LECTURE 10

Standardisation and analysis of biotechnological medicines

Recombinant proteins, monoclonal antibodies and vaccines: process control, ICH Q6B specifications, modern methods and prospects

Standardisation of Medicines and Raw Materials
Educational programme 6B10102 – Pharmacy · 4th year · Department of Fundamental Medicine, Al-Farabi Kazakh National University

OUTLINE

Lecture plan

1. Classes and features of biotechnological medicines
2. Production process and control points
3. ICH Q5 series and the Q6B specification
4. Identity, purity and potency methods
5. Safety tests and stability (ICH Q5C)
6. Biosimilars, modern approaches and prospects

Size and complexity: the process is the product

180 Da

Acetylsalicylic acid
one defined structure

5.8 kDa

Human insulin
51 amino acids, 3 disulfide bonds

~150 kDa

Monoclonal antibody (IgG)
~1300 amino acids, glycans, many variants

Consequences: heterogeneity (glycoforms, charge and size variants), sensitivity to heat, shear and freezing, activity tied to 3D structure, immunogenicity and contamination risks from cells

Main classes of biotechnological medicines

Recombinant hormones

Insulin and analogues, somatropin, epoetin

Cytokines and enzymes

Interferon alfa-2b, filgrastim, alteplase

Monoclonal antibodies

Trastuzumab, adalimumab, rituximab

Vaccines

Recombinant hepatitis B, HPV, mRNA vaccines

Advanced therapies

Gene therapy, CAR-T cell therapy

Host systems

E. coli, yeast, CHO and other mammalian cells

From cell bank to finished product

1 Cell bank	2 Upstream	3 Harvest	4 Downstream	5 DS → DP
• MCB → WCB • Identity, purity, genetic stability	• Bioreactor: pH, O_2, temperature, titre • Mycoplasma, viruses	Cell removal, clarification	• Chromatography (Protein A, IEX) • Viral inactivation and filtration	• Formulation, sterile filtration, aseptic filling • Full release testing

Three-level viral safety (ICH Q5A): test the cell banks, test the unprocessed bulk, and prove viral clearance by the process

Five groups of quality attributes

Identity

Peptide mapping (LC-MS), cIEF, glycan profile, immunoassay

Purity: product-related

Aggregates (SEC), fragments (CE-SDS), charge variants (IEX), oxidation

Purity: process-related

Host cell proteins (ELISA), host cell DNA (qPCR), Protein A

Potency

Cell-based or binding assay vs reference standard; IU or % relative potency

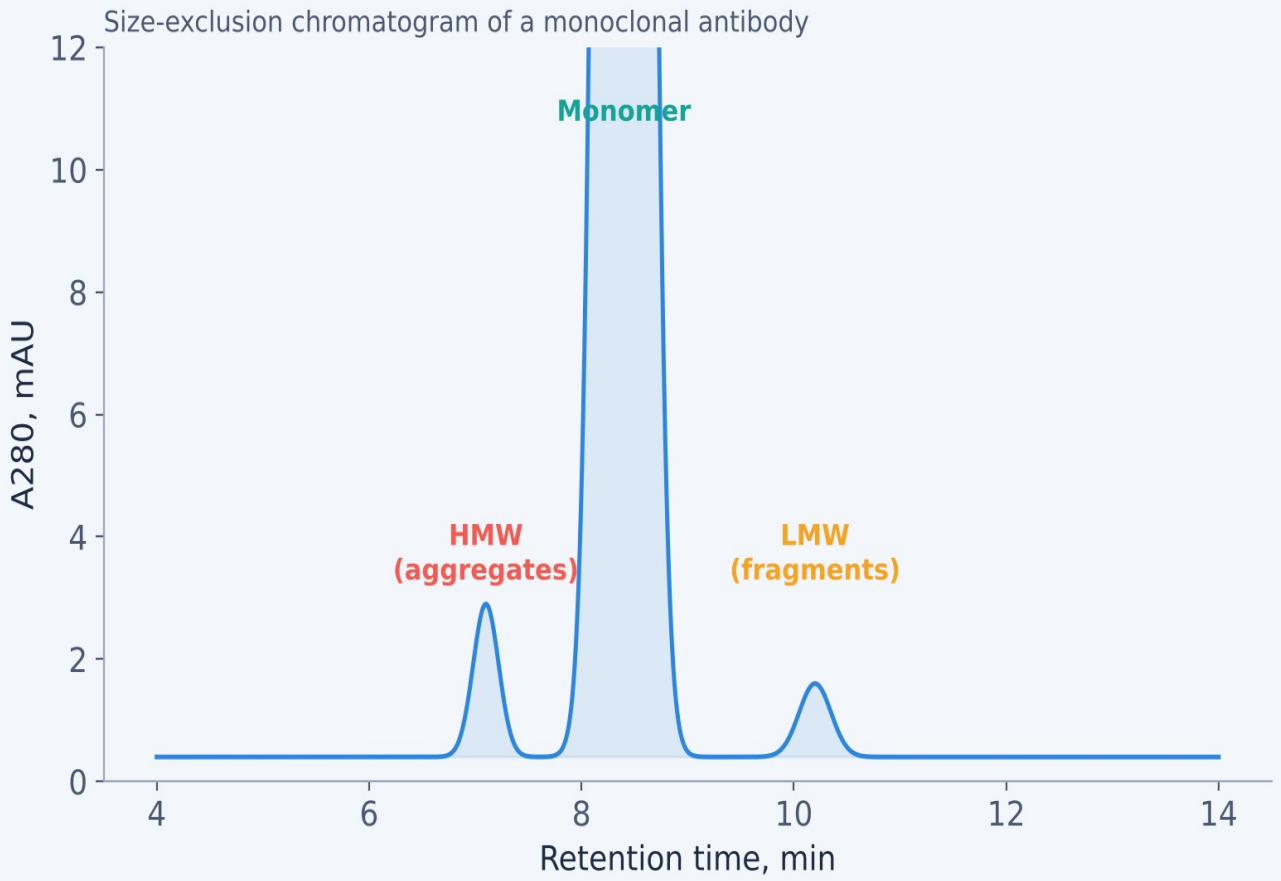
Quantity

Protein content, e.g. UV A280 with extinction coefficient

General and safety

Appearance, pH, osmolality, particles, sterility, endotoxins

Aggregates are a critical impurity



Calculations

- **Monomer, %** = A(monomer) / ΣA × 100
- **Example:** monomer 4 820 000, HMW 38 500, LMW 11 200 → 98.97%
- **HCP, ppm** = ng HCP per mg protein; 42 ng/mL ÷ 21 mg/mL = 2.0 ppm
- **Why aggregates matter:** loss of activity and risk of immunogenicity
- **Typical criteria:** monomer ≥ 95%; HCP ≤ 100 ppm

Does it work, and is it safe?

Potency assays

- **In vivo:** e.g. epoetin in mice (reticulocytes)
- **Cell-based:** proliferation, antiviral effect, reporter gene
- **Binding:** ELISA, surface plasmon resonance
- Relative to a reference standard (often WHO IS)
- **Specific activity:** IU per mg protein

Safety tests

- **Sterility** (2.6.1), endotoxins (2.6.14, rFC 2.6.32)
- **Pyrogens:** MAT (2.6.30) replaces the rabbit test
- **Mycoplasma** (2.6.7) in unprocessed bulk
- **Adventitious viruses:** cell culture, NAT, high-throughput sequencing
- **Endotoxin per mg** = EU/mL ÷ mg/mL

Real-time data and analytical similarity

Stability (ICH Q5C)

- Shelf life from real-time data, usually 2–8 °C
- No extrapolation without strong justification
- Profile of methods: SEC, IEX, potency, particles
- Cold chain; “Do not freeze”

Biosimilars and comparability (ICH Q5E)

- Similar, not identical, to the reference product
- Step 1: analytical similarity (the base)
- Step 2: functional and non-clinical
- Step 3: targeted clinical data (PK, immunogenicity)

Key message: the stronger the analytical evidence, the less clinical data is needed

MODERN APPROACHES

How quality control of biologics is changing

Multi-attribute method

One LC-MS peptide map measures oxidation, deamidation, glycans at once

Animal-free tests (3Rs)

rFC instead of LAL, MAT instead of rabbits, in vitro vaccine potency

High-throughput sequencing

Broad detection of adventitious viruses

QbD and PAT

In-line sensors (e.g. Raman) and real-time process control

Real-time release

Release based on process data, fewer end-product tests

mRNA, cell and gene therapy

New attributes: mRNA integrity, LNP size, cell viability, vector copy number

Key take-aways

1. **For biologics the process is the product:** control starts at the cell bank
2. **ICH Q6B:** identity, purity, potency, quantity and safety
3. **Potency** by bioassay against a reference standard is unique to biologics
4. **Stability (Q5C) and biosimilarity (Q5E)** rely on extensive analytical data
5. **Trends:** MS-based, animal-free and real-time methods

SELF-CHECK

Control questions and literature

Questions

1. Why are biologics controlled differently from small molecules?
2. Describe the production scheme of a monoclonal antibody and its control points.
3. What is the three-level approach to viral safety?
4. List the five groups of attributes in ICH Q6B with one method each.
5. Calculate monomer % for areas 960 000 (monomer), 25 000 (HMW), 15 000 (LMW).
6. What is specific activity and why is it important?
7. How is biosimilarity demonstrated?
8. Name three modern approaches to quality control of biologics.

Sources

- Ph. Eur. 0784 Products of recombinant DNA technology; 2031 Monoclonal antibodies
- ICH Q5A–Q5E, Q6B
- Ph. Eur. 2.6.1, 2.6.7, 2.6.14, 2.6.30, 2.6.32
- WHO guidelines on biosimilars (2022)
- State Pharmacopoeia RK