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GUIDANCE DOCUMENTS CONTAINING THE COMMON PROVISIONS ON THE CONDUCT OF GCP INSPECTIONS BY COMPETENT AUTHORITIES OF THE DIFFERENT MEMBER STATES

Annex VII

TO GUIDANCE FOR THE CONDUCT OF GOOD CLINICAL PRACTICE INSPECTIONS

Bioanalytical part, Pharmacokinetic and Statistical Analyses of Bioequivalence Trials

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This document forms part of the guidance documents containing the common provisions on the conduct of GCP inspections . Please check for updates in the Volume 10 of the Rules Governing Medicinal Products in the European Union.

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1. INTRODUCTION

Bioequivalence trials are comprised of several parts:

– a clinical part, where the test and the reference products are administered to the trial subjects and where biological samples (generally plasma or serum, possibly blood, urine or any other suitable matrix) are collected from the subjects. This part is not addressed in this document;

– a bioanalytical part, where the concentration of the active moiety and/or its biotransformation product(s) in these biological samples is measured;

– the pharmacokinetic analysis, where pharmacokinetic parameters derived from these concentrations are calculated;

– the statistical comparison of the pharmacokinetic parameters obtained for the test and the reference products.

This annex compiles specific items that may be verified during the inspection of the bioanalytical part and of the pharmacokinetic and statistical analyses of bioequivalence trials. The selection of items to be inspected will depend on the scope of the inspection and should be detailed in the inspection plan.

The documents and data relating to the following topics are generally reviewed during the inspection:

– storage of the biological samples;

– validation of the bioanalytical method;

– performance of the assays;

– if requested, pharmacokinetic and statistical analyses of the trial data.

2. BIOANALYTICAL PART OF BIOEQUIVALENCE TRIALS

2.1. General organisation of the site

2.1.1. Activity

The main points to consider are the following:

– nature of the activities carried out at the laboratory;

– proportion of bioequivalence trials in this activity;

– command of the analytical methods used, particularly for complex methods.

2.1.2. Personnel

The main points to consider are:

– organisation charts, valid at the time of the inspection and at the time when the inspected trial was conducted;

– number and categories of people employed;

– qualification, training and experience of the personnel;

– individual work load of people involved.

2.1.3. Quality assurance system

The main points to consider are the following:

– quality assurance system in place at the laboratory;

– existence, availability, accessibility and validity of Standard Operating Procedures ("**SOPs**");

– list of SOPs used for the trial;

– SOP awareness by people in charge.

2.1.4. Installations and equipment

The suitability of the facilities and equipment available, their appropriateness for the activity of the laboratory and for the bioequivalence trial inspected should be checked during the inspection.

2.1.5. Archiving of documentation

The main points to consider are the following:

– nature of the documents kept;

– place of archiving;

– access control to that place;

– conditions of storage and of protection of the documents;

– person responsible for the archives;

– documentation of file movements;

– duration of retention of the files;

– where applicable, loan arrangements.

2.2. Sample tracking

2.2.1. Receipt

General aspects relating to sample handling at the facility may be inspected including:

– responsibilities for receipt and handling of biological samples;

– organisation of the receipt system, including outside workdays/hours;

– sample registration;

– controls performed on receipt.

The points to consider specifically for the inspected trial(s) are the following:

– dates and times of receipt of the samples, and acknowledgement of receipt;

– list of samples received for each dispatch;

– shipment conditions (temperature);

– condition of the samples on receipt;

– any anomalies noted;

– known sample stability (see validation report).

2.2.2. Storage

The following points should be checked for the samples collected for the inspected trial:

– storage conditions of the trial samples;

– compliance of these conditions with the protocol and the conditions used during method validation;

– assessment of the risk of confusion between samples;

– identification of the freezer(s) used;

– temperature records of the freezer;

– calibration of the thermometer and its traceability to national/international standards;

– alarms and other surveillance measures;

– labelling of the samples, if they are still available;

– documentation of freeze/thaw cycles undergone by the samples.

2.2.3. Destruction

Check the date of destruction or return of the samples.

2.3. Sample analysis

2.3.1. Bioanalytical method used

- **Method description**

Check the consistency of the trial report with the SOP describing the bioanalytical method and other documents available.

- **Equipment**

The main points to consider regarding the equipment used (including balances and pipettes) are the following:

– identity of the equipment (make, model);

– availability of the equipment. If the equipment is no longer visible at the site at the time of the inspection, review the documentation that could show that the equipment needed was indeed available when the trial was conducted;

– availability of instructions for use;

– compliance with specific conditions necessary for the trial, if any;

– documentation relating to the qualification, checks, and maintenance of the equipment.

- **Reagents**

The main points to consider are:

– labelling of reagents, including the expiry date;

– traceability of the reagents used;

– compliance with specific storage conditions, if any.

- **Reference substances**

The main points to consider are:

– availability and contents of the certificates of analysis;

– expiry dates;

– storage conditions;

– conditions for access to reference substances.

- **Calibration, control samples**

The main points to consider are:

– dates and conditions of preparation of the stock and working solutions and of the calibration and control samples, and the number of aliquots prepared for each sample;

– accuracy of the calculation of nominal concentrations;

– conditions and duration of storage of the stock solutions, working solutions, calibration and control samples, compared to their stability, as described in the validation report;

– matrix used, including the anticoagulant, if any.

The main points to consider regarding the calibration for each run are:

– number of calibration samples;

– response function used, including weighting, if any;

– acceptance criteria for the calibration curve;

– criteria for exclusion of calibration samples.

2.3.2. Development of the method

A quick overview of the origin and of the development of the bioanalytical method can be helpful to identify critical steps in the procedure.

2.3.3. Method validation

The main points to consider are:

– validation protocol;

– dates of the validation;

– adequate documentation of all operations;

– completeness of the validation report, when compared to the various experiments performed;

– consistency of the validation report with the source documents;

– chromatogram integrations;

– the exclusion of calibration samples, if any.

The main validation parameters are the following:

- stability:
    - of the stock solutions;
    - of the samples (bench-top, freeze/thaw cycles, long term);
    - if applicable, of extracted samples before their injection;
- specificity / selectivity;
- accuracy;
- precision;
- limit of quantification;
- response function;
- carry-over;
- in case of mass spectrometric methods: matrix effect;
- effect of a dilution, if applicable;
- if applicable, effect of the anticoagulant, if the anticoagulant used for the preparation of the calibration and/or QC samples is different from the anticoagulant used to collect samples during the trial.

2.3.4. Assays

The main points to consider are:

- nature and completeness of the documentation available;
- adequacy of the documentation of all operations;
- completeness of the analytical report;
- number, date and composition of the analytical runs;
- identification of samples and tubes;
- assessment of the risk of sample mix-ups;
- assessment of the risk of sample cross-contamination;
- chromatogram integrations;
- calculation of the concentrations;
- compliance with pre-defined criteria for the exclusion of calibration samples;

– criteria of acceptance of the runs, and compliance with pre-established criteria;

– audit trail settings and information recorded in the audit trails;

– practicalities of repeat analysis and the criteria for choosing the result to be reported;

– maintenance of blinding, if required by the protocol;

– practicalities of data transfer;

– consistency of the analytical report with the source documents.

3. PHARMACOKINETIC AND STATISTICAL ANALYSES

3.1. Pharmacokinetics

The main points to consider are:

– quality system in place;

– identity, qualification and responsibilities of the personnel involved;

– software used;

– practicalities and control of data entry;

– sampling times used;

– method used for calculation of pharmacokinetic parameters;

– selection of data for the calculation of the terminal half-life, if applicable;

– consistency of the raw data with the trial report.

Pharmacokinetic parameters can be recalculated before or during the inspection if needed.

3.2. Statistics

The main points to consider are:

– quality system in place;

– identity, qualification and responsibilities of the personnel involved;

– software used;

– practicalities and control of data entry;

– data line listings and tables of results;

– consistency of the raw data with the calculated pharmacokinetic parameters and with the trial report.

The statistical analyses can be repeated before or during the inspection if needed.

4. REFERENCES

– Note for Guidance on the investigation of bioavailability and bioequivalence (CHMP/EWP/QWP/1401/98).

– Note for Guidance "Tests on samples of biological origin" (3AB11a).

The following documents, which cannot be considered as reference texts for the purpose of the inspection, are of interest for the preparation of the inspection:

– FDA Guidance for Industry, Bioanalytical method validation, May 2001.

– Bioanalytical method validation - a revisit with a decade of progress. V.P. Shah et al., Pharmaceutical Research, vol. 17, No. 12, 2000, p.1551-1557.

– Workshop/Conference Report - Quantitative Bioanalytical Methods Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays. C.T. Vishwanathan et al., The AAPS Journal 2007;9(1), E30-E42.