



Therapeutic Equivalence: Analytical, Bioequivalence, and Surrogate Endpoint Equivalence

- **Analytical Equivalence**
 - Physico-chemical Comparisons
 - Confirmation of Primary, Secondary, and Tertiary Structures
 - Analysis of Differences: Purity vs. Impurities
 - Links with Manufacturing and Stability
 - Links to Bioassay Equivalence and Surrogate Endpoints
 - Compendial reference standards: Cross-over studies
- **Bioequivalence**
 - Bioassay Equivalence
 - Bioequivalence
- **Surrogate Endpoint Equivalence**
 - Use of Validated Surrogate Endpoints
 - Establishing New Surrogate Endpoints

- This is the holy grail. Analytical equivalence may need to be shown with multiple layers of testing - analogous to comparability testing currently done for innovator biotech products. Key aspects are safety, purity, and strength.
- Identity confirmed through MS-NMR, circular dichroism (CD), MALDI-TOF, SDS-PAGE, immunological profiles. Impurities confirmed through chromatographic methods, SDS-PAGE, TLC, but are not necessarily limited to the following:
 - amino acid analysis
 - amino acid sequencing, entire sequence or amino- and carboxy-terminal sequences
 - peptide mapping
 - determination of disulfide linkage
 - Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) (reduced and non-reduced)
 - isoelectric focusing
 - Conventional and High Pressure Liquid Chromatography (HPLC) e.g., reverse-phase, size exclusion, ion-exchange, etc.
 - mass spectroscopy
 - assays to detect product-related proteins including deamidated, oxidized, cleaved, and aggregated forms and other variants e.g., amino acid substitutions, adducts/derivatives.
 - assays to detect residual host proteins, DNA, reagents
 - immunochemical analyses
 - assays to quantitate bioburden, endotoxin
 - Be intimately familiar with analytical method applications and limitations. Search the WEB for innovative technologies to apply to your product.

Additional physicochemical characterization may be required for products undergoing post-translational modifications, for example, glycosylation, sulfation, phosphorylation, or formylation. Additional physicochemical characterization may also be required for products derivatized with other agents, including other proteins, toxins, drugs, radionuclides, or chemicals. The information submitted should include the degree of derivatization or conjugation, the amount of unmodified product, removal of free materials (e.g., toxins, radionuclides, linkers, etc.), and the stability of the modified product.

- Bioequivalence is a mystery in that most drug products given via parenteral, otic, or ophthalmic routes are made with identical formulations to innovator and usually waive BE requirements. With biologics, bioassay comparisons may substitute partially for this, but with life-threatening conditions, there may be additional need for clinical endpoints (e.g., BIO-IND as currently done for topical anti-fungal creams).




Bioassay Equivalence: Points to Consider

- **Compendial assays vs. non-compendial or proprietary assays:** Need to assess if compendial methods (if applicable) are sensitive enough for distinguishing slight variations in critical product parameters; if compendial methods exist, do crossover studies for comparison
- **Establishing critical product parameters:** Need to assess/ correlate with functional activity/ stability in bioassays (e.g., antigen content vs. immunogenicity)
- **Relative Standard Deviation (RSD):** What is noise-to-signal ratio for bioassay? > 25% RSD? Need to use statistical modeling to ensure adequate sample size for assessing wide variability (see Juran's *Handbook of Quality*, Chapter 25 - Acceptance Sampling).
- **Use "gold standards" in assays:** Compare product to compendial reference standards or lots of innovator product. Establish what physico-chemical differences relate to bioassay activity.
- **Process Development:** Using neural nets to (1) help establish critical process parameters with impact on product quality and (2) develop an "edge of failure" for any given process and establishing ranges within those critical areas. Don't depend on early stage material for market needs.
- **Statistical Process Control (SPC):** Sampling: continued analysis of product quality against long-term manufacturing history
- **Combining Tests into a Panel:** Complex moieties may require several tests combined into a conglomerate panel to assess potency (e.g., RP-HPLC, SDS PAGE, and bioassay)
- **Express Potency as Quantifiable Units:** Need to express potency in some quantifiable measure - units [of activity]/ mg of compound (anhydrous weight)

Links: Need to assess impact of CMC changes to -

- Analysis of Product Characteristics: Comparability testing
- Surrogate Equivalence: Impact on activity
- Stability: Emergence of undetected impurities
- Analytical Methods: Consistency of methodology/ data
- Setting Rational Specifications



Bioequivalence (BA/BE): Uses and Limitations of a Two-Compartment Model for Parenterals

- 21 CFR 320.21 specifies BA/BE requirements for NDA/ ANDA and/or waivers based on chemical equivalence (or pharmaceutical equivalence)
- BA/BE study requirements typically waived (21 CFR 320.22) for parenteral, ophthalmic, or otic drug products based on assumed 100% BA of chemically equivalent material
- Exception to BA/BE waiver is for pro-drugs which may have different ADME profiles prior to activation
- BA measured as: AUC, C_{max} , and T_{max}
- BA/BE may also be established via *in vitro* testing when correlated to *in vivo* parameters
- Recent proposed BCS classifications may allow expanded BA/BE waivers for solid oral dosage forms on the basis of demonstrated *in vitro* characterization (e.g., dissolution, solubility, permeability, etc.)
- Are BA/BE studies sensitive enough to measure impact of subtle physico-chemical changes for biologics? If not, then are comparable results from such a BA/BE study (comparator vs. innovator) definitive?

Although some say that subtle changes in biologics (e.g., comparator vs. innovator) would only be seen in BA/BE studies - not via physico-chemical comparisons - it's not clear that a BA/BE study would be sensitive enough to detect the changes. This is partially the reason that some innovators say the only way to detect subtle chemical changes is through clinical efficacy studies.

However, the expanded utility of comparability studies - by the innovator - seeks to avoid the very obstacles they are stipulating for the competition. The definition and application of a uniform standard for product classes remains to be seen. It will most likely be a combination of innovator-specific product/ manufacturing history and collective industry experience with that class of compounds.



Surrogate Endpoint Equivalence

- **Vaccines:** Geometric Mean Titer (GMT) and seroconversion (vs. demonstrated prophylaxis from long-term studies)
- **Fibrinolytics:** in vitro clot lysis, post-MI (myocardial infarction) patency, ventricular function testing (ECG), etc. (vs. survival rates)
- **Somatotropins:** hypohysectomized rat model and comparison to external reference standards (vs. long-term growth confirmation)
- **Cystic Fibrosis Rx:** pulmonary function testing (PFT), days in ICU, days on intravenous antibiotics (vs. reduced number of exacerbations and long-term survival)
- **HIV/ AIDS:** reduction in CD4 count, viral burden, reduced rate of opportunistic infections (vs. survival rates)
- **Arthritis:** radiological imaging of joint damage, reduction of inflammatory mediators, improved joint mobility, etc.
- **Chemotherapeutics:** immunological mediators, carcinogenic antigen levels, lymph node involvement (vs. long-term survival)

Surrogate endpoints are valuable determinants when primary efficacy endpoints of morbidity or mortality (1) take too long to assess in chronic disorders, or (2) may become unethical delays in life-threatening conditions. Surrogate endpoint determinations/ equivalence is a moving target ... an evolving science. New diagnostic techniques and correlations are coming up all the time. One needs to assess the clinical condition with the most current methods available. Surrogate endpoints used 20 years ago are likely to be outdated; even some a few years old will be dated - especially when dealing with life-threatening conditions like burns, smoke inhalation, trauma, AIDS, cancer, sepsis, ALS, and others.

Also note that the innovator manufacturing may have changed substantially since approval and the clinical endpoints outlined in a SBA or referenced articles may not reflect the most current product. You will need to research this area thoroughly and be able to explain/ defend the scientific relevance (or not) of any observed microheterogeneity in your product vs. surrogate endpoint analysis. There is already precedence for therapeutic equivalence of two different manufacturers of menotropins, despite the microheterogeneity of the two materials. Thus, there can be analytical or physico-chemical differences with little or no clinical impact - but it's up to the sponsor to show this.

Remember that you cannot rely on an innovator's clinical efficacy data to support your application; yours must stand alone. Unless the innovator compound is approved under Section 505 of the FD&C Act and subject to a 505(j) or 505(b)(2) application, there is no generic biologic mechanism available. All applications must contain sufficient preclinical, clinical, and CMC data to stand alone. Thus, you need to make the best scientific case for analytical equivalence + bioequivalence + surrogate endpoint equivalence of your compound versus an innovator compound or external reference standard.

• Development of fast-track surrogate endpoints facilitated quicker reviews. Will need to do extensive research of the innovator application, reviewing division thoughts, and clinical practice to determine the most clinically relevant and achievable surrogate endpoints.

• If no surrogate endpoints were done with innovator, may need to establish them. As with mathematical equation, If $A = B$ and $B = C$, then $A = C$. Development of surrogate endpoints may be achieved through limited clinical trials comparing innovator to generic for key clinical parameters.

• Examples of surrogate endpoints that have been developed for various conditions include:

- **Alteplase in coronary infarction**, primary endpoint was global ventricular function (patency assessed by radioventriculography) but additional endpoints were artery patency and clinical outcome (death, congestive heart failure, and recurrent ischemia) (274 patients)

- **pulmonary emboli**, primary endpoint was embolysis assessed by repeat pulmonary angiography at two hours; secondary endpoints included change in pulmonary hemodynamics at two hours and lung perfusion as assessed by radionuclear methods at 24 hours (45 patients)

- **cystic fibrosis therapy**, primary endpoints were pulmonary function testing, days in ICU, days on IV antibiotic; secondary endpoints in quality of life,

• What if your product doesn't match innovator in surrogate endpoints? You need to establish noise-to-signal ratio for the clinical benefit and see where your product fits in. Also consider doing multiple