In vitro BE Studies – Inspectional approach

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Outline

- Types of in vitro BE studies
- Methods used in the in vitro BE studies
- Analytical methods for quantitation of analytes
- Documentation and archival
- Blinding of drug products
- Reserve sample requirements
- Closing remarks

Types of In vitro Bioequivalence Testing

- In vitro phosphate / bile acid binding studies
  - *Locally acting drugs*
- In vitro / in situ permeability studies
  - *Drugs for biowaiver applications*
- In vitro Nasal Aerosol or Spray Testing
  - *Nasal / aerosol products intended for release*
- In vitro release testing (IVRT) and *In vitro* permeability testing (IVPT)
  - *For dissolution, transdermal and topical delivery*

Phosphate / Bile Acid Drugs for BE Testing

Cholestyramine / Colesevelam HCl – *Bile acid sequestrants*

Sevelamer HCl / Sevelamer Carbonate – *Polymer for phosphate binding*

Calcium Acetate / Calcium Succinate – *Salt for phosphate binding*

Lanthanum Carbonate – *Salt for phosphate binding*

Nasal Aerosol or Spray Testing

- Single Actuation Content (SAC)Through Container Life
- Droplet Size Distribution by Laser Diffraction
- Drug in Small Particles/Droplets, or Particle/Droplet Size Distribution by Cascade Impactor
- Drug Particle Size Distribution by Microscopy (only for suspension products)
- Spray Pattern
- Plume Geometry (side view of aerosol cloud)
- Priming and Re-priming (delivery of labeled dose, based on SAC)

Nasal Aerosol or Spray Testing

- During inspection, the instrument setup and operation conditions will be reviewed.
- Modern instruments can provide plots of obscuration or % transmission and droplet size distribution (D10, D50, D90) over the entire life of a single spray. The plots need to be retained.
- Protocol or SOP should include criteria for defining the testing parameters.
- The instrumental output include photographs (manual), or digital images (automated imaging) should be archived.
- The analytical runs include at least three or more concentrations of QC samples.

In Vitro Release / Permeability Testing

- The IVPT utilize a balanced design comparing the test and reference on skin from the same set of donors, same number of replicate skin sections per donor per treatment group.

IVPT Membrane (Skin) Qualification:

- Trans-epidermal water loss (TEWL)
- Electrical impedance/conductance
- Tritiated water permeation

IVPT Receptor Solution Sampling Qualification:

- Accuracy and precision of receptor solution sample collection at each time point should be appropriately qualified

In Vitro Release / Permeability Testing

IVPT Qualification and Control of Study Procedures:

- Skin preparation (e.g., skin thickness membranes by dermatome)
- Thickness of skin sections mounted on diffusion cells
- Skin storage conditions
- Duration for which the skin was frozen
- Number of freeze-thaw cycles to which the skin was exposed
- Documentation of anatomical location and demographics (age, race, sex)
- Control of dose amount, the dosing technique, the dose duration
- Sample handling, storage and tracking
- Ambient laboratory conditions

Analytical Instrumentals

PO_4^{-3}, Ca^{+2} and bile salts

HPLC with Ion Conductivity

Inductively coupled plasma atomic emission spectroscopy (ICP-AES)

HPLC with UV/PDA

LC-MS/MS

Caco-2 / In situ rat perfusion, SAC / drug in particles or droplets, IVRT / IVPT

LC-MS/MS

HPLC with UV/PDA

Analytical Instrumental Methods

- Instruments, methodologies, and study conditions appropriately qualified, validated, and verified.
- Instrument settings established during pre-study validation would be used in the study.
- For comparative studies, use of the same settings will ensure that test and reference are studied under the same instrumental conditions.
- Validate all in vitro tests for accuracy and precision prior to the study.

What we cover during inspection?

Facilities & Site Operations

- Sample Storage Areas
- Drug Product Receipts and Accountability (storage, handling & processing)
- Reserve Samples
- SOPs, Protocols & Protocol Deviations
- Training Records
- Audit Trails & Data Security
- Instrument Calibration & Maintenance
- Documentation (including correspondence folder)

What we cover during inspection?

Method validation for in vitro experiments

- Precision & accuracy
- Selectivity
- Recovery and matrix effect

Sample analysis from in vitro experiments

- Sample Processing Procedures
- Sample Reassay and criterion
- Method Performance

What we cover during inspection?

Method validation for analytical methods

- Precision & Accuracy in receptor matrix
- Sensitivity
- Selectivity
- Dilution Integrity
- Stability Assessments (bench top, extended storage and F/T matrix stabilities)
- Stock solution stability
- Robustness

Documentation Expectations

- Separate reports for method validation and pivotal studies
- Maintenance and control documents for study facility environment and systems
- Experiment dates and information related to Principal Investigator including contact details including site address.
- Documentation indicating who conducted the study with dates.
- Archival of study records including source documents and made available during audit.
- Turn on audit trail feature and electronic data and is secured for audit.

Documentation Expectations

- Follow good documentation practices that include contemporaneous recording of procedures, observations and deviations to allow for reconstruction of the study.
- Study personnel training records and qualifications.
- Unexpected results and deviations from protocol or SOPs, with justification for deviations are documented.
- The original and reanalyzed data, with the reason for reanalysis, would be tabulated in the study report.

Documentation Expectations

- SOP with objective criteria for repeat testing.
- Drug accountability records for receipt, storage, and use for test and reference products.
- Documentation includes instrument output reports and photographic or graphic material in the viewable format.
- The documents be clearly labeled to indicate the drug product, batch number, and test conditions.
- If manual actuation used in the priming/re-priming testing, documentation indicating secondary analyst review and confirm the number of actuations used in these experiments.

Blinding of Drug Product

- SOP or protocol describe the procedure for sample blinding of Test and reference product with different codes and its repackaging into identical containers.
- The product will have to be masked and to result in blinding the identify of drug product.
- Analyst performing the experiments and data evaluations be blinded to the identity of the samples.

Reserve Sample Requirement

- The applicant or contract research organization shall retain reserve samples of any test and reference used in conducting an in vitro bioequivalence study required for approval of the application, per 21 CFR 320.38 and 21 CFR 320.63.
- Reserve samples from test and reference products should have adequate quantities retained from each shipment.
- Have the procedures to randomly select the drug products for reserve samples from each shipment.
- Stored in a segregated area with access limited to authorized personnel, and in original containers with proper label.

Reserve Sample Requirement

- Most common deficiency (observation) for in vitro BE studies include:
  - Reserve samples not retained
  - Improper handling, storage
  - Reserve samples retained under 21 CFR 211 (CGMP* regulations, not BE)

References:

- *Guidance for Industry: Handling and Retention of BA and BE Testing Samples (May 2004, OGD)*
- *Guidance for Industry: Compliance Policy for the Quantity of Bioavailability and Bioequivalence Samples Retained Under 21 CFR 320.38(c) (August 2020)*

Summary

- Methods used in the in vitro bioequivalence studies should be properly validated prior to the study
- Analytical methods should be sensitive and selective for quantitation of the analyte
- Follow good documentation practices that include contemporaneous recording, observations and deviations to allow for reconstruction of the study.
- Drug products are blinded to the identity of the samples to analyst for proper evaluation of data.
- Reserve sample are selected, stored in proper conditions and made available during inspection.

Challenge Question #1

Which of the following are example of membrane (Skin) barrier integrity test conducted in IVPT studies?

A. Trans-epidermal water loss (TEWL)

B. Electrical impedance/conductance

C. Tritiated water permeation

D. All of the above

Challenge Question #2

Which of the following documents are maintained at the study site and are made available at the time of inspection?

A. Contemporaneous documentation

B. Analyst's training records

C. Instrument calibration and maintenance records

D. Chromatograms and audit trails

E. All of the above

Challenge Question #3

Per 21 CFR 320.38 and 21 CFR 320.63, the applicant or CRO shall retain the reserve samples of test and reference used in conducting an *in vitro* bioequivalence study.

A. True

B. False

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QUESTIONS…