

Annex 9

Guidance for organizations performing in vivo bioequivalence studies

Background

During an informal consultation held in 2014, and at the forty-ninth meeting of the World Health Organization (WHO) Expert Committee on Specifications for Pharmaceutical Preparations, discussion took place regarding the possible revision of the guidance for organizations performing in vivo bioequivalence studies (WHO Technical Report Series, No.937, Annex 9, 2006). The WHO Expert Committee on Specifications for Pharmaceutical Preparations agreed that in light of the new developments a draft for revision would be prepared.

These new guidelines take into consideration the revision of the multisource guidelines, as well as the creation of new guidance on good data management. The revision will also take into account the experience accumulated in the area of assessing and inspecting bioequivalence (BE) studies since 2006. In areas where the same problems are repeatedly identified by inspectors, the new guidelines provide clarifications, and supplementary details have been added on bioanalysis. The guidelines also put increased emphasis on subject safety and data integrity.

Based on the first working document:¹ this second version incorporates the numerous comments and the feedback received from the public consultation, the WHO Prequalification Team (PQT) and from the Consultation on data management, bioequivalence, GMP and medicines' inspection held in 2015.

WHO/PQT was set up in 2001 to assure that medicinal products supplied for procurement meet WHO norms and standards with respect to quality, safety and efficacy (<http://www.who.int/prequal/>). Specifically, there is a requirement that the submitted product dossier with all its necessary contents is assessed and found acceptable, and that the manufacturing sites for the finished pharmaceutical product (FPP), as well as the active pharmaceutical ingredient (API), are inspected and found to comply with WHO good manufacturing practices (GMP). Since products submitted to WHO/PQT are usually multisource (generic) products, therapeutic equivalence is generally demonstrated by performing a BE study, for example in a contract research organization (also known as a clinical research organization) (CRO). For prequalification of such

¹ http://www.who.int/medicines/areas/quality_safety/quality_assurance/BE-in-vivo-studies-guidance-QAS15-622_21052015.pdf?ua=1.

a product it is vital that, in addition to the above-mentioned requirements, the CRO used by the sponsor for BE studies complies with WHO good clinical practices (GCP) and considers relevant elements from WHO good laboratory practices (GLP) and good practices for quality control (QC) laboratories to ensure integrity and traceability of data. In addition, if local legal provisions exist, CROs should be licensed by the respective national medicines authority. Where required by national regulations, BE studies should be authorized by the national regulatory authority. Those involved in the conduct and analysis of BE studies on products to be submitted for prequalification therefore need to ensure that they comply with the relevant WHO norms and standards so that they can be prepared for any inspections by WHO.

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Introduction

Multisource pharmaceutical products need to conform to the same standards of quality, efficacy and safety as the originator's (comparator) product. Specifically, the multisource product should be therapeutically equivalent and interchangeable with the comparator product. Testing the BE between a product and a suitable comparator (pharmaceutically equivalent or a pharmaceutical alternative) in a pharmacokinetic study with a limited number of subjects is one way of demonstrating therapeutic equivalence without having to perform a clinical trial involving many patients. In such a pharmacokinetic study any statement about the safety and efficacy of the test product will be a prediction based on measurement of systemic concentrations, assuming that essentially similar plasma concentrations of the active pharmaceutical ingredient (API) and/or of its metabolite will result in essentially similar concentrations at the site of action and therefore an essentially similar therapeutic outcome. The BE study thus provides indirect evidence of the efficacy and safety of a multisource pharmaceutical product. Often this will be the only evidence that the product is safe and efficacious. It is therefore crucial that the BE study is performed in an appropriate manner. Several guidance documents stress the importance of on-site inspections to verify compliance with standards of GCP (1–3).

1. Scope

The objective of this document is to provide guidance to organizations that are involved in the conduct and analysis of *in vivo* BE studies. This guidance supersedes the version published in the WHO Technical Report Series, No. 937, 2006 (4).

BE studies should be performed in compliance with the general regulatory requirements and good practices recommendations as specified in the WHO BE guidelines (5), GCP (1) and GLP (2) guidelines. It is acknowledged that GLP formally apply only to nonclinical safety studies. However the WHO BE guidelines require that the validation of bioanalytical methods and the analysis of BE study samples be performed following the principles of GLP. This does not imply that the laboratory in charge of the bioanalytical part of the study should be monitored as part of a national GLP compliance programme.

These guidelines provide advice on the conduct of BE studies and the bioanalysis of study samples. Particular consideration is given to premises, equipment, organization and management. Recommended documents, standard operating procedures (SOPs) and records are listed in Appendix 1, but this is

not to be considered an exhaustive list – other documents may be necessary depending on each individual CRO's functional and compliance needs.

These guidelines provide information on:

- organization and management;
- study protocols;
- clinical phase of a study;
- bioanalytical phase of a study;
- pharmacokinetic and statistical analysis;
- study report;
- quality management system.

This document does not replace the above-mentioned GCP or GLP guidelines. It is therefore not a stand-alone document.

2. Glossary

The definitions given below apply to the terms used in this guidance. They may have different meanings in other contexts. Unless otherwise stated, the definitions are reproduced from *Guidelines for good clinical practice for trials on pharmaceutical products* (1).

adverse event. Any untoward medical occurrence in a clinical trial subject administered a pharmaceutical product; it does not necessarily have a causal relationship with the treatment.

audit of a trial. A systematic examination, carried out independently of those directly involved in the trial, to determine whether the conduct of a trial complies with the agreed protocol and whether the data reported are consistent with the records on site, e.g. whether data reported or recorded in the case-report forms are consonant with those found in hospital files and other original records.

bioequivalence. Two pharmaceutical products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives, and their bioavailabilities, in terms of rate ($C_{\max}$ and $t_{\max}$) and extent of absorption (area under the curve), after administration of the same molar dose under the same conditions, are similar to such a degree that their effects can be expected to be essentially the same.

calibration curve samples (or calibration standards). A matrix to which a known amount of analyte has been added or spiked. Calibration standards are used to construct calibration curves.

case-report form. A document that is used to record data on each trial subject during the course of the trial, as defined by the protocol. The data should

be collected by procedures which guarantee preservation, retention and retrieval of information and allow easy access for verification, audit and inspection.

comparator product (or reference product). The comparator product is a pharmaceutical product with which the multisource product is intended to be interchangeable in clinical practice. The comparator product will normally be the innovator product for which efficacy, safety and quality have been established. If the innovator product is no longer marketed in the jurisdiction, the selection principle as described in *Guidance on the selection of comparator pharmaceutical products for equivalence assessment of interchangeable multisource (generic) products* (5) should be used to identify a suitable alternative comparator product.

contract. A document, dated and signed by the investigator, institution and sponsor, that sets out any agreements on financial matters and delegation/distribution of responsibilities. The protocol may also serve as a contract when it contains such information and is signed. Contracts can also be signed with other parties such as vendors supplying services to the contract research organization.

contract research organization (CRO). A scientific organization (commercial, academic or other) to which a sponsor may transfer some of its tasks and obligations. Any such transfer should be defined in writing.

In the context of this guidance document, bioequivalence studies are often contracted by the sponsor to a CRO, which will perform some of the tasks of the sponsor, but which will also perform the trial. The investigator (clinical part of the study) and the study director (bioanalytical part of the study) are then employees of the CRO.

To facilitate reading, the term “CRO” is used throughout this document to designate any organization performing the trial, even though it is acknowledged that part or all of the study may be performed in-house by the sponsor itself or at a hospital.

ethics committee (6). An independent body (a review board or a committee, institutional, regional or national), constituted of medical professionals and non-medical members, whose responsibility is to verify that the safety, integrity and human rights of the subjects participating in a particular trial are protected and to consider the general ethics of the trial, thereby providing public reassurance. Ethics committees should be constituted and operated so that their tasks can be executed free from bias and from any influence of those who are conducting the trial.

final report. A comprehensive description of the trial after its completion including a description of experimental methods (including statistical methods) and materials, a presentation and evaluation of the results, statistical analysis and a critical, ethical, statistical and clinical appraisal.

good clinical practice. A standard for clinical studies which encompasses the design, conduct, monitoring, termination, audit, analysis, reporting and documentation of the studies and which ensures that the studies are scientifically

and ethically sound and that the clinical properties of the pharmaceutical product (diagnostic, therapeutic or prophylactic) under investigation are properly documented.

good laboratory practice. A quality system concerned with the organizational process and the conditions under which nonclinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported.

informed consent. A subject's voluntary confirmation of willingness to participate in a particular trial and the documentation thereof. This consent should be sought only after all appropriate information has been given about the trial, including an explanation of its status as research, its objectives, potential benefits, risks and inconveniences, alternative treatment that may be available, and of the subject's rights and responsibilities in accordance with the current revision of the Declaration of Helsinki.

inspection. An officially conducted examination (i.e. review of the conduct of the trial, including quality assurance, personnel involved, any delegation of authority and audit) by relevant authorities at the site of investigation and/or at the site of the sponsor in order to verify adherence to good clinical practices and good laboratory practices as set out in this document.

internal standard. Test compound(s) (e.g. a structurally similar analogue or stable isotope-labelled compound) added to calibration standards, quality control samples and study samples at a known and constant concentration to correct for experimental variability during sample preparation and analysis.

investigational labelling. Labelling developed specifically for products involved in a clinical trial.

investigational product (or study product). Any pharmaceutical product (see definition) or placebo being tested or used as a reference in a clinical trial.

investigator. A person responsible for the trial and for the rights, health and welfare of the subjects in the trial. The investigator should have qualifications and competence in accordance with local laws and regulations as evidenced by up-to-date curriculum vitae and other credentials. Decisions relating to, and the provision of, medical or dental care must always be the responsibility of a clinically competent person legally allowed to practise medicine or dentistry.

lower limit of quantification. The lower limit of quantification of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with predefined precision and accuracy.

metadata. Metadata are data that describe the attributes of other data, and provide context and meaning. Typically, these are data that describe the structure, data elements, interrelationships and other characteristics of data. They also permit data to be attributable to an individual. Examples of metadata are the audit trails provided by certain types of software.

monitor. A person appointed by, and responsible to, the sponsor or contract research organization for the monitoring and reporting of progress of the trial and for verification of data.

pharmaceutical product. Any substance or combination of substances which has a therapeutic, prophylactic or diagnostic use, or is intended to modify physiological functions, and is presented in a dosage form suitable for administration to humans.

principal investigator. The investigator serving as coordinator for certain kinds of clinical trials, e.g. multicentre trials.

Note: “principle investigator” also has a specific, but different meaning in good laboratory practices, which is seldom used in bioequivalence studies. To avoid any misunderstanding, the term “principal investigator” will only be used in this guidance document with its good clinical practices meaning.

protocol. A document that states the background, rationale and objectives of the trial and describes its design, methodology and organization, including statistical considerations, and the conditions under which it is to be performed and managed. The protocol should be dated and signed by the investigator, the institution involved and the sponsor. It can also function as a contract.

quality assurance relating to clinical trials. Systems and quality control procedures that are established to ensure that the trial is performed and the data are generated in compliance with good clinical practices and good laboratory practices. These include procedures to be followed which apply to ethical and professional conduct, standard operating procedures, reporting, and professional qualifications or skills of personnel.

quality control samples. A spiked sample used to monitor the performance of a bioanalytical method and to assess the integrity and validity of the results of the unknown samples analysed in an individual batch.

raw data. All records or certified copies of original observations, clinical findings or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Such material includes laboratory notes, memoranda, calculations and documents, as well as all records of data from automated instruments or exact, verified copies, e.g. in the form of photocopies or microfiches. Raw data can also include photographic negatives, microfilm, magnetic media (e.g. computer diskettes) and optical media (CD-ROMs).

serious adverse event. An event that is associated with death, admission to hospital, prolongation of a hospital stay, persistent or significant disability or incapacity, or is otherwise life-threatening in connection with a clinical trial.

sponsor. An individual, a company, an institution or an organization that takes responsibility for the initiation, management and/or financing of a clinical trial. When an investigator initiates and takes full responsibility for a trial, the investigator then also assumes the role of the sponsor.

standard operating procedures. Standard, detailed, written instructions for the management of clinical trials. They provide a general framework enabling the efficient implementation and performance of all the functions and activities for a particular trial as described in this document.

study director. According to the Organisation for Economic Co-operation and Development principles of good laboratory practice: the individual responsible for the overall conduct of the nonclinical health and environmental safety study. In a bioequivalence study the individual responsible for the conduct of the bioanalytical part of the study.

study product. *see investigational product.*

test product. Any pharmaceutical product (see definition) or placebo being tested against the reference in a clinical trial. In a bioequivalence study, this is the multisource product being tested against the comparator product.

trial subject. An individual who participates in a clinical trial, either as a recipient of the pharmaceutical product under investigation or as a control. The individual may be:

- a healthy person who volunteers to participate in a trial;
- a person with a condition unrelated to the use of the investigational product;
- a person (usually a patient) whose condition is relevant to the use of the investigational product.

upper limit of quantification. The upper limit of quantification of an individual analytical procedure is the highest amount of analyte in a sample which can be quantitatively determined with predefined precision and accuracy.

validation. Action of proving and documenting, in accordance with the principles of good clinical practices and good laboratory practices, that any procedure, process, equipment (including the software or hardware used), material, activity or system actually and consistently leads to the expected results.

verification of data. The procedures carried out to ensure that the data contained in the final report match original observations. These procedures may apply to raw data, data in case-report forms (in hard copy or electronic form), computer printouts and statistical analysis and tables.

A. GENERAL SECTION

3. Organization and management

Note: the acronym “CRO” is used throughout this document to refer not only to a contract research organization, but also to any organization involved in the conduct of in vivo BE studies or in the analysis of samples or of data from such in vivo BE studies

- 3.1 Where national requirements exist as to the legal status of a CRO these have to be complied with. This also applies to the research unit which is a subsidiary of the manufacturer.
- 3.2 The CRO should have an organization chart depicting key positions and the names of responsible persons. The organization chart should be dated, authorized and kept up to date.
- 3.3 There should be job descriptions for all personnel, including a description of their responsibilities. Every job description should be signed and dated by the staff member to whom it applies.
- 3.4 There should be a list of signatures of the authorized personnel performing tasks during each study.
- 3.5 For the bioanalytical part of the trial, the principles of GLP clearly establish the responsibilities of the test facility management. For the clinical part of the trial, the CRO management should be aware that as the investigator is an employee of the CRO, some of the responsibilities usually assigned to the investigator would in a similar way reside with the CRO management. At a minimum, the CRO management should:
 - ensure that the principles of GCP and GLP, as appropriate, are complied with in the CRO;
 - ensure that a sufficient number of qualified personnel, appropriate facilities, equipment and materials are available for the timely and proper conduct of the study;
 - ensure the maintenance of a record of the qualifications, training, experience and job description for each professional and technical individual;
 - ensure that personnel clearly understand the functions they are to perform and, where necessary, provide training for these functions;
 - ensure that appropriate and technically valid SOPs are established and followed, and approve all original and revised SOPs and ensure the maintenance of a historical file of all SOPs;
 - ensure that there is a quality assurance (QA) programme with designated personnel and assure that the QA responsibility is being performed in accordance with the principles of GLP and GCP, as appropriate;
 - ensure that an individual is identified as responsible for the management of the archive(s), and ensure that the documents

transferred to the archives are kept under adequate conditions for the appropriate duration;

- ensure that supplies meet requirements appropriate to their use in a study;
- establish procedures to ensure that computerized systems are suitable for their intended purpose, and are validated, operated and maintained in accordance with the principles of GCP and GLP, as appropriate.

4. Computer systems

Note: this section highlights only some of the requirements for computer systems that are specific to BE studies. Organizations involved in BE studies should ensure that the relevant principles of the following guidelines are appropriately followed:

- GAMP 5: A risk-based approach to compliant GxP computerized systems (7);
- *Good practices for computerised systems in regulated “GXP” environments, PIC/S guidance* (8);
- US Food and Drug Administration (FDA) *Guidance for industry: part 11* (9);
- *EU guidelines for good manufacturing practice and medicinal products for human and veterinary use Annex 11, Computerised systems* (10);
- *WHO Guidance on good data and record management practices* (11).

General

- 4.1 Computer systems should be qualified and validated (hardware, software, networks, data storage systems and interfaces (7–10). Qualification is the planning, carrying out and recording of tests on equipment and systems which form part of the validated process, to demonstrate that the equipment or system will perform as intended.

Hardware

- 4.2 There should be a sufficient number of computers to enable personnel to perform data entry and data handling, required calculations and compilation of reports.
- 4.3 Computers should have sufficient capacity and memory for the intended use.

Software

- 4.4 There should be access control to the trial-related information entered and stored in computers. The method of access control should be specified (e.g. password protection) and a list of people who have access to the database should be maintained. Secure and unique, individual-specific identifiers and passwords should be used.
- 4.5 The software programs used to perform key steps detailed in these guidelines should be suitable and validated for the intended use. Whether standard, off-the-shelf software is purchased or bespoke software is developed, developer, vendor and/or service provider qualification and/or validation certificates may be provided but it is the user's responsibility to ensure that the software is validated for its intended use and that it was developed in a controlled manner in accordance with a QA system.
- 4.6 Formal qualification and validation should generally be carried out by the developer. Performance qualification should take account of the specific user's requirements, of regulatory/guideline requirements for BE studies, of the operating environment in which it will be used, and of how it will be used by an organization's staff in the context of a study. Quality risk management should be applied when deciding which components need to be validated. All phases of their life cycle should be considered. For example, when a CRO decommissions the software in use for high-performance liquid chromatography (HPLC) and mass spectrometric (MS) analysis (e.g. HPLC-MS/MS), it should ensure that the data collected by the system using this software remain fully readable. This could be done, for instance, by having the old software installed on a workstation for inspection and/or verification purposes only.
- 4.7 There should be SOPs in place for usage of each software program that is used to perform activities of a BE study.
- 4.8 There should be a system in place for the implementation of regular updates to key software programs (e.g. those used for control and data processing of chromatographic and MS systems) whenever required, following an appropriate risk assessment on the potential impact that it could have on current data and on qualification or validation status.
- 4.9 Software programs used, frequency of virus testing, storage of data and the procedure for backups and long-term archiving of all relevant electronic data should be specified in writing. The frequency of backups and archiving should be specified. If back-up data are periodically rewritten as part of the back-up procedure, the data from the backups should be archived regularly, preferably before rewriting is done.

- 4.10 The programs used should be able to provide the required quality and management information, reliably and accurately. Programs necessary for data management include word processing, data entry, databases, graphics, pharmacokinetics and statistical programs. Self-designed software programs must be suitable and validated for their intended use.
- 4.11 Since data for BE studies are often transferred electronically between organizations involved in the studies, verification that the software used by each organization is compatible with the others and that there is no impact on the data so-transferred, should be conducted prior to commencing key study-related tasks.
- 4.12 These requirements apply to all systems used in clinical BE studies, e.g. subject database, electronic case report forms, electrocardiogram (ECG) recording software, HPLC-MS/MS software, software used for pharmacokinetic analysis, for statistical analysis and any other relevant system.

Networks

- 4.13 Networks, including the full client/server architecture and interfaces such as laboratory information management systems, when used, should be appropriately designed, qualified, managed and controlled.
- 4.14 Access to each component of the system by the different users at any given organization involved in the studies, should be appropriately defined, controlled and documented.
- 4.15 There should be a documented inventory of all computerized systems on the network, with a clear identification of those which are GXP regulated. Any changes to the network, including the temporary addition or removal of systems from the network, should be documented.

Data management

- 4.16 Data entry includes transfer of the data from case report forms (CRFs), analytical data and any other data relevant to the reliability and integrity of a study, to the computerized system.
- 4.17 Data entry procedures should be designed to prevent errors. The data entry process should be specified in the SOP.
- 4.18 Data validation methodology (proofreading, double data entry, electronic logical control) should be specified in writing and performed.

- 4.19 Changes to data entered in the database should be made by authorized persons only. Changes should be specified and documented.
- 4.20 Electronic data should be backed up at regular intervals. The reliability and completeness of these backups should be verified – data should not be selected, rather all data should be comprehensively backed up.
- 4.21 All of the raw electronic data must be kept. This includes:
- all metadata associated with a computerized system and the equipment associated with it (which includes the audit trails for integration, for results, projects and for the entire instrument);
 - validation data and metadata in the form of their source electronic files.

PDF copies are not sufficient on their own, unless it can be demonstrated that these are the raw data and that no alteration was possible after they were generated.

- 4.22 All electronic records obtained from HPLC and MS analysis (e.g. HPLC-MS/MS) are required to be retained, maintained and backed up. It should be ensured that backup data are exact and complete and that they are secure against alteration, inadvertent erasures or loss. The printed paper copy of the chromatogram would not be considered a “true, exact and complete copy” of all the electronic raw data used to create that chromatogram. Printed chromatograms do not generally include, for example, the sample sequence, instrument method, processing method, integration settings or the full audit trail, all of which were used to create the chromatogram or are associated with its validity. Therefore there should be a greater emphasis on conservation of electronic data than paper data, as paper data are usually not considered the true source data, except, for instance, in the case of paper logbooks where the original record was handwritten.
- 4.23 If data are transformed during processing steps (such as in the example of re-integration of chromatographic data), it should always be possible to compare the original data with the processed data.

5. Quality management

- 5.1 The CRO should have appropriate QA and QC systems with written SOPs to ensure that trials are conducted and data are generated, documented and reported in compliance with the protocol, GCP, GLP, GMP and the applicable regulatory requirements.

5.2 QA personnel should be independent of the work they are quality assuring, including:

- conducting or monitoring of the trial;
- conducting bioanalysis;
- performing reporting and pharmacokinetic and statistical analyses.

As a consequence, QA personnel should not be directly involved in trial-related activities, and an in-process audit by QA personnel does not replace oversight by another person when required.

5.3 The QA unit should be responsible for:

- verifying all activities undertaken during the study;
- ensuring that the quality management systems, are followed, reviewed and updated;
- determining that the protocol and SOPs are made available to study personnel and are being followed;
- checking all the study data for reliability and traceability;
- planning and performing self-inspections (internal audits) at regular and defined intervals in accordance with an SOP, and following up on any corrective action as required, to determine if all studies are conducted in accordance with GCP and GLP;
- ensuring that contract facilities adhere to GCP and, if applicable, to GLP: this would include auditing of such facilities, and following up on any corrective action required;
- verifying that the trial report accurately and completely reflects the data from the study and the methods and procedures followed;
- promptly reporting audit findings in writing to management, to the investigator and to the study director, as applicable.

5.4 The CRO should allow the sponsor to monitor the studies and to perform audits of the clinical and analytical study and sites and should provide suitable office space for these activities.

5.5 Both in-process and retrospective QA verifications (e.g. in bioanalysis, as the samples and standards are being prepared and tested) should be performed.

5.6 The quality management system should include root cause analysis, tracking for trends, ensuring all aspects of data integrity and the implementation of appropriate corrective and preventive action (CAPA).

6. Archive facilities

- 6.1 The CRO should have sufficient and appropriately secure storage space, which should be fireproof, relative humidity-controlled and pest-controlled, for archiving of the trial-related documentation. Archives should also be protected from flooding.
- 6.2 An SOP should be in place for archiving.
- 6.3 Access to archive storage areas should be controlled and restricted to authorized personnel.
- 6.4 Records of document access and return should be maintained.
- 6.5 The length of time for which study documentation, including raw data, is kept in the archive should be defined in the SOP and may vary depending on country requirements. This period should be specified in the contract between the sponsor and the CRO, which should include provisions for financing of the archiving.
- 6.6 All data, including both paper and electronic versions, should be easy to retrieve and traceable.

7. Premises

- 7.1 The facilities should be kept clean and should have adequate lighting, ventilation and, if required, environmental control. Floors, walls and working bench surfaces should be easy to clean and to decontaminate.
- 7.2 Clinical trials must be carried out under conditions that ensure adequate safety for the subjects. The site selected should be appropriate to the potential risk involved.
- 7.3 The CRO should have sufficient space to accommodate the personnel and activities required to perform the studies. The trial site must have adequate facilities, including laboratories, and equipment. The facilities used for the clinical phase of the study, including areas listed in paragraph 9.6, should be well organized in order to carry out the activities in a logical order.
- 7.4 Entry to the facility should be restricted and controlled. There should be alarm systems to detect the exit of subjects from clinical facilities, or the doors should be locked (but only if emergency evacuation can still be ensured). Any entry to and exit from the facility should be recorded.

- 7.5 Sites where clinical activities take place should include a pharmacy where investigational products should be stored under appropriate conditions with entry and exit restricted by access control. Appropriate entry/exit records of each visit to the pharmacy should be maintained.
- 7.6 Utilities such as water, air, gas and electricity should be adequate, stable and uninterrupted.
- 7.7 Access to telephone, email and facsimile facilities should be available to ensure proper communication. The CRO should have the necessary office equipment (printer, copy machine) to perform the required activities.
- 7.8 Laboratory premises should be designed to suit the operations to be carried out in them. Sufficient space should be provided to avoid mix-ups, contamination and cross-contamination. Adequate storage space suitable for samples, standards, solvents, reagents and records should be available.
- 7.9 Laboratory premises should be designed to provide adequate protection to all employees and authorized external personnel, including inspectors or auditors, by ensuring their safety while handling or working in the presence of chemicals and biological samples. Inappropriate working conditions can have a negative impact on the quality of the work performed and of the data generated.

The general rules for safe working in accordance with national regulations and SOPs normally include the following requirements.

- Safety data sheets should be available to staff before testing is carried out. Staff working in the laboratory should be familiar with and knowledgeable about the material safety data sheets for the chemicals and solvents that they are handling.
- Smoking, eating and drinking in the laboratory should be prohibited.
- Staff should know how to use the firefighting equipment, including fire extinguishers, fire blankets and gas masks.
- Staff should wear laboratory coats or other protective clothing, including eye protection.
- Appropriate care should be taken when handling, for example, highly potent, infectious or volatile substances.
- Highly toxic and/or genotoxic samples should be handled in a specially designed facility to avoid the risk of contamination.
- All containers of chemicals should be fully labelled and include prominent warnings (e.g. “poison”, “flammable” or “radioactive”) whenever appropriate.

- Adequate insulation and spark-proofing should be provided for electrical wiring and equipment, including refrigerators.
- Rules on safe handling of cylinders of compressed gases should be observed and staff should be familiar with the relevant colour identification codes.
- Staff should be aware of the need to avoid working alone in the laboratory.
- First-aid materials should be provided and staff instructed in first-aid techniques, emergency care and the use of antidotes.
- Containers containing volatile organic solvents, such as mobile phases or liquid/liquid extraction solvents should be closed with an appropriate seal.
- Volatile organic chemicals should be handled under certified fume-hoods or air extractors and safety and eye showers should be available in the laboratory.

7.10 Premises should have suitable systems in place to dispose of waste, to treat fumes and to protect the environment in conformance with local or national regulations.

8. Personnel

- 8.1 There should be a sufficient number of medical, paramedical, technical and clerical staff with the appropriate qualifications, training and experience to support the trial and to be able to respond effectively to all reasonably foreseeable emergencies. The number of members of staff required depends on the number and complexity of the trials performed by the CRO. At all stages of the trial, including at night, there should be a sufficient number of appropriately qualified and trained personnel to ensure that the rights, safety and well-being of the subjects are safeguarded, and to care for the subjects in emergency situations.
- 8.2 The delegation of significant trial-related duties should be documented in writing.
- 8.3 Contract workers may be employed to perform certain activities. All contract workers who have access to the clinical or bioanalytical areas or who are performing trial-related activities should be provided with adequate information, training and job descriptions. Their contracts should be signed before beginning their work.
- 8.4 Current curricula vitae and training records should be kept for full-time and contract workers.

- 8.5 The personnel responsible for the planning and conduct of the study should have appropriate qualifications and sufficient knowledge and experience in the relevant field. They should receive the study-specific information and training required for the performance of their work.
- 8.6 Records of training and assessment of knowledge of GCP, GLP and any other relevant area or technique should be maintained.
- 8.7 There should be adequate measures in place to protect personnel from accidental infection (e.g. from accidental needle pricks) while obtaining blood samples from subjects or while handling samples that are derived from blood products (e.g. plasma and its extracts) or while handling or disposing of infectious waste.

B. CLINICAL SECTION

9. Clinical phase

Note: As in vivo BE trials are considered as clinical trials, specifically as a Phase I study, the general requirements and recommendations of GCP apply to all BE trials. Clinical trials must be carried out under conditions that ensure adequate safety of the subjects. The clinical phase of the study can be performed on the premises of a CRO or by contracting suitable premises in a hospital.

- 9.1 A CRO should have rooms meeting the requirements listed in the sections below.
- 9.2 There should be sufficient space to accommodate the study subjects.
- 9.3 Where appropriate, beds should be available for the subjects. The necessity for beds and for overnight stays depends on the type of trial and investigational product and should be specified in the trial protocol. Overnight stays are usually required for the night prior to dosing to ensure adequately controlled conditions and that there is no intake of food or medication within the number of hours that is specified in the trial protocol.
- 9.4 Systems should be in place in the accommodation facilities so that subjects can alert CRO staff in case of need.
- 9.5 Facilities for changing and storing clothes and for washing and toilet purposes should be clean, well ordered, easily accessible and appropriate for the number of users. Lockable toilets should be alarmed and doors should be designed to ensure that they can be opened from the outside should there be a medical emergency.

9.6 The study site should have rooms or areas, as appropriate, for the following:

- subjects' registration and screening;
- obtaining informed consent of individual subjects without compromising privacy;
- subjects' housing;
- subjects' recreation;
- pharmaceutical operations (restricted access room, e.g. for storage, repacking, dispensing, documentation) (see also section 14);
- administration of the investigational products and sample collection;
- sample processing (e.g. plasma separation) and storage (freezer);
- controlled access storage of study materials, medication and documentation including CRFs;
- preparation of standardized meals and a dining hall;
- proper care of subjects who require emergency or other medical care, with emergency or first-aid equipment and appropriate medication for use in emergencies;
- archiving.

9.7 Provisions should be made for the urgent transportation of subjects to a hospital or clinic equipped for their emergency care, if required.

9.8 Access to key documents, such as the randomization list, should be restricted to specific personnel, such as the pharmacist in charge of the study. Such documents should be password-secured (if electronic) or kept under lock and key (if in the form of a hard copy) and their distribution should be documented.

9.9 Equipment used should be appropriately calibrated at predefined intervals.

9.10 The adequate function and performance of emergency-use equipment (e.g. defibrillators) should be verified at appropriate intervals.

10. Clinical laboratory

10.1 A suitable clinical laboratory should be used for analysing samples. Whenever possible this should be an accredited laboratory.

10.2 Haematological tests, urine analysis and other tests should be performed during the clinical trial as specified in the study protocol.

10.3 Sample labelling, receipt, storage and chain of custody should ensure full traceability and sample integrity (9).

- 10.4 The CRO should receive information about the analytical methods used in the laboratory, a dated list of laboratory normal ranges and, if available, the accreditation certificate of the laboratory. These should be available for inspection by regulatory authorities upon request.
- 10.5 The laboratory should provide the CRO with current and signed curricula vitae of the responsible individuals.
- 10.6 Individual reports should be created by the laboratory for each subject and should be included in the CRFs. Source or raw data for all tests performed should be archived by the laboratory in electronic or paper formats, depending on their source and the laboratory's storage capacity. Electronic formats are preferred.
- 10.7 Data integrity requirements apply to all tests related to the study (11). For instance, raw data should be adequately protected from modification or deletion.

11. Ethics

11.1 Independent ethics committee

Trials must be approved by an independent ethics committee (IEC) (or equivalent) before any study is conducted, according to *WHO operational guidelines for ethics committees that review biomedical research* (6), and to the legislation in force. This Committee must be independent from the sponsor, the investigator and the CRO. Detailed minutes should be kept of the discussions, recommendations and decisions of the IEC meetings. The IEC should be given sufficient time for reviewing protocols, informed consent forms (ICFs) and related documentation.

11.2 Informed consent

The following points should be borne in mind in relation to informed consent.

- Information for study participants should be given to them in a language and at a level of complexity appropriate to their understanding, both orally and in writing.
- Informed consent must always be given by the subject and documented in writing before the start of any trial-related activities, in accordance with GCP. If informed consent is also recorded by video, this recording should be retained in accordance with local legal requirements.

- The information must make clear that participation is voluntary and that the subject has the right to withdraw from the study on his or her own initiative at any time, without having to give a reason (compensation should be paid *pro rata temporis*). If subjects who withdraw from the study offer their reasons for doing so, those reasons should be included in the study records.
- The subject must have access to information about insurance and other procedures for compensation or treatment should he or she be injured or disabled by participating in the trial or during screening.
- The volunteers or subjects should be given the opportunity to discuss with a physician their concerns regarding potential side effects or reactions from the use of the investigational products before participating in the trial. They should also be given the opportunity and sufficient time to discuss their concerns about participating in the trial with individuals outside the CRO, such as friends and family members, if they wish.
- If the ICF is available in several languages (e.g. in English and in the local language, or in several vernacular languages) care should be taken to ensure that all versions of the form contain the same information.

12. Monitoring

Note: monitoring is an essential activity to ensure the quality of the clinical trial.

- 12.1 The monitor should be appropriately qualified (see section 8: Personnel). The main responsibility of the monitor for a BE study is to ensure that the study is conducted in accordance with the protocol, GCP, GLP and applicable ethical and regulatory requirements. This includes verification of the use of correct procedures for completion of CRFs and verification of the accuracy of data obtained.
- 12.2 The sponsor can delegate the monitoring function to the CRO. In such cases the CRO should be able to arrange for the monitoring of the trial according to regulatory requirements. In this situation, attention should be paid to the independence of the monitoring function to avoid conflicts of interest and pressure on the monitors. The monitoring reports should always be provided to the sponsor.
- 12.3 A risk-based approach to monitoring can be considered. However, a pre- and post-study visit, as well as a monitoring visit during the conduct of the trial, are usually performed. The monitor should prepare a written report

after each site visit and communicate any issues to the CRO and to the sponsor as quickly as possible, even while the study is being conducted, if possible, to enable prompt corrective action. Such communications and corrective actions should be documented.

12.4 When the monitoring is delegated to the CRO, SOPs should be available to describe:

- the designation of monitors, who should be independent from the personnel performing the trial;
- procedures for the monitoring visit;
- the extent of source data verification, including with regard to accountability of the investigational products and adherence to the protocol.

The extent of the monitoring, including the number of visits to be performed, should be agreed with the sponsor.

12.5 Separate SOPs (with checklists for the monitor) for the initiation visit, routine monitoring visits and a closing visit are recommended.

12.6 Appropriate entry/exit records of each monitoring visit should be maintained.

13. Investigators

13.1 The principal investigator (PI) should have the overall responsibility for the clinical conduct of the study, including clinical aspects of study design, administration of the products under investigation, contacts with local authorities and the ethics committee and for signing the protocol and the final study report.

13.2 The investigator(s) should have appropriate qualifications, be suitably trained and have experience in the conduct of BE studies (the legal status of persons authorized to act as investigators differs between countries) and at least one investigator must be legally allowed to practise medicine.

13.3 The medically-qualified investigator should be responsible for the integrity, health and welfare of the subjects during the trial and for the accurate documentation of all trial-related clinical data.

13.4 The CRO is responsible for selecting investigator(s). If the investigators are not permanent employees of the CRO, external investigators should be contracted and adequately trained.

14. Receiving, storage and handling of investigational products

- 14.1 CROs should record all the information concerning the receipt, storage, handling and accountability of investigational products at every stage of the trial. CROs must keep records of information about the shipment, delivery, receipt, description, storage (including storage conditions), dispensing, administration, reconciliation, return and/or destruction of any remaining pharmaceutical products. Details of the pharmaceutical product used should include dosage form and strength, lot number, expiry date and any other coding that identifies the specific characteristics of the product tested.
- 14.2 A suitably qualified person within the CRO or a local pharmacy or hospital pharmacy should assume responsibility for storage, delivery, return and keeping records of the investigational products.
- 14.3 Pharmaceutical products should be stored under appropriate conditions as specified in the official product information provided by the sponsor.
- 14.4 All study medication should be kept in a securely locked area accessible only to authorized personnel.
- 14.5 Randomization should be performed in accordance with an SOP and records should be maintained, including the randomization list and seed, if applicable. The randomization list should normally be accessible only to the person who generates it, a dispensing pharmacist and the statistician, and should not be circulated or made available to other staff members via any medium. A system should be in place to allow the PI or delegated staff to access the randomization list in case of emergency.
- 14.6 Labelling should be performed in accordance with the following requirements.
 - The printing step should be done in a manner that reduces potential risks of mislabelling and in accordance with an SOP.
 - Each label should include the following information:
 - name of the sponsor,
 - a statement reading “for clinical trial use only”,
 - trial reference number or study number,
 - batch number,
 - subject identification number (to whom the product is destined to be given),

- study period,
 - active ingredient and dosage,
 - the storage conditions,
 - expiry date (month/year) or retest date,
 - identification of the product (i.e. test or reference).
- Compliance of all labels with the randomization list should be verified once they have been printed and prior to labelling of the containers.
 - Labels should be pasted onto the container, not on the lid, to ensure that the information is not lost once the lid is removed.
 - The system used for labelling and documenting the administration of the product should make it possible to verify that each subject did receive the product dispensed for him or her, for instance, by using labels with a tear-off portion. In this case, labels should be designed in such a way that two identical labels are pasted onto the container and the second label can be easily cut or detached and pasted onto the CRF at the time of dosing (e.g. two labels printed side by side, with only one that is actually pasted onto the container and another that remains attached but unpasted. Using two independent labels – one stuck on the container, one kept loose – should be avoided owing to the risk of mix-ups).
 - The empty containers should be labelled separately for the test and the reference investigational products and should remain adequately segregated in a secure area under lock and key to avoid the risk of any potential mix-ups, until the dispensing stage.
 - Label reconciliation should be performed.
 - Appropriate, detailed records should be maintained for each of the above steps.

14.7 Dispensing and packaging should be performed in accordance with the following requirements.

- The surface on which the product will be handled should be thoroughly cleaned before bringing bottles of the product into the area. Any product containers (full or empty), lone dosage formulations, labelling materials, contaminants, dirt and debris should be removed from the area.
- A second person should verify that the surface area (otherwise referred to as the “line”) is indeed clear and clean before bringing in and opening containers of the product.

- Test and reference products should be handled using an appropriate instrument, such as a spatula or spoon, as opposed to gloved hands.
- Tablets should be distributed into each container in accordance with the randomization list for the comparator or for the test product as appropriate. The two products should never be handled at the same time. This also applies to the labelled containers.
- Records should be made of this step in a manner similar to that used for manufacturing batch records, as described in WHO GMP guidelines, i.e. each and every step should be recorded sequentially in detail.
- The surface upon which the product is handled and its surroundings should be cleared and cleaned immediately before and after initiating the dispensing of the next product. It is important to note that this also applies to different products used in the same study.
- Investigational product accountability and dispensing records should be maintained at all times. Each activity should be documented at the time it is performed. This includes:
 - records of doses dispensed and returned or destroyed,
 - records of cleaning and clearance of the area before dispensing,
 - record of verification of adequate cleaning and clearance of the area,
 - record of verification by a second person of each step.

Any factors that could affect the integrity of the data relating to investigational medicinal products and comparators should be recorded, monitored and controlled.

For further guidance on labelling and dispensing, please refer to the *WHO good manufacturing practices: supplementary guidelines for the manufacture of investigational pharmaceutical products for clinical trials in humans* (12).

14.8 Dosing should meet the following requirements.

- Dosing should be performed in accordance with an SOP.
- Dosing should be performed under the supervision of the investigator or of a qualified staff member to whom this task has been explicitly delegated in writing.
- Whenever possible, just prior to dosing, a check should be performed to ensure that vial contents match the information on the label.

- The exact time of dosing should be documented.
- To ensure that the subject has swallowed the product, a mouth check should be performed by looking under the tongue, under the lips, in the corners of the mouth and between gums and cheeks, using a tongue depressor or a spatula and a penlight, in the case of solid oral dosage forms. For other dosage forms, verification of adequate administration should be performed by other suitable means. This should be documented.
- If more than one dosage unit is administered, this should be clearly documented.
- Dosing can be documented directly in the CRFs. If re-transcribed in the case of report forms from other documents, the original documents should be retained.
- Investigational product reconciliation after dosing should be verified by a second responsible person.

14.9 The investigator should follow the protocol requirements, the randomization scheme and, where required, blinding. The investigator should ensure that the use of the investigational product is documented in such a way as to ensure appropriate dosage.

14.10 Samples of the product in the original container should be retained for possible confirmatory testing in the future for a period of at least one year after the expiry date of the newest product (test or reference) or in compliance with the applicable national requirements or international recommendations, as appropriate. Sample retention should be defined and described in an SOP and be specified in the contract between the sponsor and the CRO. Dispensed products that were not administered should also be retained.

15. Case report forms

- 15.1 CRFs should be used to record data on each subject during the course of the trial.
- 15.2 The CRO should have a procedure for designing CRFs if the sponsor requests the CRO to do so. The use of a standardized format or template is recommended. This should be adapted for each study protocol in accordance with the requirements for that particular study. The CRF should be reviewed against other trial documentation, such as the protocol

- and trial database, to ensure that appropriate information and data are captured and that the CRF is consistent with other trial documentation.
- 15.3 The data to be collected on each volunteer should be specified in the trial protocol. Any data to be recorded directly on the CRF (i.e. no prior written or electronic record of data), and to be considered to be source data, should be identified in the protocol.
 - 15.4 CRFs should reflect the actual results obtained during the study and allow easy access for verification, audit and inspection of the data.
 - 15.5 Appropriate procedures should be established and followed to document the investigator's certification of the accuracy of CRFs. Any errors or omissions should be clarified with the investigator, corrected, dated and signed and explained on the CRF.
 - 15.6 Copies of the clinical laboratory reports and all ECGs should be included with the CRFs for each subject and should be submitted together with the dossier, if applicable, in accordance with the requirements of the regulatory authority to which the dossier is submitted.

16. Volunteers and recruitment methods

Note: The selection of subjects should be performed sufficiently far in advance to ensure that a sufficient number of subjects will be available for the study. The last-minute selection of additional subjects may result in noncompliance with the inclusion and exclusion criteria, possibly compromising the safety of the subjects and the integrity of the trial data. The use of a generic screening process to select a pool of subjects that can be enrolled in any BE study conducted at the CRO (unless the protocol foresees specific inclusion or exclusion criteria) can help to achieve this goal.

- 16.1 Procedures for the recruitment of volunteers should be available and should include a description of the potential methods that can be used by the CRO for this purpose. A database should be maintained on volunteers, to avoid cross-participation and to specify a minimum time that should elapse between a volunteer's participation in one study and the next. Access to the database should be password controlled in order to secure confidential information on volunteers or subjects.
- 16.2 Identification of volunteers and subjects should be ensured by reliable means. If a biometric system is used, this system should be periodically validated, as well as after any change made to the validated system that could affect its function.

- 16.3 The informed consent of potential subjects should be obtained for any screening procedures required to determine eligibility for the study in addition to informed consent for participation in the research portion of the study.
- 16.4 Criteria for subject selection (inclusion and exclusion criteria) and screening procedures should be described in the clinical trial protocol.
- 16.5 The results of subject screening and of trial participation should be recorded in a validated database maintained by the CRO. If a regional or national volunteer database exists, then this should be checked to find out whether any of the subjects have participated in a previous trial and participation data should be uploaded to this central repository to prevent over-volunteering. Access to the database should be password controlled in order to secure confidential subject information.
- 16.6 Ideally the CRO's database should record and allow the users to query:
- contact details;
 - sex;
 - status: e.g. eligible, disqualified, not eligible, quarantined, and the reason for this status if applicable;
 - date and place of last study participation, if applicable/if known;
 - date of last screening;
 - a unique code assigned to the subject which will never change;
 - outcome of last trial: e.g. completed, randomized but not dosed, withdrawn for personal reasons, withdrawn for medical reasons.

These data should be backed up daily and be available for review at any time.

- 16.7 Medical records should be generated for each subject and should include information obtained during each screening visit and from each study in which the subject has participated, which could be relevant for the inclusion and follow-up of the subject in subsequent trials. Access to previous medical records for individual subjects should be available and a consistency check conducted where trial-specific medical records are generated. This is important to ensure that safety issues can be assessed before a subject's enrolment in a study.

17. Food and fluids

- 17.1 As meals can significantly affect absorption of active pharmaceutical ingredients, fasting and meals should be standardized and adequately controlled and scheduled during the study days. The CRO should be able to arrange for standardized meals, snacks and drinks for the study subjects as described in the clinical trial protocol.
- 17.2 Records should be maintained of the timing, duration and amount of food and fluids consumed. Prior to samples being obtained from ambulatory subjects, they should be asked about their food and drink consumption, if the protocol contains specific requirements.
- 17.3 Standardized meals should be designed by a dietitian with appropriate qualifications, training and experience. If such services are contracted out, a formal contract with terms of reference should be available.

18. Safety, adverse events and adverse event reporting

- 18.1 Appropriate study planning includes adequate evaluation of risk to the subjects. The study should be planned, organized, performed and monitored so that the safety profile will be acceptable, including to the volunteers.
- 18.2 First-aid equipment and appropriate rescue medication should be available and ready for emergency use at the study site where there should be adequate facilities for the proper care of subjects who require emergency or other medical treatment. Any treatment given to a subject should be documented and included in the CRF and in the supporting documentation, as necessary.
- 18.3 A medical doctor should be responsible for medical decisions in the case of adverse events and for notifying the relevant health authorities, the sponsor and, when applicable, the ethics committee, without delay in the case of serious adverse events. Appropriate timelines should be respected in accordance with national regulations.
- 18.4 The CRO should have appropriate adverse event registration and reporting forms, which should be provided to the investigator; these forms can be part of the CRF. If required the sponsor's forms may be used.

C. BIOANALYTICAL SECTION

Note: The measurement of analyte concentrations (API or metabolites) may be performed by the same CRO as conducted the clinical study, or this work may be contracted to another laboratory or CRO.

19. Method development

- 19.1 The bioanalytical laboratory should provide a detailed description of how a bioanalytical method was developed. The laboratory should keep a copy of any publications used in developing the bioanalytical method. The modifications and adaptations to the published method made by the laboratory should be documented.
- 19.2 Selection of the internal standard should be justifiable by sound scientific principles. In general, the chemical and physical properties of the internal standard should be as close to those of the analyte as possible. Both stable isotope-labelled and non-isotope-labelled internal standards are acceptable, although the use of a stable isotope-labelled internal standard is recommended when MS methods are used. The selection of a stable isotope-labelled internal standard should take into consideration factors such as the isotope labelling positions in order to limit the risk of exchange reactions.
- 19.3 The procedure for method development should ensure that methods are created in a manner that will minimize any potential human error.

20. Method validation

The most up-to-date guidelines available from stringent regulatory authorities (SRAs) on the topic of bioanalytical method validation should be followed.

- 20.1 Validation requirements for the analytical method should be described in the protocol. There should be separate SOPs for analytical method validation.
- 20.2 Data to support the stability of the samples under the stated conditions and period of storage should be available, preferably before the start of the study.
- 20.3 Method validation should be performed with at least one run that is comparable in length to those that are expected to be used for analysis of samples.

21. Sample collection, storage and handling of biological material

- 21.1 The specification of the samples (serum, plasma or urine), sampling method, volume and number of samples should be stated in the clinical trial protocol and in the information provided to the volunteers.
- 21.2 There should be documented procedures for the collection, preparation, transport or shipping and storage of samples.
- 21.3 Any specific lighting conditions foreseen by the protocol or other documents should be complied with. This should be documented.
- 21.4 Actual sampling times and deviations from the prespecified sampling times should be recorded. Deviations should be noted in the study report and should be taken into consideration when calculating the pharmacokinetic parameters.
- 21.5 Labelling of collected samples should be clear to ensure correct identification and traceability of each sample.
- 21.6 The conditions for the storage of samples depend on the analyte. However, all storage conditions (e.g. freezer temperature) should be specified in the study protocol, controlled, monitored and recorded throughout the storage period and during transportation. Procedures should be in place to ensure maintenance of sample integrity in case of system failures.
- 21.7 Records of the storage and retrieval of samples should be maintained.
- 21.8 It is recommended to keep duplicate or back-up samples, and to store and ship them separately.
- 21.9 The duration of storage of bioanalytical samples should be specified in the contract between the sponsor and the CRO.
- 21.10 Local requirements for the handling and destruction of any remaining biological materials should be complied with.

22. Analysis of study samples

The most up-to-date guidelines from SRAs on the topic of bioanalytical method validation should be followed. Additionally:

- 22.1 The results of the method validation should be available before the initiation of study sample analysis, with the possible exception of the

evaluation of the long-term stability of the analyte in matrix. However, these results should be available before the study report is issued and should be submitted with the validation report in the application.

- 22.2 Each analytical run should include calibration curve (CC) standards, QC samples and subject samples processed simultaneously. The exact sequence of processing should be documented. All samples collected from a given subject during all trial periods should be analysed in the same run unless scientifically justified (e.g. where the limited stability of samples necessitates the analysis of period one samples before period two is conducted).
- 22.3 Equipment with an adequate capacity should be used to enable all samples in a run to be processed simultaneously, rather than splitting the samples into several extraction batches. However, if using several extraction batches within a single analytical run cannot be avoided, each batch should include QC samples. The acceptance criteria for the analytical run should be defined in an SOP first for the full run, then if the run is acceptable, for each individual extraction batch.
- 22.4 Every effort should be made during method development to avoid carry-over effects. If carry-over cannot be avoided, procedures should be implemented to limit its influence, for instance, by inserting wash samples into runs after samples with a high concentration.
- 22.5 With regard to the use of blank plasma in the preparation of CCs and QCs:
 - the number of freeze–thaw cycles and the duration of storage that a given blank plasma sample can be submitted to should be limited as much as possible to ensure that there is no degradation and/or any change of its properties. Freezing blank plasma in small volumes should be considered to help limit the number of freeze–thaw cycles for any given blank plasma sample;
 - the anticoagulant that was used for the blank plasma should be documented. It should match the anticoagulant that was used in study samples, in nature and in proportion.
- 22.6 With regard to incurred sample reanalysis:
 - incurred sample reanalysis should be performed in line with the European Medicines Agency (EMA) *Guidelines on bioanalytical method validation* (13);
 - large differences between results may indicate analytical issues and should be investigated.

23. Data processing and documentation

- 23.1 Integration settings should be science-based and fully justifiable. Smoothing should be kept low enough not to mask possible interferences and changes in peak geometry.
- 23.2 The different iterations used to obtain a CC should be saved – if a given CC fails, it is not acceptable to exclude CCs which meet acceptance criteria or, similarly, to include CC standards that do not meet criteria, just to make the calibration or the QC standards pass. The source data should contain the original, first evaluation of runs (containing all calibration samples). If several calibration samples are excluded sequentially, the CC obtained at each step should be retained to document that the criteria for excluding the next sample were met. If electronic raw data are used it is acceptable to save only the final calibration if it is possible to revert to the initial calibration during an inspection. The process and criteria for acceptance and exclusion of CC standards should be described in an SOP.
- 23.3 If the first or last calibration sample is rejected, the calibration range should be truncated, i.e. the second calibration sample becomes the lower limit of quantification (LLOQ) in that run (or the penultimate calibration sample becomes the upper limit of quantification (ULOQ)). Samples with a concentration below the revised LLOQ (or above the revised ULOQ) should be reanalysed. Alternatively, the whole run may be repeated, but this is not the preferred option.
- 23.4 Internal standard variation should be trended and used as part of the verifications of result validity. Significant changes in internal standard response could signal an analytical problem that requires an investigation and/or sample reanalysis. Significant differences between the internal standard results of CC standards or QC standards versus samples could also signal problems affecting the reliability of the results.
- 23.5 Full audit trails should be activated at all times and on all analytical instruments in a given facility, before, during and after the method validation and the study of interest.
- 23.6 All original analytical raw data (e.g. calculations, chromatograms and their associated audit trails) should be documented in a manner that will ensure traceability with respect to the sample number, equipment used, date and time of analysis and the name(s) of the technician(s). If several audit trail files are generated, all should be retained (e.g. results table audit trail, project audit trail and instrument audit trail).

- 23.7 Each data point should be traceable to a specific sample, including sample number, time of collection of the sample, time of centrifugation, if applicable, time when the sample was placed in the freezer and time of sample analysis, to be able to determine whether any aberrant results might have been caused by sample mishandling.

24. Good laboratory practices

- 24.1 Although most GLP guidelines (2) apply formally only to nonclinical safety studies, general principles of GLP should also be followed during the bioanalytical part of BE studies.
- 24.2 Analysis should be performed in a laboratory with established QA systems (14).
- 24.3 Key sample storage systems or other areas requiring environmental controls should be adequately qualified, calibrated and maintained. There should be an alarm system or an adequate monitoring system to control the temperature of the critical stage areas and key sample storage systems, such as freezers. If there is an automatic alarm system it has to be tested regularly to check its functionality. The daily monitoring and all the alarm checks should be documented. There should be a system in place to ensure that timely and appropriate action is taken following an alarm.
- 24.4 For the purposes of qualification and requalification, the temperature-mapping of the freezers and refrigerators should be run for between 24 and 72 hours, or more if justified. Remapping should be done after any significant modifications to the storage units.
- 24.5 Appropriate repairs and/or transfer of samples to other equivalent storage units should be considered whenever an analysis of temperature monitoring records shows unexplained variability outside normal operating limits.
- 24.6 Balances, other measuring devices and equipment and instruments used during the conduct of a trial should be periodically calibrated and verified before use. They should be fit for their intended purpose.
- 24.7 There should be SOPs for the operation, use, calibration, checks and preventive maintenance of equipment. Records should be maintained. Items of equipment used during the course of the trial should be identified to enable verification that they have been appropriately qualified and calibrated.

- 24.8 Chemicals, reference substances, reagents, solvents and solutions should be labelled to indicate identity, purity, concentration (if appropriate), expiry date and specific storage instructions. Information concerning source, preparation date and stability should be available.

D. PHARMACOKINETIC, STATISTICAL CALCULATIONS AND REPORTING SECTION

25. Pharmacokinetic and statistical calculations

- 25.1 The statistical model underlying any primary BE analysis should be stated in the protocol and/or a statistical analysis plan. It should be made clear which factors are fixed and which are random and whether the model is a mixed effects model, a normal linear model, or another type. If the methods of statistical analysis are amended following approval of the protocol then this should be documented in a protocol amendment and should also be reported in the clinical study report together with the reason for change.
- 25.2 Calculations should be made by suitably qualified personnel (see section 8: Personnel).
- 25.3 The means of performing pharmacokinetic and statistical calculations (both software and scripts) should be specified in the study protocol and/or a pharmacokinetic analysis plan and a statistical analysis plan. Data analysis should conform to these requirements. This should include the manner in which area under the curve from time zero to infinity (AUC_{inf}) is derived (i.e. how the points used for extrapolation are selected).
- 25.4 Calculations should be made using validated software and scripts. Software and scripts should be validated or qualified using an SOP, ideally with datasets of varying complexity and with the alpha level(s) actually in use. Self-designed software should be demonstrated as suitable for intended use. For guidance on the use of computerized systems (see section 4: Computer systems) (8).
- 25.5 Data values input should be double-checked by a second qualified person in accordance with an SOP.
- 25.6 A database of trial records should be maintained and should ideally be locked as soon as possible after completion of the study. Once it is locked the study can be unblinded and statistical analysis performed. The dates of locking and statistical analysis should be documented and mentioned in the study report, and the process should be defined in a suitable procedure.

26. Study report

- 26.1 The clinical study report should accurately reflect all the study procedures and results.
- 26.2 The clinical study report should be well written and presented. All deviations from the protocol in the performance of the study should be reported.
- 26.3 There should be no discrepancies between the results stated in the report and the actual original (raw) data.
- 26.4 The report should comply with regulatory requirements as applicable and be presented in a standard format.
- 26.5 The study report should include a report on the bioanalytical part of the trial, including a description of the bioanalytical method used and the report of the validation of this method.
- 26.6 The clinical study report should be approved by the investigator and sponsor. The bioanalytical report should be approved by the study director.
- 26.7 The report should be approved (signed and dated) by the responsible personnel.
- 26.8 All monitoring and audit reports should be available before release of the final study report.

References

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Appendix 1

Example list of standard operating procedures at a contract research organization

The following is an example list of the standard operating procedures (SOPs) that should be used at contract research organizations (CROs). This list is not exhaustive as additional procedures may be necessary depending on the functional and compliance requirements at the facility concerned.

All of the documents at the CRO related to a bioequivalence (BE) clinical trial should be controlled (e.g. version date, date approved, etc.) documents. This control is easier if the documents are in the SOP format or are appended to SOPs.

SOPs should be in place at least for all the critical and major operations in the BE/clinical trial.

Number and name of SOP

1. Conduct of BE study
2. Archiving and retrieval of documents related to a BE study
3. Quality assurance of a BE study; audits of clinical and bioanalytical part of the study and the study report
4. Study files
5. Preparation and review of the protocol for the study
6. Amendment to the protocol for the study
7. Protocol deviations/violation recording and reporting
8. Sponsor/CRO quality assurance agreement on conducting the BE study
9. Process for approval of study by ethical committee
10. Bioavailability (BA)/BE report
11. Study report
12. Written informed consent
13. Obtaining written informed consent for screening from study volunteers
14. Allocation of identification numbers to volunteers at various stages in BE study
15. Investigator's brochure
16. Case report form (CRF)
17. Preparation of CRF, review and completion

18. Data collection and CRF completion
19. Adverse/serious adverse event monitoring, recording and reporting
20. Organizational chart for the study
21. Training of personnel
22. Responsibilities of the members of the research team
23. Monitoring of the study by the sponsor
24. Conduct of pre-study meeting.
25. Study start-up
26. Subject management
27. SOP on mobilization of individuals for registration in volunteer bank
28. Eligibility criteria for registration and registration of individuals in volunteer bank
29. Handling of subject withdrawal
30. Allocation of identification numbers to volunteers at various stages in the biostudy
31. Screening of volunteers enrolled for the study
32. Collection of urine samples from subjects for detection of drugs of abuse and transportation of samples to pathology laboratory
33. Custodian duties
34. Payments to research subjects for BE studies
35. Procedures for entry into and exit from clinical unit
36. Handling of subject check-in and check-out
37. Housekeeping at clinical unit
38. Planning, preparation, evaluation and service of standardized meals for bio-studies
39. Distribution of meals to study subjects
40. Operation and maintenance of nurse call system
41. Administration of oral solid dosage form of the investigational product to human subjects during BE study
42. Cannulation of study subjects
43. Collection of blood samples from study subjects
44. Identification of biological samples
45. Recording of vital signs of subjects
46. Operation and verification of fire alarm system
47. Administration of oxygen to subject from medical oxygen cylinder

48. Emergency care of subjects during BA/BE study
49. Availability of ambulance during BA/BE study
50. Centrifugation and separation of blood samples
51. Storage of plasma and serum samples
52. Segregation of bio-samples
53. Transfer of plasma and serum samples to bioanalytical laboratory
54. Procedures for washing glassware
55. Recording temperature and relative humidity of rooms
56. Instructions on operation and maintenance procedures for all the equipment in the clinical unit
57. Numbering the equipment and logbooks for use in the clinical unit
58. Control of access to pharmacy
59. Pharmacy area requirements
60. Authorization related to investigational product storage, dispensing and retrieval from storage for BE study
61. Investigational product receipt, return and accountability documentation
62. Investigational product receipt and return procedures
63. Storage of investigational products in the pharmacy
64. Line clearance before and after dispensing
65. Documentation of line clearance and dispensing; packaging records and release of dispensed products
66. Retention of samples of investigational products
67. Disposal of archived investigational products
68. Disposal of biological materials
69. Procedures for bioanalytical laboratory (SOPs for the different items of equipment, analytical methods, reagent preparation)
70. Out-of-specification in the laboratory
71. Acceptance criteria for analytical runs: acceptance of calibration curves, acceptance of the runs based on quality control samples results
72. Chromatographic acceptance criteria and chromatogram integration
73. Sample re-assay
74. Pharmacokinetic data from bioanalytical data
75. Procedure for statistical analysis in a BE study