7: Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients.
- Recommendations for regulatory submissions
  - Describe the manufacturing process
    - Operating ranges for relevant process parameters
    - Reaction times, quantities, conditions, purification processes
  - Describe changes to synthesis/process through development
  - Consider meeting with the Agency to discuss regulatory starting materials

Elements of a Control Strategy



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Characterization: Standard Tests

Characteristic	Analytical Method
Sequence/Structure confirmation	MS, MS/MS NMR Amino acid analysis (AAA) Peptide mapping
Enantiomeric purity	Chiral AAA
Counter ion identity/content	RP-HPLC, ion chromatography

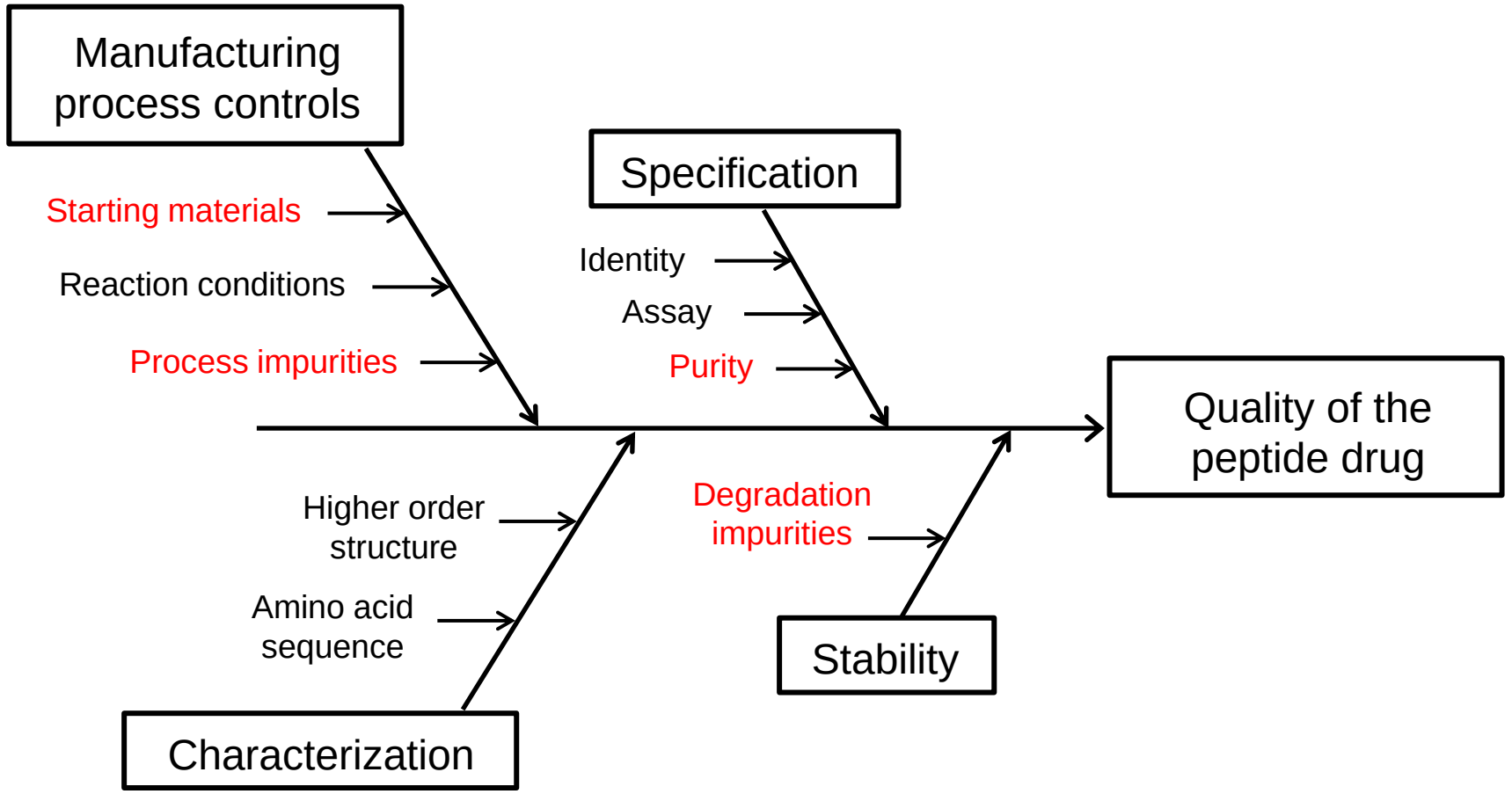
Characterization: Additional Considerations

Characteristic	Analytical Method
Higher order structure	Circular dichroism FT-IR NMR
Oligomers/ Aggregation	Size Exclusion Chromatography (SEC) Dynamic Light Scattering Gel electrophoresis
Biological Activity	Cell-based and other biological assays

Characterization: Risk of Immunogenicity

- Depends on the source, sequence, size, route of administration, and mechanism of action of the drug substance.
- Refer to FDA guidance “Immunogenicity assessment of therapeutic protein products” (Final 2015): principles apply to peptides

Impurities



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Types of Impurities

- Diastereomers
- Substitution
- Insertion

- Diastereomers
- Deletion
- Insertion
- Truncation
- Functional group modifications
- Disulfide modifications

- Impurity purging

- Disulfide modifications

- Functional group modifications
- Disulfide modifications

- Resolution of impurities may be challenging
  - Consider using orthogonal methods
  - Methods should be appropriately validated

Impurity Thresholds

	ICH Q3A [1] (MDD ≤ 2 g)	Ph.Eur.2034: Limits for Synthetic Peptides	Certain Generic Peptides[2]
Reporting Threshold	0.05%	0.1%	N/A
Identification Threshold	0.10% (or 1.0 mg/day)	0.5%	0.10%
Qualification Threshold	0.15% (or 1.0 mg/day)	1.0%	0.5%

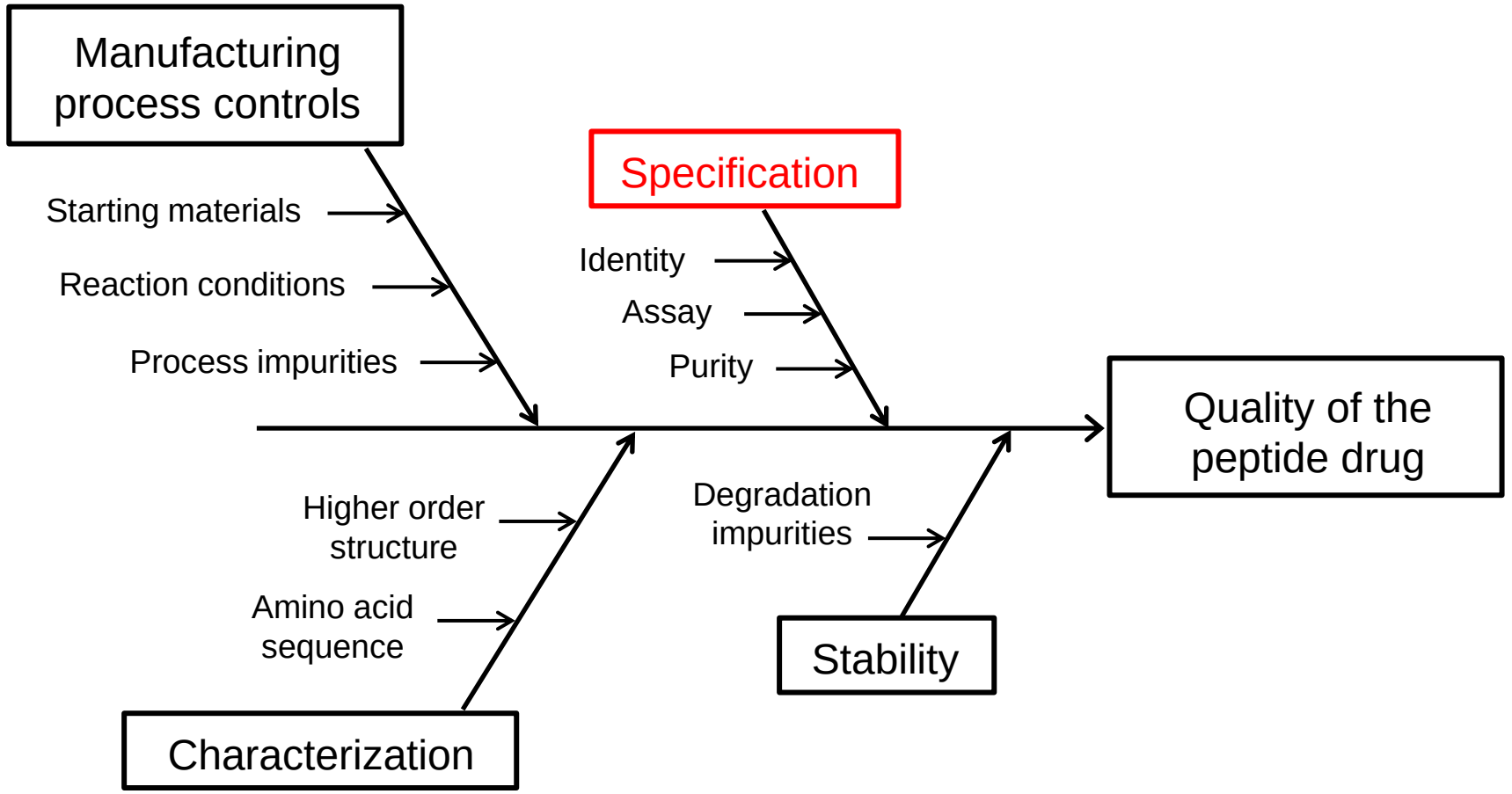
(1) Specifically excludes peptides

(2) See "ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin Guidance for Industry." Refers to 5 peptide drug products: glucagon, liraglutide, nesiritide, teriparatide, and teduglutide.

Impurity Controls

- Proposed reporting, identification, and qualification thresholds for impurities are evaluated on a case-by-case basis
- Considerations:
  - Principles of ICH Q3A(R2) and ICH Q3B(R2)
  - Provided batch data and stability data
  - Provided toxicology data

Elements of a Control Strategy



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Representative Specification

Test
Appearance
Solubility
Identification
Assay (of total mass) - HPLC
Assay (anhydrous and counter ion free substance) - Calculation
Purity Related substance impurities Residual solvents Elemental impurities
Water content
Counter ion content
Bacterial endotoxins
Microbial limits

Refer to USP chapter <1503> Quality Attributes of Synthetic Peptide Drug Substances

Specification: Identity Tests

ICH Topic Q 6 A
Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances

of closely related structure which are likely to be present. Identity tests should be specific for the new drug substance, e.g., infrared spectroscopy. Identification solely by a single chromatographic retention time, for example, is not regarded as being specific. However, the use of two chromatographic procedures, where the separation is based on different principles, or combination of tests into a single procedure, such as HPLC/UV diode array, HPLC/MS, or GC/MS, is generally acceptable.

- Generally, a combination of methods is used for peptide identification testing:
  - Mass (MS)
  - RRT (HPLC)
  - AAA
  - NMR
  - LC-MS/MS
  - Peptide mapping

Specification: Purity/Impurities

In summary, the new drug substance specification should include, where applicable, the following list of impurities:

Organic Impurities

- Each specified identified impurity
- Each specified unidentified impurity
- Any unspecified impurity with an acceptance criterion of not more than (≤) the identification threshold
- Total impurities

Residual Solvents

Inorganic Impurities

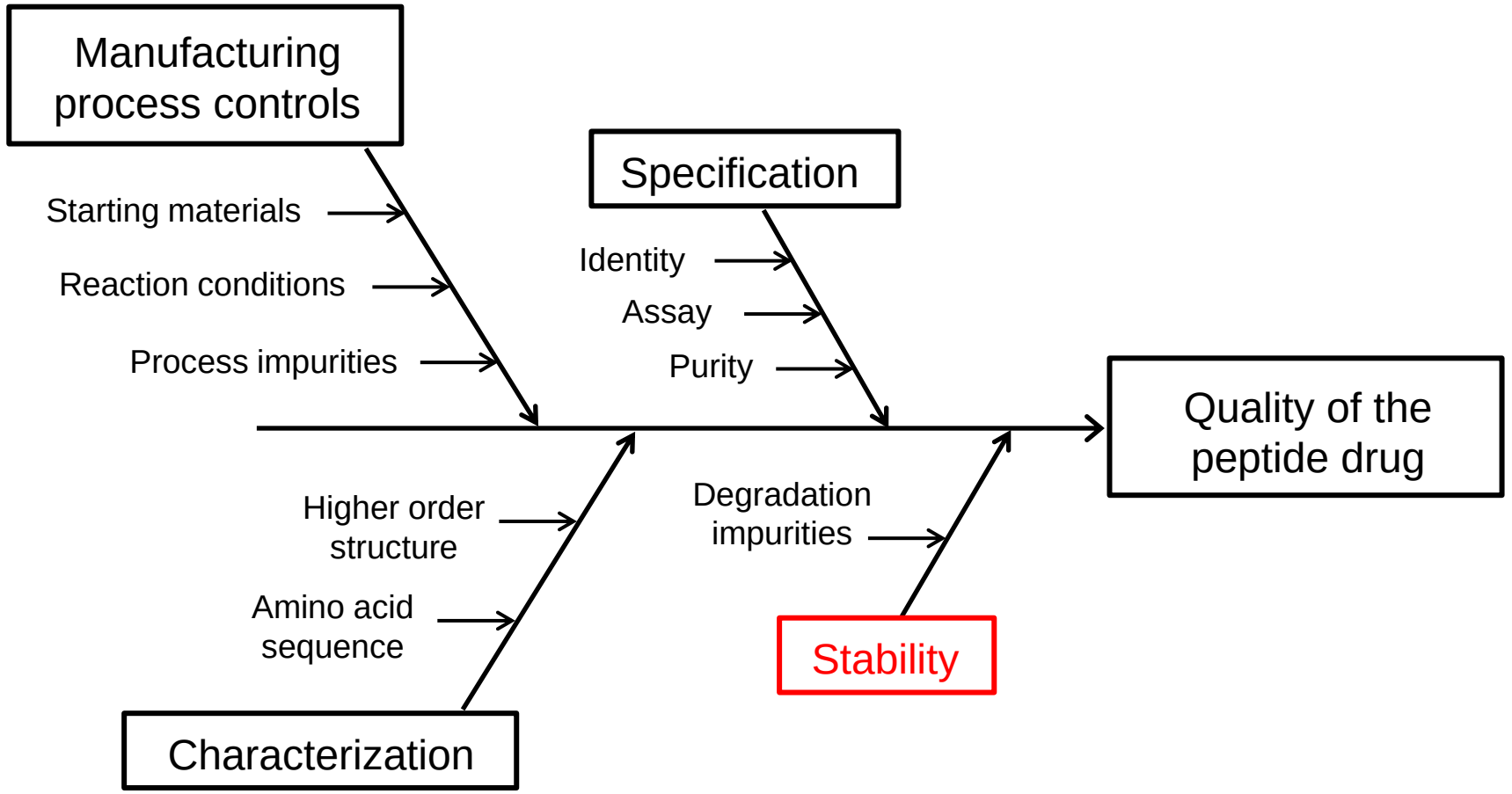
Guidance for Industry

Q3A Impurities in New Drug Substances

Specification: Assay Test

- A specific stability-indicating test that can determine the content of the new drug substance
  - A weight-based assay against a reference standard of known purity
  - Calculation of mass balance (purity, water, counter ion)
- Bioassay/potency typically not required for peptides
  - For complex peptide APIs, justification for the omission of a bioassay may be requested
  - Provide available bioassay data obtained during development and higher order structure characterization data

Elements of a Control Strategy



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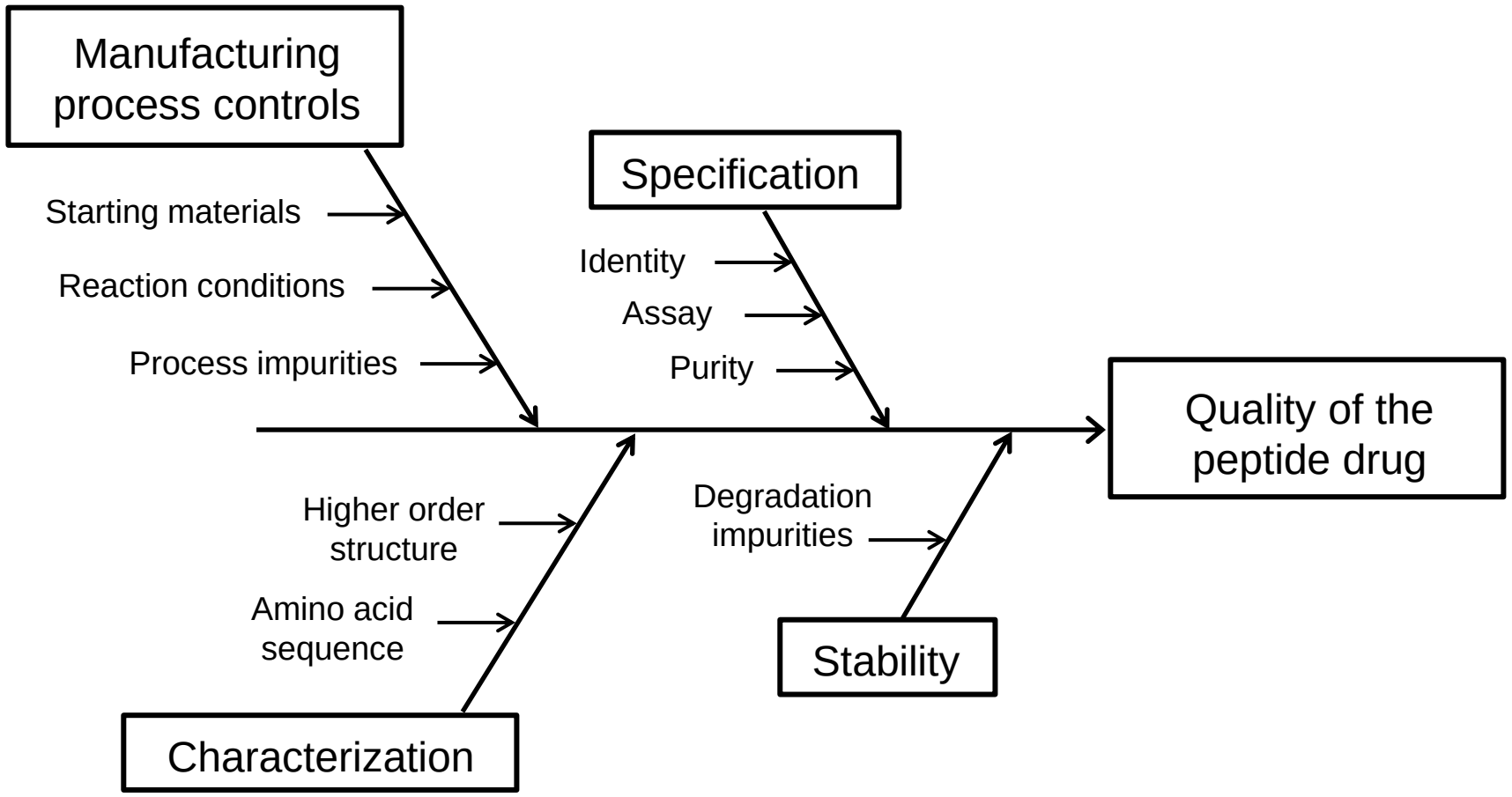
Stability Studies

- Stability studies should be in line with ICH Q1 guidelines
- Per ICH Q1E, no extrapolation allowed for drug substances to be stored at -20 °C
- Recommend performing forced degradation studies to elucidate degradation pathways and identify major degradants

Conclusions

- Provide increasing information as development proceeds to ensure the **identity**, **strength**, **quality**, and **purity** of the peptide drug

- **Reach out to the Agency** for guidance on CMC-related topics early and throughout development



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Guidances

For the most recent version of a guidance, check the FDA Drugs guidance webpage at: https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm

- Development and manufacture of DS: ICH Q11
- cGMP: ICH Q7
- Setting specifications: ICH Q6A
- Impurities: ICH Q3A-D
- Stability: ICH Q1A-E
- FDA Salt Policy: “Naming of Drug Products Containing Salt Drug Substances Guidance for Industry”
- MAPP 5017.2 Rev. 1: Establishing Impurity Acceptance Criteria as Part of Specifications for NDAs, ANDAs, and BLAs Based on Clinical Relevance
- For peptides from biological sources: ICH Q5A, Q5B, Q5D, and Q6B

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