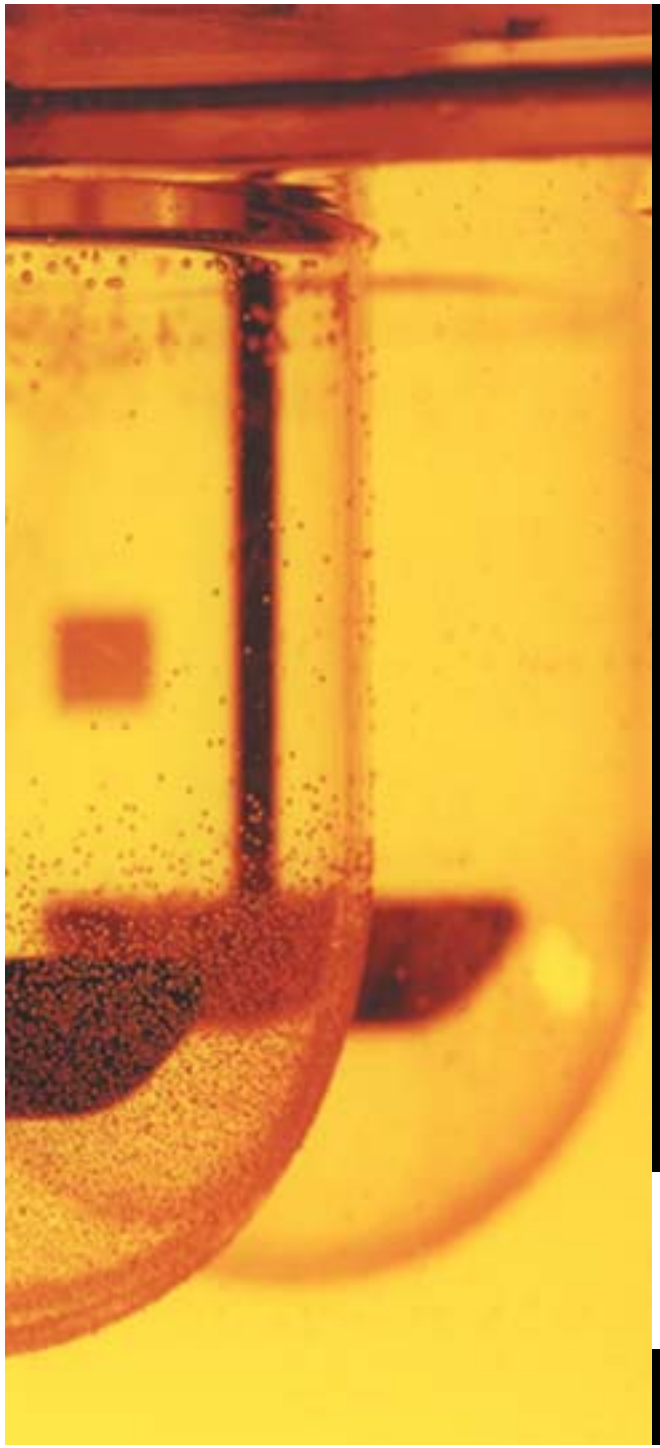

Bioavailability and Bioequivalence

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Design and Validation of Bioanalytical Test Methods for BA/BE Studies

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PHAST Pharmaceutical Quality Standards

Topics

- General aspects and regulatory requirements
- Structure of GLP – general QMS
- Methods and Equipment
  - technical aspects
  - equipment
- Method development
- Method validation
  - pre-study
  - in-study
- Documentation

How to Identify the Optimum CRO?

Prerequisites according to CPMP/ICH/135/95 (ICH Topic E6):

- **Parts of the sponsor's duties may be delegated to CRO**
- **But ultimate responsibility for the quality and integrity of the trial data always resides with the sponsor**
- **Does the CRO perform the complete study?**
  - **yes: than performance fully according to GCP**
  - **no:**
    - **performance according to GMP for pharmaceutical QC**
    - **performance according to GLP for bioanalytical tests**
- **Audits are mandatory**

Prerequisite: GxP requirements met

- **no preference for particular country in general**
- **lab's in Eastern Europe (outside EUR) or India may be accepted if already audited by EU-authorities**
- **BE-study results accepted in EU even if performed in non-Caucasians**
- **with regard to study goal population aspects may be relevant**

Validation Regulated under GLP

- for Bioavailability and
- Bioequivalence studies
- as well as for pharmacokinetic studies in humans and animals

- source: CPMP/QWP/1401/98 chapter 3.4
  - "…should be conducted according to applicable principles of GLP...!
  - six characteristics essential:
    - stability
    - specificity
    - accuracy
    - precision
    - LOQ (limit of quantification)
    - response function
  - all procedures should be performed according to pre-established SOPs

experience required

Most Valuable Source: FDA Guidance for Industry

www.fda.gov/cder/guidance/index.htm

“Bioanalytical method validation”

- Valuable source:
  - –definition of parameters and acceptance criteria
  - –practical instructions for performance
  - –documentation
    - » …also for auditors!

Further Regulatory Definition for Human Studies

ICH Q 2 A - CPMP/ICH/381/95

- Validation of Analytical Methods: Definitions and Terminology

ICH Q 2 B – CPMP/ICH/281/95

- Validation of Analytical Methods: Methodology

But primary intention is implementation in GMP

Typical GLP-Structure

- Test facilities organization and personnel
- Quality assurance program
- Facilities
- Instruments, Materials, and Reagents
- Test Systems
- Test and reference materials
- Standard Operating Procedures (SOPs)
- Performance of the study
- Reporting of study results
- Storage and retention of records and samples

Description given a priori in GCP Study Protocol

- Description given as relevant for GLP:
  - responsible institute and persons
  - sample collection, processing, and storage
  - analytical method
  - description of validation parameters
  - acceptance criteria
  - rejection criteria
  - special quality procedures
    - repeated analysis
    - reintegration
  - documentation
    - report
    - archiving

Methods and Equipment

- technical improvement within the past years is leading to
  - better sensitivity and selectivity performance
  - progressing automation of sample preparation and measurement
  - electronic records

- most commonly used methods
  - LC
  - GC
  - with
    - mass detection (e.g. LOD 1pg/ml)
    - fluorescence detection
    - electrochemical detection
- less common
  - immunological assays
  - microbiological assays

Development and Validation of Methods

Exemplified for

- human BA/BE study
- analysis of blood, serum, plasma, urine
- chemical assay, e.g. chromatography, HPLC

Concept of validation also applicable to other methods such as microbiological tests

Chemical Reference Standard Substances

- Authenticated analytical reference standard substances of known identity, purity, and content should be used for reference solutions with known concentrations

- The CRS should be completely identical to the analyte. If not relevant a stable form (free base, salt, ester) may be used

- Three types of standard are being used:
  - pharmacopeial standards (cave: potency sometimes method related)
  - commercially supplied CRS with certificate of analysis
  - custom-synthesized CRS with documented ID, purity

- The source must be declared, specifications must be given for individual lot number, expiry date must be indicated, storage conditions should be labeled

Method Development

Principle aspects

- Sample preparation
  - precipitation
  - extraction (e.g. liquid-liquid, solid phase extraction)

- Analytical method
  - HPLC, LC-MS/MS, GC
    - materials and reagents
    - chromatographic conditions (e.g. temperature, mobile phase, stationary phase, flow rate) with emphasis on robustness
    - detection

- Validation of the method
  - pre-study
  - in-study

Validation Program

- **Full Validation**
  - Establishment of all validation parameters for each analyte
- **Partial validation**
  - where no full validation is required:
    - method transfer
    - method changes, e.g. detector
    - sample matrix changes, e.g. plasma to urine
    - extension of concentration range
    - co-medication given in study
- **Cross-validation**
  - comparison of methods for selected parameters

Validation Pre-Study

- Selectivity
- Linearity
- Accuracy
- Precision
  - within run = repeatability
  - inter run = intermediate
- Stability
- Recovery

Validation Pre-Study Selectivity

Selectivity is the ability of analytical method to differentiate and quantify the analyte in the presence of other sample components

Potential other components:

- endogenous matrix components
- metabolites
- decomposition products
- concomitantly given medication
- exogenous xenobiotics

How to:

- Analyses of blank matrix samples for appropriate biological matrix as obtained from at least 6 individual sources. Each blank sample should be tested for interference and selectivity should be ensured at the lower limit of quantification (LLOQ)

Validation Pre-Study Linearity, Standard Curve

- A standard curve is the mathematical functional relationship between instrument response and known concentrations of the analyte
- Concentrations of the CRS should be justified on the basis of the expected range

- A standard curve should include:
  - blank sample (without internal standard)
  - zero sample (with internal standard)
  - at minimum 6 spiked samples including LLOQ
- Acceptance criteria
  - 20 % deviation of LLOQ from nominal value
  - 15 % deviation at other concentration levels
  - Four out of six non-zero samples including LLOQ and ULOQ (upper limit of quantitation) should meet the criteria

Validation Pre-Study Accuracy

The accuracy describes the closeness of mean test results obtained by the method to the true vaue of the analyte

Proof of validation should include:

- minimum three levels of the expected concentration range
- minimum five replicates at each level

Acceptance criteria:

- LLOQ +/- 20 % from nominal value
- other levels +/- 15 % from nominal value

Validation Pre-Study Precision

The precision describes the closeness of individual measurements of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix

Within run, intra-batch precision or repeatability:

- precision during a single run

Between-run, inter-batch precision or repeatability:

- precision with time, may involve different analysts, equipment, reagents, laboratories

Investigations of precision should include:

- minimum three levels of the expected concentration range
- minimum five replicates at each level

Acceptance criteria:

- LLOQ CV $\leq$ 20 %
- other levels CV $\leq$ 15 %

Validation Pre-Study Recovery

- The recovery of an analyte in an assay is the detector response obtained from an amount of the analyte added to and extracted form the biological matrix compared to the detector response obtained for the true concentration of the pure authentic standard

- Recovery pertains to the extraction efficacy of an analytical method within the limits of variability

- Proof of recovery should include:
  - three concentrations (low, medium, and high of the range)
  - analyte and internal standard

- Acceptance criteria:
  - extent of recovery should be consistent, precise, and reproducible
  - needs not to be 100 %

Validation Pre-Study Stability

- The stability of an analyte in a biological matrix is a function of storage conditions, the chemical properties of the drug, the matrix, and the container system.

- Proof of stability investigations should include:

1. Freeze and thaw stability
2. Short-term temperature stability
3. Long-term temperature stability
4. Stock solution stability
5. Post-preparative stability

Validation Pre-Study Stability cont'd

1. Freeze and thaw stability

   Investigations after three freeze -thaw cycles three aliquots of each concentrations (low and high level of range) stored at the intended storage temperature for 24 h and thawed unassisted at room temperature, refrozen for 12 – 24 h under the same conditions. The cycle should be repeated two more times, then analyzed on the third cycle.

   If analyte is unstable at the intended storage temperature, the stability sample should be frozen at -70 °C during the three freeze and thaw cycles

Validation Pre-Study Stability cont'd

2. Short-term temperature stability

   Investigations on short-term stability based on the expected duration that samples will be kept at room temperature.

   - three aliquots of each of the low and high concentrations should be thawed at room temperature and kept at this temperature for 4 - 24 h.

3. Long-term temperature stability

   Investigations on long-term stability should exceed the time between 6the date of first sample collection and the date of last sample analysis

   - three aliquots of each of the low and high concentrations should be stored under the same conditions as the study samples.

Validation Pre-Study Stability cont'd

4. Stock solution stability

   The stability of the stock solution of the analyte and the internal standard should be evaluated at room temperature at room temperature for at least 6 h and for the relevant period if the stock solution are refrigerated or frozen.

   - the stored stock solutions will be compared to freshly prepared solutions

5. Post-preparative stability

   The stability of processed samples including the time in the auto sampler should be determined.

   - three aliquots of each of the low and high concentrations should be analyzed for analyte and internal standard

Validation In-Study

Before starting the analytical run with the study samples the size of the run, the injection sequence and how many subject‘s samples have to be analyzed

- rule of thumb
  - all samples of one subject
  - not more samples than one day capacity
- in-study validation should include
  - samples of the standard curve
  - QC samples
  - samples of subjects

Validation In-Study – the Standard Curve

The standard curve within one run should include:

- blank sample (without internal standard)
- zero sample (with internal standard)
- at minimum 6 standard point samples including LLOQ

Acceptance criteria

- 75 % or a minimum of six standards should fall within ± 15% deviation from nominal concentration except for LLOQ which should fall within ± 20 % of nominal value

Validation In-Study QC-Samples

The analytical run should include QC-samples

- replicated at least once at a minimum of three concentrations
  - one with 3x of the LLOQ (low QC)
  - one in the midrange (middle QC)
  - and one approaching the high end of he range (high QC)
- at least 5 % of total number of unknown samples to be analyzed or six total QCs whichever is greater

Acceptance criteria

- 67 % (four of six) should be within ± 15 % of the nominal value
- 33 % (two of six, but not at the same concentration) may be outside the + 15 % nominal value

Documentation of Validation

Validation report according to the FDA note for guidance:

- summary information
- documentation for method establishment
- application to routine drug analysis
- other information

Additionally for each bioanalytical report

- …storage and preparation of samples
- in-study validation data…

Conclusions:

- fully validated methods are the prerequisite to evaluate product differences expressed as the quantitative intra-individual bioavailability relationship obtained with a variable biological model

- the general validation requirements as being proposed by ICH are a sound basis

- the FDA guidance for industry Bioanalytical Method Validation is of great use for routine

Acknowledgments

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www.yes-services.de