الجمهورية اللبنانية

وزارة الصحة العامة

الـوزيـر

رقم المحفوظات : ص

بيروت في ١٩/١٠/٢٠٢٣

قرار رقم ١٢٤٨/١

الدليل الإرشادي (Guideline) لل Good Clinical Practice (GCP) وال Bioanalytical Method Validation وال Study Sample Analysis

ان وزير الصحة العامة

بناء على المرسوم رقم ٨٣٧٦ تاريخ ٢٠٢١/٩/١٠ (تشكيل الحكومة)

بناء على قانون مزاولة مهنة الصيدلة في لبنان رقم ٣٦٧ تاريخ ١٩٩٤/٨/١ و تعديلاته

بناء على الكتاب رقم ٢٠٢٣/٢/٢١٨٧٩ والموجّه الى كل من نقابة مصانع الأدوية، نقابة مستوردي الادوية وأصحاب المستودعات في لبنان، نقابة صيادلة لبنان ونقابة الأطباء في بيروت والشمال

بناء على توصية منظّمة الصحة العالمية التي اعتمدت أصول ومقاييس تواكب التطوّر الحاصل في المجالات العلمية والتقنيّة والتكنولوجيا والدراسات السريريّة

بناء على توصية منظّمة الصحة العالميّة لاعتماد الـ H M10 guidelines for Bioanalytical Method Validation and Study Sample Analysis

واعتماد أصول ال Clinical Practice وفق ال International Council of Harmonisation (ICH)

بناء على المبادئ الأخلاقية (Ethical Principles) في اتّفاقيّة هلسنكي ١٩٩٦ (Declaration of Helsinki 1996)

بناء على اقتراح مدير عام وزارة الصحة العامة

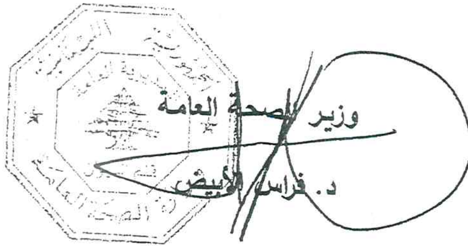
وبعد استطلاع رأي مجلس شورى الدولة (الرأي رقم ٢١٠ /٢٠٢٢-٢٠٢٣ تاريخ ٢٠٢٣/٩/١٤)

يقرر ما يأتـي

المادة الأولى: تُعتمد ال Guidelines المتعلّقة بال Good Clinical Practice (GCP) وال Bioanalytical Method Validation وال Study Sample Analysis المرفقة بهذا القرار.

المادة الثانية: على جميع المؤسسات المعنيّة، الإلتزام بالدلائل الإرشادية المرفقة بهذا القرار على ان يصار الى إجراء الكشف اللازم من قبل وزارة الصحة العامة على هذه المؤسّسات للتأكّد من التزامها بتطبيقها.

المادة الثالثة: يبلغ هذا القرار حيث تدعو الحاجة ويعمل به فور نشره في الجريدة الرسمية.



وزير الصحة العامة

د. فراس الأبيض

يبلغ: - المديرية العامة للصحة
- مكتب منظمة الصحة العالمية
- نقابة صيادلة لبنان
- نقابة مصانع الادوية
- نقابة مستوردي الادوية وأصحاب المستودعات في لبنان
- نقابة المستشفيات في لبنان
- نقابتي أطباء بيروت و الشمال
- مصلحة الصيدلة والدوائر التابعة لها
- الموقع الإلكتروني
- المحفوظات

GUIDELINES FOR GOOD CLINICAL PRACTICE

Guidance for the Clinical Investigations that Involve Human Subjects

Adopted from the International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use
(Updated version)

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GUIDELINES FOR GOOD CLINICAL PRACTICE

INTRODUCTION

The Ministry of Public Health (MOPH) recognizes the importance of the International Council for Harmonisation (ICH) guidelines and adopts them as part of its regulatory framework. By aligning with these guidelines, the MOPH aims to ensure that the regulatory processes and standards for pharmaceuticals in their jurisdiction are consistent with international best practices.

Good Clinical Practice (GCP) is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety and well-being of trial subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki, and that the clinical trial data are credible.

The objective of this ICH GCP Guideline is to provide a unified standard for the European Union (EU), Japan and the United States to facilitate the mutual acceptance of clinical data by the regulatory authorities in these jurisdictions.

The guideline was developed with consideration of the current good clinical practices of the European Union, Japan, and the United States, as well as those of Australia, Canada, the Nordic countries and the World Health Organization (WHO).

This guideline is recommended to be followed when generating clinical trial data that are intended to be submitted to regulatory authorities.

The principles established in this guideline may also be applied to other clinical investigations that may have an impact on the safety and well-being of human subjects.

1. GLOSSARY

1.1 Adverse Drug Reaction (ADR)

In the pre-approval clinical experience with a new medicinal product or its new usages, particularly as the therapeutic dose(s) may not be established: all noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions. The phrase responses to a medicinal product means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

Regarding marketed medicinal products: a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases or for modification of physiological function (see the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

1.2 Adverse Event (AE)

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (see the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

1.3 Amendment (to the protocol)

See Protocol Amendment.

1.4 Applicable Regulatory Requirement(s)

Any law(s) and regulation(s) addressing the conduct of clinical trials of investigational products.

1.5 Approval (in relation to Institutional Review Boards)

The affirmative decision of the IRB that the clinical trial has been reviewed and may be conducted at the institution site within the constraints set forth by the IRB, the institution, Good Clinical Practice (GCP), and the applicable regulatory requirements.

1.6 Audit

A systematic and independent examination of trial related activities and documents to determine whether the evaluated trial related activities were conducted, and the data were recorded, analyzed and accurately reported according to the protocol, sponsor's standard operating procedures (SOPs), Good Clinical Practice (GCP), and the applicable regulatory requirement(s).

1.7 Audit Certificate

A declaration of confirmation by the auditor that an audit has taken place.

1.8 Audit Report

A written evaluation by the sponsor's auditor of the results of the audit.

1.9 Audit Trail

Documentation that allows reconstruction of the course of events.

1.10 Blinding/Masking

A procedure in which one or more parties to the trial are kept unaware of the treatment assignment(s). Single-blinding usually refers to the subject(s) being unaware, and double-blinding usually refers to the subject(s), investigator(s), monitor, and, in some cases, data analyst(s) being unaware of the treatment assignment(s).

1.11 Case Report Form (CRF)

A printed, optical, or electronic document designed to record all of the protocol required information to be reported to the sponsor on each trial subject.

1.12 Clinical Trial/Study

Any investigation in human subjects intended to discover or verify the clinical, pharmacological and/or other pharmacodynamic effects of an investigational product(s), and/or to identify any adverse reactions to an investigational product(s), and/or to study absorption, distribution, metabolism, and excretion of an investigational product(s) with the object of ascertaining its safety and/or efficacy. The terms clinical trial and clinical study are synonymous.

1.13 Clinical Trial/Study Report

A written description of a trial/study of any therapeutic, prophylactic, or diagnostic agent conducted in human subjects, in which the clinical and statistical description, presentations, and analyses are fully integrated into a single report (see the ICH Guideline for Structure and Content of Clinical Study Reports).

1.14 Comparator (Product)

An investigational or marketed product (i.e., active control), or placebo, used as a reference in a clinical trial.

1.15 Compliance (in relation to trials)

Adherence to all the trial-related requirements, Good Clinical Practice (GCP) requirements, and the applicable regulatory requirements.

1.16 Confidentiality

Prevention of disclosure, to other than authorized individuals, of a sponsor's proprietary information or of a subject's identity.

1.17 Contract

A written, dated, and signed agreement between two or more involved parties that sets out any arrangements on delegation and distribution of tasks and obligations and, if appropriate, on financial matters. The protocol may serve as the basis of a contract.

1.18 Coordinating Committee

A committee that a sponsor may organize to coordinate the conduct of a multicentre trial.

1.19 Coordinating Investigator

An investigator assigned the responsibility for the coordination of investigators at different centres participating in a multicentre trial.

1.20 Contract Research Organization (CRO)

A person or an organization (commercial, academic, or other) contracted by the sponsor to perform one or more of a sponsor's trial-related duties and functions.

1.21 Direct Access

Permission to examine, analyze, verify, and reproduce any records and reports that are important to evaluation of a clinical trial. Any party (e.g., domestic and foreign regulatory authorities, sponsor's monitors and auditors) with direct access should take all reasonable precautions within the constraints of the applicable regulatory requirement(s) to maintain the confidentiality of subjects' identities and sponsor’s proprietary information.

1.22 Documentation

All records, in any form (including, but not limited to, written, electronic, magnetic, and optical records, and scans, x-rays, and electrocardiograms) that describe or record the methods, conduct, and/or results of a trial, the factors affecting a trial, and the actions taken.

1.23 Essential Documents

Documents which individually and collectively permit evaluation of the conduct of a study and the quality of the data produced (see 8. Essential Documents for the Conduct of a Clinical Trial).

1.24 Good Clinical Practice (GCP)

A standard for the design, conduct, performance, monitoring, auditing, recording, analyses, and reporting of clinical trials that provides assurance that the data and reported results are credible and accurate, and that the rights, integrity, and confidentiality of trial subjects are protected.

1.25 Independent Data-Monitoring Committee (IDMC) (Data and Safety Monitoring Board, Monitoring Committee, Data Monitoring Committee)

An independent data-monitoring committee that may be established by the sponsor to assess at intervals the progress of a clinical trial, the safety data, and the critical efficacy endpoints, and to recommend to the sponsor whether to continue, modify, or stop a trial.

1.26 Impartial Witness

A person, who is independent of the trial, who cannot be unfairly influenced by people involved with the trial, who attends the informed consent process if the subject or the subject’s legally acceptable representative cannot read, and who reads the informed consent form and any other written information supplied to the subject.

1.27 Independent Ethics Committee (IEC)

An independent body (a review board or a committee, institutional, regional, national, or supranational), constituted of medical professionals and non-medical members, whose responsibility it is to ensure the protection of the rights, safety and well-being of human subjects involved in a trial and to provide public assurance of that protection, by, among other things, reviewing and approving/providing favourable opinion on, the trial protocol, the suitability of the investigator(s), facilities, and the methods and material to be used in obtaining and documenting informed consent of the trial subjects.

The legal status, composition, function, operations and regulatory requirements pertaining to Independent Ethics Committees may differ among countries, but should allow the Independent Ethics Committee to act in agreement with GCP as described in this guideline.

1.28 Informed Consent

A process by which a subject voluntarily confirms his or her willingness to participate in a particular trial, after having been informed of all aspects of the trial that are relevant to the subject's decision to participate. Informed consent is documented by means of a written, signed and dated informed consent form.

1.29 Inspection

The act by a regulatory authority(ies) of conducting an official review of documents, facilities, records, and any other resources that are deemed by the authority(ies) to be related to the clinical trial and that may be located at the site of the trial, at the sponsor's and/or contract research organization’s (CRO’s) facilities, or at other establishments deemed appropriate by the regulatory authority(ies).

1.30 Institution (medical)

Any public or private entity or agency or medical or dental facility where clinical trials are conducted.

1.31 Institutional Review Board (IRB)

An independent body constituted of medical, scientific, and non-scientific members, whose responsibility is to ensure the protection of the rights, safety and well-being of human subjects involved in a trial by, among other things, reviewing, approving, and providing continuing review of trial protocol and amendments and of the methods and material to be used in obtaining and documenting informed consent of the trial subjects.

1.32 Interim Clinical Trial/Study Report

A report of intermediate results and their evaluation based on analyses performed during the course of a trial.

1.33 Investigational Product

A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.

1.34 Investigator

A person responsible for the conduct of the clinical trial at a trial site. If a trial is conducted by a team of individuals at a trial site, the investigator is the responsible leader of the team and may be called the principal investigator. See also Subinvestigator.

1.35 Investigator/Institution

An expression meaning "the investigator and/or institution, where required by the applicable regulatory requirements".

1.36 Investigator's Brochure

A compilation of the clinical and nonclinical data on the investigational product(s) which is relevant to the study of the investigational product(s) in human subjects (see 7. Investigator’s Brochure).

1.37 Legally Acceptable Representative

An individual or juridical or other body authorized under applicable law to consent, on behalf of a prospective subject, to the subject's participation in the clinical trial.

1.38 Monitoring

The act of overseeing the progress of a clinical trial, and of ensuring that it is conducted, recorded, and reported in accordance with the protocol, Standard Operating Procedures (SOPs), Good Clinical Practice (GCP), and the applicable regulatory requirement(s).

1.39 Monitoring Report

A written report from the monitor to the sponsor after each site visit and/or other trial-related communication according to the sponsor's SOPs.

1.40 Multicentre Trial

A clinical trial conducted according to a single protocol but at more than one site, and therefore, carried out by more than one investigator.

1.41 Nonclinical Study

Biomedical studies not performed on human subjects.

1.42 Opinion (in relation to Independent Ethics Committee)

The judgement and/or the advice provided by an Independent Ethics Committee (IEC).

1.43 Original Medical Record

See Source Documents.

1.44 Protocol

A document that describes the objective(s), design, methodology, statistical considerations, and organization of a trial. The protocol usually also gives the background and rationale for the trial, but these could be provided in other protocol referenced documents. Throughout the ICH GCP Guideline the term protocol refers to protocol and protocol amendments.

1.45 Protocol Amendment

A written description of a change(s) to or formal clarification of a protocol.

1.46 Quality Assurance (QA)

All those planned and systematic actions that are established to ensure that the trial is performed and the data are generated, documented (recorded), and reported in compliance with Good Clinical Practice (GCP) and the applicable regulatory requirement(s).

1.47 Quality Control (QC)

The operational techniques and activities undertaken within the quality assurance system to verify that the requirements for quality of the trial-related activities have been fulfilled.

1.48 Randomization

The process of assigning trial subjects to treatment or control groups using an element of chance to determine the assignments in order to reduce bias.

1.49 Regulatory Authorities

Bodies having the power to regulate. In the ICH GCP Guidelines the expression Regulatory Authorities includes the authorities that review submitted clinical data and those that conduct inspections (see 1.29). These bodies are sometimes referred to as competent authorities.

1.50 Serious Adverse Event (SAE) or Serious Adverse Drug Reaction (Serious ADR)

Any untoward medical occurrence that at any dose:

- results in death,

- is life-threatening,

- requires inpatient hospitalization or prolongation of existing hospitalization,

- results in persistent or significant disability/incapacity,

or

- is a congenital anomaly/birth defect

(see the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

1.51 Source Data

All information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

1.52 Source Documents

Original documents, data, and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial).

1.53 Sponsor

An individual, company, institution, or organization which takes responsibility for the initiation, management, and/or financing of a clinical trial.

1.54 Sponsor-Investigator

An individual who both initiates and conducts, alone or with others, a clinical trial, and under whose immediate direction the investigational product is administered to, dispensed to, or used by a subject. The term does not include any person other than an individual (e.g., it does not include a corporation or an agency). The obligations of a sponsor-investigator include both those of a sponsor and those of an investigator.

1.55 Standard Operating Procedures (SOPs)

Detailed, written instructions to achieve uniformity of the performance of a specific function.

1.56 Subinvestigator

Any individual member of the clinical trial team designated and supervised by the investigator at a trial site to perform critical trial-related procedures and/or to make important trial-related decisions (e.g., associates, residents, research fellows). See also Investigator.

1.57 Subject/Trial Subject

An individual who participates in a clinical trial, either as a recipient of the investigational product(s) or as a control.

1.58 Subject Identification Code

A unique identifier assigned by the investigator to each trial subject to protect the subject's identity and used in lieu of the subject's name when the investigator reports adverse events and/or other trial related data.

1.59 Trial Site

The location(s) where trial-related activities are actually conducted.

1.60 Unexpected Adverse Drug Reaction

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product) (see the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

1.61 Vulnerable Subjects

Individuals whose willingness to volunteer in a clinical trial may be unduly influenced by the expectation, whether justified or not, of benefits associated with participation, or of a retaliatory response from senior members of a hierarchy in case of refusal to participate. Examples are members of a group with a hierarchical structure, such as medical, pharmacy, dental, and nursing students, subordinate hospital and laboratory personnel, employees of the pharmaceutical industry, members of the armed forces, and persons kept in detention. Other vulnerable subjects include patients with incurable diseases, persons in nursing homes, unemployed or impoverished persons, patients in emergency situations, ethnic minority groups, homeless persons, nomads, refugees, minors, and those incapable of giving consent.

1.62 Well-being (of the trial subjects)

The physical and mental integrity of the subjects participating in a clinical trial.

1.63 Certified Copy

A copy (irrespective of the type of media used) of the original record that has been verified (i.e., by a dated signature or by generation through a validated process) to have the same information, including data that describe the context, content, and structure, as the original.

1.64 Monitoring Plan

A document that describes the strategy, methods, responsibilities, and requirements for monitoring the trial.

1.65 Validation of Computerized Systems

A process of establishing and documenting that the specified requirements of a computerized system can be consistently fulfilled from design until decommissioning of the system or transition to a new system. The approach to validation should be based on a risk assessment that takes into consideration the intended use of the system and the potential of the system to affect human subject protection and reliability of trial results.

2. THE PRINCIPLES OF ICH GCP

2.1 Clinical trials should be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with GCP and the applicable regulatory requirement(s).

2.2 Before a trial is initiated, foreseeable risks and inconveniences should be weighed against the anticipated benefit for the individual trial subject and society. A trial should be initiated and continued only if the anticipated benefits justify the risks.

2.3 The rights, safety, and well-being of the trial subjects are the most important considerations and should prevail over interests of science and society.

2.4 The available nonclinical and clinical information on an investigational product should be adequate to support the proposed clinical trial.

2.5 Clinical trials should be scientifically sound, and described in a clear, detailed protocol.

2.6 A trial should be conducted in compliance with the protocol that has received prior institutional review board (IRB)/independent ethics committee (IEC) approval/favourable opinion.

2.7 The medical care given to, and medical decisions made on behalf of, subjects should always be the responsibility of a qualified physician or, when appropriate, of a qualified dentist.

2.8 Each individual involved in conducting a trial should be qualified by education, training, and experience to perform his or her respective task(s).

2.9 Freely given informed consent should be obtained from every subject prior to clinical trial participation.

2.10 All clinical trial information should be recorded, handled, and stored in a way that allows its accurate reporting, interpretation and verification.

This principle applies to all records referenced in this guideline, irrespective of the type of media used.

2.11 The confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).

2.12 Investigational products should be manufactured, handled, and stored in accordance with applicable good manufacturing practice (GMP). They should be used in accordance with the approved protocol.

2.13 Systems with procedures that assure the quality of every aspect of the trial should be implemented.

Aspects of the trial that are essential to ensure human subject protection and reliability of trial results should be the focus of such systems.

3. INSTITUTIONAL REVIEW BOARD/INDEPENDENT ETHICS COMMITTEE (IRB/IEC)

3.1 Responsibilities

3.1.1 An IRB/IEC should safeguard the rights, safety, and well-being of all trial subjects. Special attention should be paid to trials that may include vulnerable subjects.

3.1.2 The IRB/IEC should obtain the following documents:

trial protocol(s)/amendment(s), written informed consent form(s) and consent form updates that the investigator proposes for use in the trial, subject recruitment procedures (e.g., advertisements), written information to be provided to subjects, Investigator's Brochure (IB), available safety information, information about payments and compensation available to subjects, the investigator's current curriculum vitae and/or other documentation evidencing qualifications, and any other documents that the IRB/IEC may need to fulfil its responsibilities.

The IRB/IEC should review a proposed clinical trial within a reasonable time and document its views in writing, clearly identifying the trial, the documents reviewed and the dates for the following:

- approval/favourable opinion;

- modifications required prior to its approval/favourable opinion;

- disapproval / negative opinion; and

- termination/suspension of any prior approval/favourable opinion.

3.1.3 The IRB/IEC should consider the qualifications of the investigator for the proposed trial, as documented by a current curriculum vitae and/or by any other relevant documentation the IRB/IEC requests.

3.1.4 The IRB/IEC should conduct continuing review of each ongoing trial at intervals appropriate to the degree of risk to human subjects, but at least once per year.

3.1.5 The IRB/IEC may request more information than is outlined in paragraph 4.8.10 be given to subjects when, in the judgement of the IRB/IEC, the additional information would add meaningfully to the protection of the rights, safety and/or well-being of the subjects.

3.1.6 When a non-therapeutic trial is to be carried out with the consent of the subject's legally acceptable representative (see 4.8.12, 4.8.14), the IRB/IEC should determine that the proposed protocol and/or other document(s) adequately addresses relevant ethical concerns and meets applicable regulatory requirements for such trials.

3.1.7 Where the protocol indicates that prior consent of the trial subject or the subject's legally acceptable representative is not possible (see 4.8.15), the IRB/IEC should determine that the proposed protocol and/or other document(s) adequately addresses relevant ethical concerns and meets applicable regulatory requirements for such trials (i.e., in emergency situations).

3.1.8 The IRB/IEC should review both the amount and method of payment to subjects to assure that neither presents problems of coercion or undue influence on the trial subjects. Payments to a subject should be prorated and not wholly contingent on completion of the trial by the subject.

3.1.9 The IRB/IEC should ensure that information regarding payment to subjects, including the methods, amounts, and schedule of payment to trial subjects, is set forth in the written informed consent form and any other written information to be provided to subjects. The way payment will be prorated should be specified.

3.2 Composition, Functions and Operations

3.2.1 The IRB/IEC should consist of a reasonable number of members, who collectively have the qualifications and experience to review and evaluate the science, medical aspects, and ethics of the proposed trial. It is recommended that the IRB/IEC should include:

(a) At least five members.

(b) At least one member whose primary area of interest is in a nonscientific area.

(c) At least one member who is independent of the institution/trial site.

Only those IRB/IEC members who are independent of the investigator and the sponsor of the trial should vote/provide opinion on a trial-related matter.

A list of IRB/IEC members and their qualifications should be maintained.

3.2.2 The IRB/IEC should perform its functions according to written operating procedures, should maintain written records of its activities and minutes of its meetings, and should comply with GCP and with the applicable regulatory requirement(s).

3.2.3 An IRB/IEC should make its decisions at announced meetings at which at least a quorum, as stipulated in its written operating procedures, is present.

3.2.4 Only members who participate in the IRB/IEC review and discussion should vote/provide their opinion and/or advise.

3.2.5 The investigator may provide information on any aspect of the trial, but should not participate in the deliberations of the IRB/IEC or in the vote/opinion of the IRB/IEC.

3.2.6 An IRB/IEC may invite nonmembers with expertise in special areas for assistance.

3.3 Procedures

The IRB/IEC should establish, document in writing, and follow its procedures, which should include:

3.3.1 Determining its composition (names and qualifications of the members) and the authority under which it is established.

3.3.2 Scheduling, notifying its members of, and conducting its meetings.

3.3.3 Conducting initial and continuing review of trials.

3.3.4 Determining the frequency of continuing review, as appropriate.

3.3.5 Providing, according to the applicable regulatory requirements, expedited review and approval/favourable opinion of minor change(s) in ongoing trials that have the approval/favourable opinion of the IRB/IEC.

3.3.6 Specifying that no subject should be admitted to a trial before the IRB/IEC issues its written approval/favourable opinion of the trial.

3.3.7 Specifying that no deviations from, or changes of, the protocol should be initiated without prior written IRB/IEC approval/favourable opinion of an appropriate amendment, except when necessary to eliminate immediate hazards to the subjects or when the change(s) involves only logistical or administrative aspects of the trial (e.g., change of monitor(s), telephone number(s)) (see 4.5.2).

3.3.8 Specifying that the investigator should promptly report to the IRB/IEC:

(a) Deviations from, or changes of, the protocol to eliminate immediate hazards to the trial subjects (see 3.3.7, 4.5.2, 4.5.4).

(b) Changes increasing the risk to subjects and/or affecting significantly the conduct of the trial (see 4.10.2).

(c) All adverse drug reactions (ADRs) that are both serious and unexpected.

(d) New information that may affect adversely the safety of the subjects or the conduct of the trial.

3.3.9 Ensuring that the IRB/IEC promptly notify in writing the investigator/institution concerning:

(a) Its trial-related decisions/opinions.

(b) The reasons for its decisions/opinions.

(c) Procedures for appeal of its decisions/opinions.

3.4 Records

The IRB/IEC should retain all relevant records (e.g., written procedures, membership lists, lists of occupations/affiliations of members, submitted documents, minutes of meetings, and correspondence) for a period of at least 3-years after completion of the trial and make them available upon request from the regulatory authority(ies).

The IRB/IEC may be asked by investigators, sponsors or regulatory authorities to provide its written procedures and membership lists.

4. INVESTIGATOR

4.1 Investigator's Qualifications and Agreements

4.1.1 The investigator(s) should be qualified by education, training, and experience to assume responsibility for the proper conduct of the trial, should meet all the qualifications specified by the applicable regulatory requirement(s), and should provide evidence of such qualifications through up-to-date curriculum vitae and/or other relevant documentation requested by the sponsor, the IRB/IEC, and/or the regulatory authority(ies).

4.1.2 The investigator should be thoroughly familiar with the appropriate use of the investigational product(s), as described in the protocol, in the current Investigator's Brochure, in the product information and in other information sources provided by the sponsor.

4.1.3 The investigator should be aware of, and should comply with, GCP and the applicable regulatory requirements.

4.1.4 The investigator/institution should permit monitoring and auditing by the sponsor, and inspection by the appropriate regulatory authority(ies).

4.1.5 The investigator should maintain a list of appropriately qualified persons to whom the investigator has delegated significant trial-related duties.

4.2 Adequate Resources

4.2.1 The investigator should be able to demonstrate (e.g., based on retrospective data) a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

4.2.2 The investigator should have sufficient time to properly conduct and complete the trial within the agreed trial period.

4.2.3 The investigator should have available an adequate number of qualified staff and adequate facilities for the foreseen duration of the trial to conduct the trial properly and safely.

4.2.4 The investigator should ensure that all persons assisting with the trial are adequately informed about the protocol, the investigational product(s), and their trial-related duties and functions.

4.2.5 The investigator is responsible for supervising any individual or party to whom the investigator delegates trial-related duties and functions conducted at the trial site.

4.2.6 If the investigator/institution retains the services of any individual or party to perform trial-related duties and functions, the investigator/institution should ensure this individual or party is qualified to perform those trial-related duties and functions and should implement procedures to ensure the integrity of the trial-related duties and functions performed and any data generated.

4.3 Medical Care of Trial Subjects

4.3.1 A qualified physician (or dentist, when appropriate), who is an investigator or a sub-investigator for the trial, should be responsible for all trial-related medical (or dental) decisions.

4.3.2 During and following a subject's participation in a trial, the investigator/institution should ensure that adequate medical care is provided to a subject for any adverse events, including clinically significant laboratory values, related to the trial. The investigator/institution should inform a subject when medical care is needed for intercurrent illness(es) of which the investigator becomes aware.

4.3.3 It is recommended that the investigator inform the subject's primary physician about the subject's participation in the trial if the subject has a primary physician and if the subject agrees to the primary physician being informed.

4.3.4 Although a subject is not obliged to give his/her reason(s) for withdrawing prematurely from a trial, the investigator should make a reasonable effort to ascertain the reason(s), while fully respecting the subject's rights.

4.4 Communication with IRB/IEC

4.4.1 Before initiating a trial, the investigator/institution should have written and dated approval/favourable opinion from the IRB/IEC for the trial protocol, written informed consent form, consent form updates, subject recruitment procedures (e.g., advertisements), and any other written information to be provided to subjects.

4.4.2 As part of the investigator's/institution’s written application to the IRB/IEC, the investigator/institution should provide the IRB/IEC with a current copy of the Investigator's Brochure. If the Investigator's Brochure is updated during the trial, the investigator/institution should supply a copy of the updated Investigator’s Brochure to the IRB/IEC.

4.4.3 During the trial the investigator/institution should provide to the IRB/IEC all documents subject to review.

4.5 Compliance with Protocol

4.5.1 The investigator/institution should conduct the trial in compliance with the protocol agreed to by the sponsor and, if required, by the regulatory authority(ies) and which was given approval/favourable opinion by the IRB/IEC. The investigator/institution and the sponsor should sign the protocol, or an alternative contract, to confirm agreement.

4.5.2 The investigator should not implement any deviation from, or changes of the protocol without agreement by the sponsor and prior review and documented approval/favourable opinion from the IRB/IEC of an amendment, except where necessary to eliminate an immediate hazard(s) to trial subjects, or when the change(s) involves only logistical or administrative aspects of the trial (e.g., change in monitor(s), change of telephone number(s)).

4.5.3 The investigator, or person designated by the investigator, should document and explain any deviation from the approved protocol.

4.5.4 The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB/IEC approval/favourable opinion. As soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted:

(a) to the IRB/IEC for review and approval/favourable opinion,

(b) to the sponsor for agreement and, if required,

(c) to the regulatory authority(ies).

4.6 Investigational Product(s)

4.6.1 Responsibility for investigational product(s) accountability at the trial site(s) rests with the investigator/institution.

4.6.2 Where allowed/required, the investigator/institution may/should assign some or all of the investigator's/institution’s duties for investigational product(s) accountability at the trial site(s) to an appropriate pharmacist or another appropriate individual who is under the supervision of the investigator/institution..

4.6.3 The investigator/institution and/or a pharmacist or other appropriate individual, who is designated by the investigator/institution, should maintain records of the product's delivery to the trial site, the inventory at the site, the use by each subject, and the return to the sponsor or alternative disposition of unused product(s). These records should include dates, quantities, batch/serial numbers, expiration dates (if applicable), and the unique code numbers assigned to the investigational product(s) and trial subjects. Investigators should maintain records that document adequately that the subjects were provided the doses specified by the protocol and reconcile all investigational product(s) received from the sponsor.

4.6.4 The investigational product(s) should be stored as specified by the sponsor (see 5.13.2 and 5.14.3) and in accordance with applicable regulatory requirement(s).

4.6.5 The investigator should ensure that the investigational product(s) are used only in accordance with the approved protocol.

4.6.6 The investigator, or a person designated by the investigator/institution, should explain the correct use of the investigational product(s) to each subject and should check, at intervals appropriate for the trial, that each subject is following the instructions properly.

4.7 Randomization Procedures and Unblinding

The investigator should follow the trial's randomization procedures, if any, and should ensure that the code is broken only in accordance with the protocol. If the trial is blinded, the investigator should promptly document and explain to the sponsor any premature unblinding (e.g., accidental unblinding, unblinding due to a serious adverse event) of the investigational product(s).

4.8 Informed Consent of Trial Subjects

4.8.1 In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. Prior to the beginning of the trial, the investigator should have the IRB/IEC's written approval/favourable opinion of the written informed consent form and any other written information to be provided to subjects.

4.8.2 The written informed consent form and any other written information to be provided to subjects should be revised whenever important new information becomes available that may be relevant to the subject’s consent. Any revised written informed consent form, and written information should receive the IRB/IEC's approval/favourable opinion in advance of use. The subject or the subject’s legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject’s willingness to continue participation in the trial. The communication of this information should be documented.

4.8.3 Neither the investigator, nor the trial staff, should coerce or unduly influence a subject to participate or to continue to participate in a trial.

4.8.4 None of the oral and written information concerning the trial, including the written informed consent form, should contain any language that causes the subject or the subject's legally acceptable representative to waive or to appear to waive any legal rights, or that releases or appears to release the investigator, the institution, the sponsor, or their agents from liability for negligence.

4.8.5 The investigator, or a person designated by the investigator, should fully inform the subject or, if the subject is unable to provide informed consent, the subject's legally acceptable representative, of all pertinent aspects of the trial including the written information and the approval/ favourable opinion by the IRB/IEC.

4.8.6 The language used in the oral and written information about the trial, including the written informed consent form, should be as non-technical as practical and should be understandable to the subject or the subject's legally acceptable representative and the impartial witness, where applicable.

4.8.7 Before informed consent may be obtained, the investigator, or a person designated by the investigator, should provide the subject or the subject's legally acceptable representative ample time and opportunity to inquire about details of the trial and to decide whether or not to participate in the trial. All questions about the trial should be answered to the satisfaction of the subject or the subject's legally acceptable representative.

4.8.8 Prior to a subject’s participation in the trial, the written informed consent form should be signed and personally dated by the subject or by the subject's legally acceptable representative, and by the person who conducted the informed consent discussion.

4.8.9 If a subject is unable to read or if a legally acceptable representative is unable to read, an impartial witness should be present during the entire informed consent discussion. After the written informed consent form and any other written information to be provided to subjects, is read and explained to the subject or the subject’s legally acceptable

representative, and after the subject or the subject's legally acceptable representative has orally consented to the subject's participation in the trial and, if capable of doing so, has signed and personally dated the informed consent form, the witness should sign and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the subject or the subject's legally acceptable representative, and that informed consent was freely given by the subject or the subject's legally acceptable representative.

4.8.10 Both the informed consent discussion and the written informed consent form and any other written information to be provided to subjects should include explanations of the following:

(a) That the trial involves research.

(b) The purpose of the trial.

(c) The trial treatment(s) and the probability for random assignment to each treatment.

(d) The trial procedures to be followed, including all invasive procedures.

(e) The subject's responsibilities.

(f) Those aspects of the trial that are experimental.

(g) The reasonably foreseeable risks or inconveniences to the subject and, when applicable, to an embryo, fetus, or nursing infant.

(h) The reasonably expected benefits. When there is no intended clinical benefit to the subject, the subject should be made aware of this.

(i) The alternative procedure(s) or course(s) of treatment that may be available to the subject, and their important potential benefits and risks.

(j) The compensation and/or treatment available to the subject in the event of trial-related injury.

(k) The anticipated prorated payment, if any, to the subject for participating in the trial.

(l) The anticipated expenses, if any, to the subject for participating in the trial.

(m) That the subject's participation in the trial is voluntary and that the subject may refuse to participate or withdraw from the trial, at any time, without penalty or loss of benefits to which the subject is otherwise entitled.

(n) That the monitor(s), the auditor(s), the IRB/IEC, and the regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations and that, by signing a written informed consent form, the subject or the subject's legally acceptable representative is authorizing such access.

(o) That records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. If the results of the trial are published, the subject's identity will remain confidential.

(p) That the subject or the subject's legally acceptable representative will be informed in a timely manner if information becomes available that may be relevant to the subject's willingness to continue participation in the trial.

(q) The person(s) to contact for further information regarding the trial and the rights of trial subjects, and whom to contact in the event of trial-related injury.

(r) The foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated.

(s) The expected duration of the subject's participation in the trial.

(t) The approximate number of subjects involved in the trial.

4.8.11 Prior to participation in the trial, the subject or the subject's legally acceptable representative should receive a copy of the signed and dated written informed consent form and any other written information provided to the subjects. During a subject's participation in the trial, the subject or the subject's legally acceptable representative should receive a copy of the signed and dated consent form updates and a copy of any amendments to the written information provided to subjects.

4.8.12 When a clinical trial (therapeutic or non-therapeutic) includes subjects who can only be enrolled in the trial with the consent of the subject's legally acceptable representative (e.g., minors, or patients with severe dementia), the subject should be informed about the trial to the extent compatible with the subject's understanding and, if capable, the subject should sign and personally date the written informed consent.

4.8.13 Except as described in 4.8.14, a non-therapeutic trial (i.e., a trial in which there is no anticipated direct clinical benefit to the subject), should be conducted in subjects who personally give consent and who sign and date the written informed consent form.

4.8.14 Non-therapeutic trials may be conducted in subjects with consent of a legally acceptable representative provided the following conditions are fulfilled:

(a) The objectives of the trial can not be met by means of a trial in subjects who can give informed consent personally.

(b) The foreseeable risks to the subjects are low.

(c) The negative impact on the subject's well-being is minimized and low.

(d) The trial is not prohibited by law.

(e) The approval/favourable opinion of the IRB/IEC is expressly sought on the inclusion of such subjects, and the written approval/ favourable opinion covers this aspect.

Such trials, unless an exception is justified, should be conducted in patients having a disease or condition for which the investigational product is intended. Subjects in these trials should be particularly closely monitored and should be withdrawn if they appear to be unduly distressed.

4.8.15 In emergency situations, when prior consent of the subject is not possible, the consent of the subject's legally acceptable representative, if present, should be requested. When prior consent of the subject is not possible, and the subject's legally acceptable representative is not available, enrolment of the subject should require measures described in the protocol and/or elsewhere, with documented approval/favourable opinion by the IRB/IEC, to protect the rights, safety and well-being of the subject and to ensure compliance with applicable regulatory requirements. The subject or the subject's legally acceptable representative should be informed about the trial as soon as possible

and consent to continue and other consent as appropriate (see 4.8.10) should be requested.

4.9. Records and Reports

4.9.0 The investigator/institution should maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial subjects. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained if necessary (e.g., *via* an audit trail).

4.9.1 The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor in the CRFs and in all required reports.

4.9.2 Data reported on the CRF, that are derived from source documents, should be consistent with the source documents or the discrepancies should be explained.

4.9.3 Any change or correction to a CRF should be dated, initialed, and explained (if necessary) and should not obscure the original entry (i.e., an audit trail should be maintained); this applies to both written and electronic changes or corrections (see 5.18.4 (n)). Sponsors should provide guidance to investigators and/or the investigators' designated representatives on making such corrections. Sponsors should have written procedures to assure that changes or corrections in CRFs made by sponsor's designated representatives are documented, are necessary, and are endorsed by the investigator. The investigator should retain records of the changes and corrections.

4.9.4 The investigator/institution should maintain the trial documents as specified in Essential Documents for the Conduct of a Clinical Trial (see 8.) and as required by the applicable regulatory requirement(s). The investigator/institution should take measures to prevent accidental or premature destruction of these documents.

4.9.5 Essential documents should be retained until at least 2-years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2-years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period however if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained (see 5.5.12).

4.9.6 The financial aspects of the trial should be documented in an agreement between the sponsor and the investigator/institution.

4.9.7 Upon request of the monitor, auditor, IRB/IEC, or regulatory authority, the investigator/institution should make available for direct access all requested trial-related records.

4.10. Progress Reports

4.10.1. The investigator should submit written summaries of the trial status to the IRB/IEC annually, or more frequently, if requested by the IRB/IEC.

4.10.2. The investigator should promptly provide written reports to the sponsor, the IRB/IEC (see 3.3.8) and, where applicable, the institution on any changes significantly affecting the conduct of the trial, and/or increasing the risk to subjects.

4.11. Safety Reporting

4.11.1. All serious adverse events (SAEs) should be reported immediately to the sponsor except for those SAEs that the protocol or other document (e.g., Investigator's Brochure) identifies as not needing immediate reporting. The immediate reports should be followed promptly by detailed, written reports. The immediate and follow-up reports should identify subjects by unique code numbers assigned to the trial subjects rather than by the subjects' names, personal identification numbers, and/or addresses. The investigator should also comply with the applicable regulatory requirement(s) related to the reporting of unexpected serious adverse drug reactions to the regulatory authority(ies) and the IRB/IEC.

4.11.2. Adverse events and/or laboratory abnormalities identified in the protocol as critical to safety evaluations should be reported to the sponsor according to the reporting requirements and within the time periods specified by the sponsor in the protocol.

4.11.3. For reported deaths, the investigator should supply the sponsor and the IRB/IEC with any additional requested information (e.g., autopsy reports and terminal medical reports).

4.12. Premature Termination or Suspension of a Trial

If the trial is prematurely terminated or suspended for any reason, the investigator/institution should promptly inform the trial subjects, should assure appropriate therapy and follow-up for the subjects, and, where required by the applicable regulatory requirement(s), should inform the regulatory authority(ies). In addition:

4.12.1. If the investigator terminates or suspends a trial without prior agreement of the sponsor, the investigator should inform the institution where applicable, and the investigator/institution should promptly inform the sponsor and the IRB/IEC, and should provide the sponsor and the IRB/IEC a detailed written explanation of the termination or suspension.

4.12.2. If the sponsor terminates or suspends a trial (see 5.21), the investigator should promptly inform the institution where applicable and the investigator/institution should promptly inform the IRB/IEC and provide the IRB/IEC a detailed written explanation of the termination or suspension.

4.12.3. If the IRB/IEC terminates or suspends its approval/favourable opinion of a trial (see 3.1.2 and 3.3.9), the investigator should inform the institution where applicable and the investigator/institution should promptly notify the sponsor and provide the sponsor with a detailed written explanation of the termination or suspension.

4.13. Final Report(s) by Investigator

Upon completion of the trial, the investigator, where applicable, should inform the institution; the investigator/institution should provide the IRB/IEC with a summary of the trial’s outcome, and the regulatory authority(ies) with any reports required.

5. SPONSOR

5.0 Quality Management

The sponsor should implement a system to manage quality throughout all stages of the trial process.

Sponsors should focus on trial activities essential to ensuring human subject protection and the reliability of trial results. Quality management includes the design of efficient clinical trial protocols and tools and procedures for data collection and processing, as well as the collection of information that is essential to decision making.

The methods used to assure and control the quality of the trial should be proportionate to the risks inherent in the trial and the importance of the information collected. The sponsor should ensure that all aspects of the trial are operationally feasible and should avoid unnecessary complexity, procedures, and data collection. Protocols, case report forms, and other operational documents should be clear, concise, and consistent.

The quality management system should use a risk-based approach as described below.

5.0.1 Critical Process and Data Identification

During protocol development, the sponsor should identify those processes and data that are critical to ensure human subject protection and the reliability of trial results.

5.0.2 Risk Identification

The sponsor should identify risks to critical trial processes and data. Risks should be considered at both the system level (e.g., standard operating procedures, computerized systems, personnel) and clinical trial level (e.g., trial design, data collection, informed consent process).

5.0.3 Risk Evaluation

The sponsor should evaluate the identified risks, against existing risk controls by considering:

(a) The likelihood of errors occurring.
(b) The extent to which such errors would be detectable.
(c) The impact of such errors on human subject protection and reliability of trial results.

5.0.4 Risk Control

The sponsor should decide which risks to reduce and/or which risks to accept. The approach used to reduce risk to an acceptable level should be proportionate to the significance of the risk. Risk reduction activities may be incorporated in protocol design and implementation, monitoring plans, agreements between parties defining roles and responsibilities, systematic safeguards to ensure adherence to standard operating procedures, and training in processes and procedures.

Predefined quality tolerance limits should be established, taking into consideration the medical and statistical characteristics of the variables as well as the statistical design of trial results. Detection of deviations from the predefined quality tolerance limits should trigger an evaluation to determine if action is needed.

5.0.5 Risk Communication

The sponsor should document quality management activities. The sponsor should communicate quality management activities to those who are involved in or affected by such activities, to facilitate risk review and continual improvement during clinical trial execution.

5.0.6 *Risk Review*

The sponsor should periodically review risk control measures to ascertain whether the implemented quality management activities remain effective and relevant, taking into account emerging knowledge and experience.

5.0.7 *Risk Reporting*

The sponsor should describe the quality management approach implemented in the trial and summarize important deviations from the predefined quality tolerance limits and remedial actions taken in the clinical study report (ICH E3, Section 9.6 Data Quality Assurance).

5.1 Quality Assurance and Quality Control

5.1.1 The sponsor is responsible for implementing and maintaining quality assurance and quality control systems with written SOPs to ensure that trials are conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirement(s).

5.1.2 The sponsor is responsible for securing agreement from all involved parties to ensure direct access (see 1.21) to all trial related sites, source data/documents , and reports for the purpose of monitoring and auditing by the sponsor, and inspection by domestic and foreign regulatory authorities.

5.1.3 Quality control should be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

5.1.4 Agreements, made by the sponsor with the investigator/institution and any other parties involved with the clinical trial, should be in writing, as part of the protocol or in a separate agreement.

5.2 Contract Research Organization (CRO)

5.2.1 A sponsor may transfer any or all of the sponsor's trial-related duties and functions to a CRO, but the ultimate responsibility for the quality and integrity of the trial data always resides with the sponsor. The CRO should implement quality assurance and quality control.

5.2.2 Any trial-related duty and function that is transferred to and assumed by a CRO should be specified in writing.

The sponsor should ensure oversight of any trial-related duties and functions carried out on its behalf, including trial-related duties and functions that are subcontracted to another party by the sponsor's contracted CRO(s).

5.2.3 Any trial-related duties and functions not specifically transferred to and assumed by a CRO are retained by the sponsor.

5.2.4 All references to a sponsor in this guideline also apply to a CRO to the extent that a CRO has assumed the trial related duties and functions of a sponsor.

5.3 Medical Expertise

The sponsor should designate appropriately qualified medical personnel who will be readily available to advise on trial related medical questions or problems. If necessary, outside consultant(s) may be appointed for this purpose.

5.4 Trial Design

5.4.1 The sponsor should utilize qualified individuals (e.g., biostatisticians, clinical pharmacologists, and physicians) as appropriate, throughout all stages of the trial process, from designing the protocol and CRFs and planning the analyses to analyzing and preparing interim and final clinical trial reports.

5.4.2 For further guidance: Clinical Trial Protocol and Protocol Amendment(s) (see 6.), the ICH Guideline for Structure and Content of Clinical Study Reports, and other appropriate ICH guidance on trial design, protocol and conduct.

5.5 Trial Management, Data Handling, and Record Keeping

5.5.1 The sponsor should utilize appropriately qualified individuals to supervise the overall conduct of the trial, to handle the data, to verify the data, to conduct the statistical analyses, and to prepare the trial reports.

5.5.2 The sponsor may consider establishing an independent data-monitoring committee (IDMC) to assess the progress of a clinical trial, including the safety data and the critical efficacy endpoints at intervals, and to recommend to the sponsor whether to continue, modify, or stop a trial. The IDMC should have written operating procedures and maintain written records of all its meetings.

5.5.3 When using electronic trial data handling and/or remote electronic trial data systems, the sponsor should:

(a) Ensure and document that the electronic data processing system(s) conforms to the sponsor's established requirements for completeness, accuracy, reliability, and consistent intended performance (i.e., validation).

The sponsor should base their approach to validation of such systems on a risk assessment that takes into consideration the intended use of the system and the Maintains SOPs for using these systems.

The SOPs should cover system setup, installation, and use. The SOPs should describe system validation and functionality testing, data collection and handling, system maintenance, system security measures, change control, data backup, recovery, contingency planning, and decommissioning. The responsibilities of the

sponsor, investigator, and other parties with respect to the use of these computerized systems should be clear, and the users should be provided with training in their use.

(b) Ensure that the systems are designed to permit data changes in such a way that the data changes are documented and that there is no deletion of entered data (i.e., maintain an audit trail, data trail, edit trail).

(c) Maintain a security system that prevents unauthorized access to the data.

(d) Maintain a list of the individuals who are authorized to make data changes (see 4.1.5 and 4.9.3).

(e) Maintain adequate backup of the data.

(f) Safeguard the blinding, if any (e.g., maintain the blinding during data entry and processing).

(g) Ensure the integrity of the data including any data that describe the context, content, and structure. This is particularly important when making changes to the computerized systems, such as software upgrades or migration of data.

5.5.4 If data are transformed during processing, it should always be possible to compare the original data and observations with the processed data.

5.5.5 The sponsor should use an unambiguous subject identification code (see 1.58) that allows identification of all the data reported for each subject.

5.5.6 The sponsor, or other owners of the data, should retain all of the sponsor-specific essential documents pertaining to the trial (see 8. Essential Documents for the Conduct of a Clinical Trial).

5.5.7 The sponsor should retain all sponsor-specific essential documents in conformance with the applicable regulatory requirement(s) of the country(ies) where the product is approved, and/or where the sponsor intends to apply for approval(s).

5.5.8 If the sponsor discontinues the clinical development of an investigational product (i.e., for any or all indications, routes of administration, or dosage forms), the sponsor should maintain all sponsor-specific essential documents for at least 2-years after formal discontinuation or in conformance with the applicable regulatory requirement(s).

5.5.9 If the sponsor discontinues the clinical development of an investigational product, the sponsor should notify all the trial investigators/institutions and all the regulatory authorities.

5.5.10 Any transfer of ownership of the data should be reported to the appropriate authority(ies), as required by the applicable regulatory requirement(s).

5.5.11 The sponsor specific essential documents should be retained until at least 2-years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2-years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period however if required by the applicable regulatory requirement(s) or if needed by the sponsor.

5.5.12 The sponsor should inform the investigator(s)/institution(s) in writing of the need for record retention and should notify the investigator(s)/institution(s) in writing when the trial related records are no longer needed.

5.6 Investigator Selection

5.6.1 The sponsor is responsible for selecting the investigator(s)/institution(s). Each investigator should be qualified by training and experience and should have adequate resources (see 4.1, 4.2) to properly conduct the trial for which the investigator is selected. If organization of a coordinating committee and/or selection of coordinating investigator(s) are to be utilized in multicentre trials, their organization and/or selection are the sponsor's responsibility.

5.6.2 Before entering an agreement with an investigator/institution to conduct a trial, the sponsor should provide the investigator(s)/institution(s) with the protocol and an up-to-date Investigator's Brochure, and should provide sufficient time for the investigator/institution to review the protocol and the information provided.

5.6.3 The sponsor should obtain the investigator's/institution's agreement:

(a) to conduct the trial in compliance with GCP, with the applicable regulatory requirement(s) (see 4.1.3), and with the protocol agreed to by the sponsor and given approval/favourable opinion by the IRB/IEC (see 4.5.1);

(b) to comply with procedures for data recording/reporting;

(c) to permit monitoring, auditing and inspection (see 4.1.4) and

(d) to retain the trial related essential documents until the sponsor informs the investigator/institution these documents are no longer needed (see 4.9.4 and 5.5.12).

The sponsor and the investigator/institution should sign the protocol, or an alternative document, to confirm this agreement.

5.7 Allocation of Responsibilities

Prior to initiating a trial, the sponsor should define, establish, and allocate all trial-related duties and functions.

5.8 Compensation to Subjects and Investigators

5.8.1 If required by the applicable regulatory requirement(s), the sponsor should provide insurance or should indemnify (legal and financial coverage) the investigator/the institution against claims arising from the trial, except for claims that arise from malpractice and/or negligence.

5.8.2 The sponsor's policies and procedures should address the costs of treatment of trial subjects in the event of trial-related injuries in accordance with the applicable regulatory requirement(s).

5.8.3 When trial subjects receive compensation, the method and manner of compensation should comply with applicable regulatory requirement(s).

5.9 Financing

The financial aspects of the trial should be documented in an agreement between the sponsor and the investigator/institution.

5.10 Notification/Submission to Regulatory Authority(ies)

Before initiating the clinical trial(s), the sponsor (or the sponsor and the investigator, if required by the applicable regulatory requirement(s)) should submit any required application(s) to the appropriate authority(ies) for review, acceptance, and/or permission (as required by the applicable regulatory requirement(s)) to begin the trial(s). Any notification/submission should be dated and contain sufficient information to identify the protocol.

5.11 Confirmation of Review by IRB/IEC

5.11.1 The sponsor should obtain from the investigator/institution:

(a) The name and address of the investigator's/institution's IRB/IEC.

(b) A statement obtained from the IRB/IEC that it is organized and operates according to GCP and the applicable laws and regulations.

(c) Documented IRB/IEC approval/favourable opinion and, if requested by the sponsor, a current copy of protocol, written informed consent form(s) and any other written information to be provided to subjects, subject recruiting procedures, and documents related to payments and compensation available to the subjects, and any other documents that the IRB/IEC may have requested.

5.11.2 If the IRB/IEC conditions its approval/favourable opinion upon change(s) in any aspect of the trial, such as modification(s) of the protocol, written informed consent form and any other written information to be provided to subjects, and/or other procedures, the sponsor should obtain from the investigator/institution a copy of the modification(s) made and the date approval/favourable opinion was given by the IRB/IEC.

5.11.3 The sponsor should obtain from the investigator/institution documentation and dates of any IRB/IEC reapprovals/re-evaluations with favourable opinion, and of any withdrawals or suspensions of approval/favourable opinion.

5.12 Information on Investigational Product(s)

5.12.1 When planning trials, the sponsor should ensure that sufficient safety and efficacy data from nonclinical studies and/or clinical trials are available to support human exposure by the route, at the dosages, for the duration, and in the trial population to be studied.

5.12.2 The sponsor should update the Investigator's Brochure as significant new information becomes available (see 7. Investigator's Brochure).

5.13 Manufacturing, Packaging, Labelling, and Coding Investigational Product(s)

5.13.1 The sponsor should ensure that the investigational product(s) (including active comparator(s) and placebo, if applicable) is characterized as appropriate to the stage of development of the product(s), is manufactured in accordance with any applicable GMP, and is coded and labelled in a manner that protects the blinding, if applicable. In addition, the labelling should comply with applicable regulatory requirement(s).

5.13.2 The sponsor should determine, for the investigational product(s), acceptable storage temperatures, storage conditions (e.g., protection from light), storage times, reconstitution fluids and procedures, and devices for product infusion, if any. The sponsor should inform all involved parties (e.g., monitors, investigators, pharmacists, storage managers) of these determinations.

5.13.3 The investigational product(s) should be packaged to prevent contamination and unacceptable deterioration during transport and storage.

5.13.4 In blinded trials, the coding system for the investigational product(s) should include a mechanism that permits rapid identification of the product(s) in case of a medical emergency, but does not permit undetectable breaks of the blinding.

5.13.5 If significant formulation changes are made in the investigational or comparator product(s) during the course of clinical development, the results of any additional studies of the formulated product(s) (e.g., stability, dissolution rate, bioavailability) needed to assess whether these changes would significantly alter the pharmacokinetic profile of the product should be available prior to the use of the new formulation in clinical trials.

5.14 Supplying and Handling Investigational Product(s)

5.14.1 The sponsor is responsible for supplying the investigator(s)/institution(s) with the investigational product(s).

5.14.2 The sponsor should not supply an investigator/institution with the investigational product(s) until the sponsor obtains all required documentation (e.g., approval/favourable opinion from IRB/IEC and regulatory authority(ies)).

5.14.3 The sponsor should ensure that written procedures include instructions that the investigator/institution should follow for the handling and storage of investigational product(s) for the trial and documentation thereof. The procedures should address adequate and safe receipt, handling, storage, dispensing, retrieval of unused product from subjects, and return of unused investigational product(s) to the sponsor (or alternative disposition if authorized by the sponsor and in compliance with the applicable regulatory requirement(s)).

5.14.4 The sponsor should:

(a) Ensure timely delivery of investigational product(s) to the investigator(s).

(b) Maintain records that document shipment, receipt, disposition, return, and destruction of the investigational product(s) (see 8. Essential Documents for the Conduct of a Clinical Trial).

(c) Maintain a system for retrieving investigational products and documenting this retrieval (e.g., for deficient product recall, reclaim after trial completion, expired product reclaim).

(d) Maintain a system for the disposition of unused investigational product(s) and for the documentation of this disposition.

5.14.5 The sponsor should:

(a) Take steps to ensure that the investigational product(s) are stable over the period of use.

(b) Maintain sufficient quantities of the investigational product(s) used in the trials to reconfirm specifications, should this become necessary, and maintain records of batch sample analyses and characteristics. To the extent stability permits, samples should be retained either until the analyses of the trial data are complete or as required by the applicable regulatory requirement(s), whichever represents the longer retention period.

5.15 Record Access

5.15.1 The sponsor should ensure that it is specified in the protocol or other written agreement that the investigator(s)/institution(s) provide direct access to source data/documents for trial-related monitoring, audits, IRB/IEC review, and regulatory inspection.

5.15.2 The sponsor should verify that each subject has consented, in writing, to direct access to his/her original medical records for trial-related monitoring, audit, IRB/IEC review, and regulatory inspection.

5.16 Safety Information

5.16.1 The sponsor is responsible for the ongoing safety evaluation of the investigational product(s).

5.16.2 The sponsor should promptly notify all concerned investigator(s)/institution(s) and the regulatory authority(ies) of findings that could affect adversely the safety of subjects, impact the conduct of the trial, or alter the IRB/IEC's approval/favourable opinion to continue the trial.

5.17 Adverse Drug Reaction Reporting

5.17.1 The sponsor should expedite the reporting to all concerned investigator(s)/institutions(s), to the IRB(s)/IEC(s), where required, and to the regulatory authority(ies) of all adverse drug reactions (ADRs) that are both serious and unexpected.

5.17.2 Such expedited reports should comply with the applicable regulatory requirement(s) and with the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting.

5.17.3 The sponsor should submit to the regulatory authority(ies) all safety updates and periodic reports, as required by applicable regulatory requirement(s).

5.18 Monitoring

5.18.1 Purpose

The purposes of trial monitoring are to verify that:

(a) The rights and well-being of human subjects are protected.

(b) The reported trial data are accurate, complete, and verifiable from source documents.

(c) The conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with the applicable regulatory requirement(s).

5.18.2 Selection and Qualifications of Monitors

(a) Monitors should be appointed by the sponsor.

(b) Monitors should be appropriately trained, and should have the scientific and/or clinical knowledge needed to monitor the trial adequately. A monitor's qualifications should be documented.

(c) Monitors should be thoroughly familiar with the investigational product(s), the protocol, written informed consent form and any other written information to be provided to subjects, the sponsor's SOPs, GCP, and the applicable regulatory requirement(s).

5.18.3 Extent and Nature of Monitoring

The sponsor should ensure that the trials are adequately monitored. The sponsor should determine the appropriate extent and nature of monitoring. The determination of the extent and nature of monitoring should be based on considerations such as the objective, purpose, design, complexity, blinding, size, and endpoints of the trial. In general there is a need for on-site monitoring, before, during, and after the trial; however in exceptional circumstances the sponsor may determine that central monitoring in conjunction with procedures such as investigators' training and meetings, and extensive written guidance can assure appropriate conduct of the trial in accordance with GCP. Statistically controlled sampling may be an acceptable method for selecting the data to be verified.

The sponsor should develop a systematic, prioritized, risk-based approach to monitoring clinical trials. The flexibility in the extent and nature of monitoring described in this section is intended to permit varied approaches that improve the effectiveness and efficiency of monitoring. The sponsor may choose on-site monitoring, a combination of on-site and centralized monitoring, or, where justified, centralized monitoring. The sponsor should document the rationale for the chosen monitoring strategy (e.g., in the monitoring plan).

On-site monitoring is performed at the sites at which the clinical trial is being conducted. Centralized monitoring is a remote evaluation of accumulating data, performed in a timely manner, supported by appropriately qualified and trained persons (e.g., data managers, biostatisticians).

Centralized monitoring processes provide additional monitoring capabilities that can complement and reduce the extent and/or frequency of on-site monitoring and help distinguish between reliable data and potentially unreliable data.

Review, that may include statistical analyses, of accumulating data from centralized monitoring can be used to:

(a) identify missing data, inconsistent data, data outliers, unexpected lack of variability and protocol deviations.

(b) examine data trends such as the range, consistency, and variability of data within and across sites.

(c) evaluate for systematic or significant errors in data collection and reporting at a site or across sites; or potential data manipulation or data integrity problems.

(d) analyze site characteristics and performance metrics.

(e) select sites and/or processes for targeted on-site monitoring.

5.18.4 Monitor's Responsibilities

The monitor(s) in accordance with the sponsor's requirements should ensure that the trial is conducted and documented properly by carrying out the following activities when relevant and necessary to the trial and the trial site:

(a) Acting as the main line of communication between the sponsor and the investigator.

(b) Verifying that the investigator has adequate qualifications and resources (see 4.1, 4.2, 5.6) and remain adequate throughout the trial period, that facilities, including laboratories, equipment, and staff, are adequate to safely and properly conduct the trial and remain adequate throughout the trial period.

(c) Verifying, for the investigational product(s):

  (i) That storage times and conditions are acceptable, and that supplies are sufficient throughout the trial.

  (ii) That the investigational product(s) are supplied only to subjects who are eligible to receive it and at the protocol specified dose(s).

  (iii) That subjects are provided with necessary instruction on properly using, handling, storing, and returning the investigational product(s).

  (iv) That the receipt, use, and return of the investigational product(s) at the trial sites are controlled and documented adequately.

  (v) That the disposition of unused investigational product(s) at the trial sites complies with applicable regulatory requirement(s) and is in accordance with the sponsor.

(d) Verifying that the investigator follows the approved protocol and all approved amendment(s), if any.

(e) Verifying that written informed consent was obtained before each subject's participation in the trial.

(f) Ensuring that the investigator receives the current Investigator's Brochure, all documents, and all trial supplies needed to conduct the trial properly and to comply with the applicable regulatory requirement(s).

(g) Ensuring that the investigator and the investigator's trial staff are adequately informed about the trial.

(h) Verifying that the investigator and the investigator's trial staff are performing the specified trial functions, in accordance with the protocol and any other written agreement between the sponsor and the investigator/institution and have not delegated these functions to unauthorized individuals.

(i) Verifying that the investigator is enrolling only eligible subjects.

(j) Reporting the subject recruitment rate.

(k) Verifying that source documents and other trial records are accurate, complete, kept up-to-date and maintained.

(l) Verifying that the investigator provides all the required reports, notifications, applications, and submissions, and that these documents are accurate, complete, timely, legible, dated, and identify the trial.

(m) Checking the accuracy and completeness of the CRF entries, source documents and other trial-related records against each other. The monitor specifically should verify that:

    (i) The data required by the protocol are reported accurately on the CRFs and are consistent with the source documents.

    (ii) Any dose and/or therapy modifications are well documented for each of the trial subjects.

    (iii) Adverse events, concomitant medications and intercurrent illnesses are reported in accordance with the protocol on the CRFs.

    (iv) Visits that the subjects fail to make, tests that are not conducted, and examinations that are not performed are clearly reported as such on the CRFs.

    (v) All withdrawals and dropouts of enrolled subjects from the trial are reported and explained on the CRFs.

(n) Informing the investigator of any CRF entry error, omission, or illegibility. The monitor should ensure that appropriate corrections, additions, or deletions are made, dated, explained (if necessary), and initialled by the investigator or by a member of the investigator's trial staff who is authorized to initial CRF changes for the investigator. This authorization should be documented.

(o) Determining whether all adverse events (AEs) are appropriately reported within the time periods required by GCP, the protocol, the IRB/IEC, the sponsor, and the applicable regulatory requirement(s).

(p) Determining whether the investigator is maintaining the essential documents (see 8. Essential Documents for the Conduct of a Clinical Trial).

(q) Communicating deviations from the protocol, SOPs, GCP, and the applicable regulatory requirements to the investigator and taking appropriate action designed to prevent recurrence of the detected deviations.

5.18.5 Monitoring Procedures

The monitor(s) should follow the sponsor's established written SOPs as well as those procedures that are specified by the sponsor for monitoring a specific trial.

5.18.6 Monitoring Report

(a) The monitor should submit a written report to the sponsor after each trial-site visit or trial-related communication.

(b) Reports should include the date, site, name of the monitor, and name of the investigator or other individual(s) contacted.

(c) Reports should include a summary of what the monitor reviewed and the monitor's statements concerning the significant findings/facts, deviations and deficiencies, conclusions, actions taken or to be taken and/or actions recommended to secure compliance.

(d) The review and follow-up of the monitoring report with the sponsor should be documented by the sponsor's designated representative.

(e) Reports of on-site and/or centralized monitoring should be provided to the sponsor (including appropriate management and staff responsible for trial and site oversight) in a timely manner for review and follow up. Results of monitoring activities should be documented in sufficient detail to allow verification of compliance with the monitoring plan. Reporting of centralized monitoring activities should be regular and may be independent from site visits.

5.18.7 Monitoring Plan

The sponsor should develop a monitoring plan that is tailored to the specific human subject protection and data integrity risks of the trial. The plan should describe the monitoring strategy, the monitoring responsibilities of all the parties involved, the various monitoring methods to be used, and the rationale for their use. The plan should also emphasize the monitoring of critical data and processes. Particular attention should be given to those aspects that are not routine clinical practice and that require additional training. The monitoring plan should reference the applicable policies and procedures.

5.19 Audit

If or when sponsors perform audits, as part of implementing quality assurance, they should consider:

5.19.1 Purpose

The purpose of a sponsor's audit, which is independent of and separate from routine monitoring or quality control functions, should be to evaluate trial conduct and compliance with the protocol, SOPs, GCP, and the applicable regulatory requirements.

5.19.2 Selection and Qualification of Auditors

(a) The sponsor should appoint individuals, who are independent of the clinical trials/systems, to conduct audits.

(b) The sponsor should ensure that the auditors are qualified by training and experience to conduct audits properly. An auditor's qualifications should be documented.

5.19.3 Auditing Procedures

(a) The sponsor should ensure that the auditing of clinical trials/systems is conducted in accordance with the sponsor's written procedures on what to audit, how to audit, the frequency of audits, and the form and content of audit reports.

(b) The sponsor's audit plan and procedures for a trial audit should be guided by the importance of the trial to submissions to regulatory authorities, the number of subjects in the trial, the type and complexity of the trial, the level of risks to the trial subjects, and any identified problem(s).

(c) The observations and findings of the auditor(s) should be documented.

(d) To preserve the independence and value of the audit function, the regulatory authority(ies) should not routinely request the audit reports. Regulatory authority(ies) may seek access to an audit report on a case by case basis when evidence of serious GCP non-compliance exists, or in the course of legal proceedings.

(e) When required by applicable law or regulation, the sponsor should provide an audit certificate.

5.20 Noncompliance

5.20.1 Noncompliance with the protocol, SOPs, GCP, and/or applicable regulatory requirement(s) by an investigator/institution, or by member(s) of the sponsor's staff should lead to prompt action by the sponsor to secure compliance.

If noncompliance that significantly affects or has the potential to significantly affect human subject protection or reliability of trial results is discovered, the sponsor should perform a root cause analysis and implement appropriate corrective and preventive actions.

5.20.2 If the monitoring and/or auditing identifies serious and/or persistent noncompliance on the part of an investigator/institution, the sponsor should terminate the investigator's/institution’s participation in the trial. When an investigator's/institution’s participation is terminated because of noncompliance, the sponsor should notify promptly the regulatory authority(ies).

5.21 Premature Termination or Suspension of a Trial

If a trial is prematurely terminated or suspended, the sponsor should promptly inform the investigators/institutions, and the regulatory authority(ies) of the termination or suspension and the reason(s) for the termination or suspension. The IRB/IEC should also be informed promptly and provided the reason(s) for the termination or suspension by the sponsor or by the investigator/institution, as specified by the applicable regulatory requirement(s).

5.22 Clinical Trial/Study Reports

Whether the trial is completed or prematurely terminated, the sponsor should ensure that the clinical trial reports are prepared and provided to the regulatory agency(ies) as required by the applicable regulatory requirement(s). The sponsor should also ensure that the clinical trial reports in marketing applications meet the standards of the ICH Guideline for Structure and Content of

Clinical Study Reports. (NOTE: The ICH Guideline for Structure and Content of Clinical Study Reports specifies that abbreviated study reports may be acceptable in certain cases.)

5.23 Multicentre Trials

For multicentre trials, the sponsor should ensure that:

5.23.1 All investigators conduct the trial in strict compliance with the protocol agreed to by the sponsor and, if required, by the regulatory authority(ies), and given approval/favourable opinion by the IRB/IEC.

5.23.2 The CRFs are designed to capture the required data at all multicentre trial sites. For those investigators who are collecting additional data, supplemental CRFs should also be provided that are designed to capture the additional data.

5.23.3 The responsibilities of coordinating investigator(s) and the other participating investigators are documented prior to the start of the trial.

5.23.4 All investigators are given instructions on following the protocol, on complying with a uniform set of standards for the assessment of clinical and laboratory findings, and on completing the CRFs.

5.23.5 Communication between investigators is facilitated.

6. CLINICAL TRIAL PROTOCOL AND PROTOCOL AMENDMENT(S)

The contents of a trial protocol should generally include the following topics. However, site specific information may be provided on separate protocol page(s), or addressed in a separate agreement, and some of the information listed below may be contained in other protocol referenced documents, such as an Investigator's Brochure.

6.1.General Information

6.1.1. Protocol title, protocol identifying number, and date. Any amendment(s) should also bear the amendment number(s) and date(s).

6.1.2. Name and address of the sponsor and monitor (if other than the sponsor).

6.1.3. Name and title of the person(s) authorized to sign the protocol and the protocol amendment(s) for the sponsor.

6.1.4. Name, title, address, and telephone number(s) of the sponsor's medical expert (or dentist when appropriate) for the trial.

6.1.5. Name and title of the investigator(s) who is (are) responsible for conducting the trial, and the address and telephone number(s) of the trial site(s).

6.1.6. Name, title, address, and telephone number(s) of the qualified physician (or dentist, if applicable), who is responsible for all trial-site related medical (or dental) decisions (if other than investigator).

6.1.7. Name(s) and address(es) of the clinical laboratory(ies) and other medical and/or technical department(s) and/or institutions involved in the trial.

6.2. Background Information

6.2.1. Name and description of the investigational product(s).

6.2.2. A summary of findings from nonclinical studies that potentially have clinical significance and from clinical trials that are relevant to the trial.

6.2.3. Summary of the known and potential risks and benefits, if any, to human subjects.

6.2.4. Description of and justification for the route of administration, dosage, dosage regimen, and treatment period(s).

6.2.5. A statement that the trial will be conducted in compliance with the protocol, GCP and the applicable regulatory requirement(s).

6.2.6. Description of the population to be studied.

6.2.7. References to literature and data that are relevant to the trial, and that provide background for the trial.

6.3. Trial Objectives and Purpose

A detailed description of the objectives and the purpose of the trial.

6.4. Trial Design

The scientific integrity of the trial and the credibility of the data from the trial depend substantially on the trial design. A description of the trial design, should include:

6.4.1. A specific statement of the primary endpoints and the secondary endpoints, if any, to be measured during the trial.

6.4.2. A description of the type/design of trial to be conducted (e.g., double-blind, placebo- controlled, parallel design) and a schematic diagram of trial design, procedures and stages.

6.4.3. A description of the measures taken to minimize/avoid bias, including:

(a) Randomization.

(b) Blinding.

6.4.4. A description of the trial treatment(s) and the dosage and dosage regimen of the investigational product(s). Also include a description of the dosage form, packaging, and labelling of the investigational product(s).

6.4.5. The expected duration of subject participation, and a description of the sequence and duration of all trial periods, including follow-up, if any.

6.4.6. A description of the "stopping rules" or "discontinuation criteria" for individual subjects, parts of trial and entire trial.

6.4.7. Accountability procedures for the investigational product(s), including the placebo(s) and comparator(s), if any.

6.4.8. Maintenance of trial treatment randomization codes and procedures for breaking codes.

6.4.9. The identification of any data to be recorded directly on the CRFs (i.e., no prior written or electronic record of data), and to be considered to be source data.

6.5. Selection and Withdrawal of Subjects

6.5.1. Subject inclusion criteria.

6.5.2. Subject exclusion criteria.

6.5.3. Subject withdrawal criteria (i.e., terminating investigational product treatment/trial treatment) and procedures specifying:

(a) When and how to withdraw subjects from the trial/ investigational product treatment.

(b) The type and timing of the data to be collected for withdrawn subjects.

(c) Whether and how subjects are to be replaced.

(d) The follow-up for subjects withdrawn from investigational product treatment/trial treatment.

6.6. Treatment of Subjects

6.6.1. The treatment(s) to be administered, including the name(s) of all the product(s), the dose(s), the dosing schedule(s), the route/mode(s) of administration, and the treatment period(s), including the follow-up period(s) for subjects for each investigational product treatment/trial treatment group/arm of the trial.

6.6.2. Medication(s)/treatment(s) permitted (including rescue medication) and not permitted before and/or during the trial.

6.6.3. Procedures for monitoring subject compliance.

6.7. Assessment of Efficacy

6.7.1. Specification of the efficacy parameters.

6.7.2. Methods and timing for assessing, recording, and analysing of efficacy parameters.

6.8. Assessment of Safety

6.8.1. Specification of safety parameters.

6.8.2. The methods and timing for assessing, recording, and analysing safety parameters.

6.8.3. Procedures for eliciting reports of and for recording and reporting adverse event and intercurrent illnesses.

6.8.4. The type and duration of the follow-up of subjects after adverse events.

6.9. Statistics

6.9.1. A description of the statistical methods to be employed, including timing of any planned interim analysis(ses).

6.9.2. The number of subjects planned to be enrolled. In multicentre trials, the numbers of enrolled subjects projected for each trial site should be specified. Reason for choice of sample size, including reflections on (or calculations of) the power of the trial and clinical justification.

6.9.3. The level of significance to be used.

6.9.4. Criteria for the termination of the trial.

6.9.5. Procedure for accounting for missing, unused, and spurious data.

6.9.6. Procedures for reporting any deviation(s) from the original statistical plan (any deviation(s) from the original statistical plan should be described and justified in protocol and/or in the final report, as appropriate).

6.9.7. The selection of subjects to be included in the analyses (e.g., all randomized subjects, all dosed subjects, all eligible subjects, evaluable subjects).

6.10. Direct Access to Source Data/Documents

The sponsor should ensure that it is specified in the protocol or other written agreement that the investigator(s)/institution(s) will permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspection(s), providing direct access to source data/documents.

6.11. Quality Control and Quality Assurance

6.12. Ethics

Description of ethical considerations relating to the trial.

6.13. Data Handling and Record Keeping

6.14. Financing and Insurance

Financing and insurance if not addressed in a separate agreement.

6.15. Publication Policy

Publication policy, if not addressed in a separate agreement.

6.16. Supplements

(NOTE: Since the protocol and the clinical trial/study report are closely related, further relevant information can be found in the ICH Guideline for Structure and Content of Clinical Study Reports.)

7. INVESTIGATOR'S BROCHURE

7.1. Introduction

The Investigator's Brochure (IB) is a compilation of the clinical and nonclinical data on the investigational product(s) that are relevant to the study of the product(s) in human subjects. Its purpose is to provide the investigators and others involved in the trial with the information to facilitate their understanding of the rationale for, and their compliance with, many key features of the protocol, such as the dose, dose frequency/interval, methods of administration: and safety monitoring procedures. The IB also provides insight to support the clinical management of the study subjects during the course of the clinical trial. The information should be presented in a concise, simple, objective, balanced, and non-promotional form that enables a clinician, or potential investigator, to understand it and make his/her own unbiased risk-benefit assessment of the appropriateness of the proposed trial. For this reason, a medically qualified person should generally participate in the editing of an IB, but the contents of the IB should be approved by the disciplines that generated the described data.

This guideline delineates the minimum information that should be included in an IB and provides suggestions for its layout. It is expected that the type and extent of information available will vary with the stage of development of the investigational product. If the investigational product is marketed and its pharmacology is widely understood by medical practitioners, an extensive IB may not be necessary. Where permitted by regulatory authorities, a basic product information brochure, package leaflet, or labelling may be an appropriate alternative, provided that it includes current, comprehensive, and detailed information on all aspects of the investigational product that might be of importance to the investigator. If a marketed product is being studied for a new use (i.e., a new indication), an IB specific to that new use should be prepared. The IB should be reviewed at least annually and revised as necessary in compliance with a sponsor's written procedures. More frequent revision may be appropriate depending on the stage of development and the generation of relevant new information. However, in accordance with Good Clinical Practice, relevant new information may be so important that it should be communicated to the investigators, and possibly to the Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs) and/or regulatory authorities before it is included in a revised IB.

Generally, the sponsor is responsible for ensuring that an up-to-date IB is made available to the investigator(s) and the investigators are responsible for providing the up-to-date IB to the responsible IRBs/IECs. In the case of an investigator sponsored trial, the sponsor-investigator should determine whether a brochure is available from the commercial manufacturer. If the investigational product is provided by the sponsor-investigator, then he or she should provide the necessary information to the trial personnel. In cases where preparation of a formal IB is impractical, the sponsor-investigator should provide, as a substitute, an expanded background information section in the trial protocol that contains the minimum current information described in this guideline.

7.2. General Considerations

The IB should include:

7.2.1. Title Page

This should provide the sponsor's name, the identity of each investigational product (i.e., research number, chemical or approved generic name, and trade name(s) where legally permissible and desired by the sponsor), and the release date. It is also suggested that an

edition number, and a reference to the number and date of the edition it supersedes, be provided. An example is given in Appendix 1.

7.2.2. Confidentiality Statement

The sponsor may wish to include a statement instructing the investigator/recipients to treat the IB as a confidential document for the sole information and use of the investigator's team and the IRB/IEC.

7.3. Contents of the Investigator's Brochure

The IB should contain the following sections, each with literature references where appropriate:

7.3.1. Table of Contents

An example of the Table of Contents is given in Appendix 2

7.3.2. Summary

A brief summary (preferably not exceeding two pages) should be given, highlighting the significant physical, chemical, pharmaceutical, pharmacological, toxicological, pharmacokinetic, metabolic, and clinical information available that is relevant to the stage of clinical development of the investigational product.

7.3.3. Introduction

A brief introductory statement should be provided that contains the chemical name (and generic and trade name(s) when approved) of the investigational product(s), all active ingredients, the investigational product (s) pharmacological class and its expected position within this class (e.g., advantages), the rationale for performing research with the investigational product(s), and the anticipated prophylactic, therapeutic, or diagnostic indication(s). Finally, the introductory statement should provide the general approach to be followed in evaluating the investigational product.

7.3.4. Physical, Chemical, and Pharmaceutical Properties and Formulation

A description should be provided of the investigational product substance(s) (including the chemical and/or structural formula(e)), and a brief summary should be given of the relevant physical, chemical, and pharmaceutical properties.

To permit appropriate safety measures to be taken in the course of the trial, a description of the formulation(s) to be used, including excipients, should be provided and justified if clinically relevant. Instructions for the storage and handling of the dosage form(s) should also be given.

Any structural similarities to other known compounds should be mentioned.

7.3.5. Nonclinical Studies

Introduction:

The results of all relevant nonclinical pharmacology, toxicology, pharmacokinetic, and investigational product metabolism studies should be provided in summary form. This summary should address the methodology used, the results, and a discussion of the

relevance of the findings to the investigated therapeutic and the possible unfavourable and unintended effects in humans.

The information provided may include the following, as appropriate, if known/available:

- Species tested
- Number and sex of animals in each group
- Unit dose (e.g., milligram/kilogram (mg/kg))
- Dose interval
- Route of administration
- Duration of dosing
- Information on systemic distribution
- Duration of post-exposure follow-up
- Results, including the following aspects:
  - Nature and frequency of pharmacological or toxic effects
  - Severity or intensity of pharmacological or toxic effects
  - Time to onset of effects
  - Reversibility of effects
  - Duration of effects
  - Dose response

Tabular format/listings should be used whenever possible to enhance the clarity of the presentation.

The following sections should discuss the most important findings from the studies, including the dose response of observed effects, the relevance to humans, and any aspects to be studied in humans. If applicable, the effective and nontoxic dose findings in the same animal species should be compared (i.e., the therapeutic index should be discussed). The relevance of this information to the proposed human dosing should be addressed. Whenever possible, comparisons should be made in terms of blood/tissue levels rather than on a mg/kg basis.

(a) Nonclinical Pharmacology

A summary of the pharmacological aspects of the investigational product and, where appropriate, its significant metabolites studied in animals, should be included. Such a summary should incorporate studies that assess potential therapeutic activity (e.g., efficacy models, receptor binding, and specificity) as well as those that assess safety (e.g., special studies to assess pharmacological actions other than the intended therapeutic effect(s)).

(b) Pharmacokinetics and Product Metabolism in Animals

A summary of the pharmacokinetics and biological transformation and disposition of the investigational product in all species studied should be given. The discussion of the findings should address the absorption and the local and systemic bioavailability of the investigational product and its metabolites, and their relationship to the pharmacological and toxicological findings in animal species.

(c) Toxicology

A summary of the toxicological effects found in relevant studies conducted in different animal species should be described under the following headings where appropriate:

- Single dose
- Repeated dose
- Carcinogenicity
- Special studies (e.g., irritancy and sensitisation)
- Reproductive toxicity
- Genotoxicity (mutagenicity)

7.3.6. Effects in Humans

Introduction:

A thorough discussion of the known effects of the investigational product(s) in humans should be provided, including information on pharmacokinetics, metabolism, pharmacodynamics, dose response, safety, efficacy, and other pharmacological activities. Where possible, a summary of each completed clinical trial should be provided. Information should also be provided regarding results of any use of the investigational product(s) other than from in clinical trials, such as from experience during marketing.

(a) Pharmacokinetics and Product Metabolism in Humans

- A summary of information on the pharmacokinetics of the investigational product(s) should be presented, including the following, if available:
- Pharmacokinetics (including metabolism, as appropriate, and absorption, plasma protein binding, distribution, and elimination).
- Bioavailability of the investigational product (absolute, where possible, and/or relative) using a reference dosage form.
- Population subgroups (e.g., gender, age, and impaired organ function).
- Interactions (e.g., product-product interactions and effects of food).
- Other pharmacokinetic data (e.g., results of population studies performed within clinical trial(s).

(b) Safety and Efficacy

A summary of information should be provided about the investigational product's/products' (including metabolites, where appropriate) safety, pharmacodynamics, efficacy, and dose response that were obtained from preceding trials in humans (healthy volunteers and/or patients). The implications of this information should be discussed. In cases where a number of clinical trials have been completed, the use of summaries of safety and efficacy across multiple trials by indications in subgroups may provide a clear presentation of the data. Tabular summaries of adverse drug reactions for all the clinical trials (including those for all the studied indications) would be useful. Important differences in adverse drug reaction patterns/incidences across indications or subgroups should be discussed.

The IB should provide a description of the possible risks and adverse drug reactions to be anticipated on the basis of prior experiences with the product under investigation and with related products. A description should also be provided of the precautions or special monitoring to be done as part of the investigational use of the product(s).

(c) Marketing Experience

The IB should identify countries where the investigational product has been marketed or approved. Any significant information arising from the marketed use should be summarized (e.g., formulations, dosages, routes of administration, and adverse product reactions). The IB should also identify all the countries where the investigational product did not receive approval/registration for marketing or was withdrawn from marketing/registration.

7.3.7. Summary of Data and Guidance for the Investigator

This section should provide an overall discussion of the nonclinical and clinical data, and should summarise the information from various sources on different aspects of the investigational product(s), wherever possible. In this way, the investigator can be provided with the most informative interpretation of the available data and with an assessment of the implications of the information for future clinical trials.

Where appropriate, the published reports on related products should be discussed. This could help the investigator to anticipate adverse drug reactions or other problems in clinical trials.

The overall aim of this section is to provide the investigator with a clear understanding of the possible risks and adverse reactions, and of the specific tests, observations, and precautions that may be needed for a clinical trial. This understanding should be based on the available physical, chemical, pharmaceutical, pharmacological, toxicological, and clinical information on the investigational product(s). Guidance should also be provided to the clinical investigator on the recognition and treatment of possible overdose and adverse drug reactions that is based on previous human experience and on the pharmacology of the investigational product.

7.4. APPENDIX 1:

TITLE PAGE *(Example)*

SPONSOR'S NAME

Product:

Research Number:

Name(s): Chemical, Generic (if approved)

Trade Name(s) (if legally permissible and desired by the sponsor)

INVESTIGATOR'S BROCHURE

Edition Number:

Release Date:

Replaces Previous Edition Number:

Date:

7.5. APPENDIX 2:

TABLE OF CONTENTS OF INVESTIGATOR'S BROCHURE *(Example)*

- Confidentiality Statement (optional) ....
- Signature Page (optional) ....

1 Table of Contents ....

2 Summary ....

3 Introduction ....

4 Physical, Chemical, and Pharmaceutical Properties and Formulation ....

5 Nonclinical Studies ....

5.1 Nonclinical Pharmacology ....

5.2 Pharmacokinetics and Product Metabolism in Animals ....

5.3 Toxicology ....

6 Effects in Humans ....

6.1 Pharmacokinetics and Product Metabolism in Humans ....

6.2 Safety and Efficacy ....

6.3 Marketing Experience ....

7 Summary of Data and Guidance for the Investigator ....

NB: References on 1. Publications

2. Reports

These references should be found at the end of each chapter

Appendices (if any)

8. ESSENTIAL DOCUMENTS FOR THE CONDUCT OF A CLINICAL TRIAL

8.1. Introduction

Essential Documents are those documents which individually and collectively permit evaluation of the conduct of a trial and the quality of the data produced. These documents serve to demonstrate the compliance of the investigator, sponsor and monitor with the standards of Good Clinical Practice and with all applicable regulatory requirements.

Essential Documents also serve a number of other important purposes. Filing essential documents at the investigator/institution and sponsor sites in a timely manner can greatly assist in the successful management of a trial by the investigator, sponsor and monitor. These documents are also the ones which are usually audited by the sponsor's independent audit function and inspected by the regulatory authority(ies) as part of the process to confirm the validity of the trial conduct and the integrity of data collected.

The minimum list of essential documents which has been developed follows. The various documents are grouped in three sections according to the stage of the trial during which they will normally be generated: 1) before the clinical phase of the trial commences, 2) during the clinical conduct of the trial, and 3) after completion or termination of the trial. A description is given of the purpose of each document, and whether it should be filed in either the investigator/institution or sponsor files, or both. It is acceptable to combine some of the documents, provided the individual elements are readily identifiable.

Trial master files should be established at the beginning of the trial, both at the investigator/institution's site and at the sponsor's office. A final close-out of a trial can only be done when the monitor has reviewed both investigator/institution and sponsor files and confirmed that all necessary documents are in the appropriate files.

Any or all of the documents addressed in this guideline may be subject to, and should be available for, audit by the sponsor's auditor and inspection by the regulatory authority(ies).

The sponsor and investigator/institution should maintain a record of the location(s) of their respective essential documents including source documents. The storage system used during the trial and for archiving (irrespective of the type of media used) should provide for document identification, version history, search, and retrieval.

Essential documents for the trial should be supplemented or may be reduced where justified (in advance of trial initiation) based on the importance and relevance of the specific documents to the trial.

The sponsor should ensure that the investigator has control of and continuous access to the CRF data reported to the sponsor. The sponsor should not have exclusive control of those data.

When a copy is used to replace an original document (e.g., source documents, CRF), the copy should fulfill the requirements for certified copies.

The investigator/institution should have control of all essential documents and records generated by the investigator/institution before, during, and after the trial.

8.2. Before the Clinical Phase of the Trial Commences

During this planning stage the following documents should be generated and should be on file before the trial formally starts

	Title of Document	Purpose	Located in Files of	
			Investigator/ Institution	**Sponsor**
8.2.1	**INVESTIGATOR'S BROCHURE**	To document that relevant and current scientific information about the investigational product has been provided to the investigator	X	X
8.2.2	**SIGNED PROTOCOL AND AMENDMENTS, IF ANY, AND SAMPLE CASE REPORT FORM (CRF)**	To document investigator and sponsor agreement to the protocol/amendment(s) and CRF	X	X
8.2.3	**INFORMATION GIVEN TO TRIAL SUBJECT** **- INFORMED CONSENT FORM** (including all applicable translations)	To document the informed consent	X	X
	- ANY OTHER WRITTEN INFORMATION	To document that subjects will be given appropriate written information (content and wording) to support their ability to give fully informed consent	X	X

	Title of Document	Purpose	Located in Files of	
			Investigator/ Institution	Sponsor
	- ADVERTISEMENT FOR SUBJECT RECRUITMENT (if used)	To document that recruitment measures are appropriate and not coercive	X	
8.2.4	**FINANCIAL ASPECTS OF THE TRIAL**	To document the financial agreement between the investigator/institution and the sponsor for the trial	X	X
8.2.5	**INSURANCE STATEMENT** (where required)	To document that compensation to subject(s) for trial-related injury will be available	X	X
8.2.6	**SIGNED AGREEMENT BETWEEN INVOLVED PARTIES,** e.g.: - investigator/institution and sponsor - investigator/institution and CRO - sponsor and CRO - investigator/institution and authority(ies) (where required)	To document agreements	 X X X	 X X (where required) X X

	Title of Document	Purpose	Located in Files of	
			Investigator/ Institution	Sponsor
8.2.7	**DATED, DOCUMENTED APPROVAL/FAVOURABLE OPINION OF INSTITUTIONAL REVIEW BOARD (IRB) /INDEPENDENT ETHICS COMMITTEE (IEC) OF THE FOLLOWING:** - protocol and any amendments - CRF (if applicable) - informed consent form(s) - any other written information to be provided to the subject(s) - advertisement for subject recruitment (if used) - subject compensation (if any) - any other documents given approval/ favourable opinion	To document that the trial has been subject to IRB/IEC review and given approval/favourable opinion. To identify the version number and date of the document(s)	X	X

	Title of Document	Purpose	Located in Files of	
			Investigator/ Institution	Sponsor
8.2.8	**INSTITUTIONAL REVIEW BOARD/INDEPENDENT ETHICS COMMITTEE COMPOSITION**	To document that the IRB/IEC is constituted in agreement with GCP	X	X (where required)
8.2.9	**REGULATORY AUTHORITY(IES) AUTHORISATION/APPROVAL/ NOTIFICATION OF PROTOCOL** (where required)	To document appropriate authorisation/approval/notification by the regulatory authority(ies) has been obtained prior to initiation of the trial in compliance with the applicable regulatory requirement(s)	X (where required)	X (where required)
8.2.10	**CURRICULUM VITAE AND/OR OTHER RELEVANT DOCUMENTS EVIDENCING QUALIFICATIONS OF INVESTIGATOR(S) AND SUB-INVESTIGATOR(S)**	To document qualifications and eligibility to conduct trial and/or provide medical supervision of subjects	X	X
8.2.11	**NORMAL VALUE(S)/RANGE(S) FOR MEDICAL/ LABORATORY/TECHNICAL PROCEDURE(S) AND/OR TEST(S) INCLUDED IN THE PROTOCOL**	To document normal values and/or ranges of the tests	X	X

	Title of Document	Purpose	Located in Files of	
			Investigator/ Institution	**Sponsor**
8.2.12	**MEDICAL/LABORATORY/TECHNICAL PROCEDURES /TESTS** - certification or - accreditation or - established quality control and/or external quality assessment or - other validation (where required)	To document competence of facility to perform required test(s), and support reliability of results	X (where required)	X
8.2.13	**SAMPLE OF LABEL(S) ATTACHED TO INVESTIGATIONAL PRODUCT CONTAINER(S)**	To document compliance with applicable labelling regulations and appropriateness of instructions provided to the subjects		X
8.2.14	**INSTRUCTIONS FOR HANDLING OF INVESTIGATIONAL PRODUCT(S) AND TRIAL-RELATED MATERIALS** (if not included in protocol or Investigator's Brochure)	To document instructions needed to ensure proper storage, packaging, dispensing and disposition of investigational products and trial-related materials	X	X
8.2.15	**SHIPPING RECORDS FOR INVESTIGATIONAL PRODUCT(S) AND TRIAL-RELATED MATERIALS**	To document shipment dates, batch numbers and method of shipment of investigational product(s) and trial-related materials. Allows tracking of product batch, review of shipping conditions, and accountability	X	X

	Title of Document	Purpose	Located in Files of	
			Investigator/ Institution	Sponsor
8.2.16	**CERTIFICATE(S) OF ANALYSIS OF INVESTIGATIONAL PRODUCT(S) SHIPPED**	To document identity, purity, and strength of investigational product(s) to be used in the trial		X
8.2.17	**DECODING PROCEDURES FOR BLINDED TRIALS**	To document how, in case of an emergency, identity of blinded investigational product can be revealed without breaking the blind for the remaining subjects' treatment	X	X (third party if applicable)

	Title of Document	Purpose	Located in Files of	
			Investigator/ Institution	Sponsor
8.2.18	**MASTER RANDOMISATION LIST**	To document method for randomisation of trial population		X (third party if applicable)
8.2.19	**PRE-TRIAL MONITORING REPORT**	To document that the site is suitable for the trial (may be combined with 8.2.20)		X
8.2.20	**TRIAL INITIATION MONITORING REPORT**	To document that trial procedures were reviewed with the investigator and the investigator's trial staff (may be combined with 8.2.19)	X	X

8.3. During the Clinical Conduct of the Trial

In addition to having on file the above documents, the following should be added to the files during the trial as evidence that all new relevant information is documented as it becomes available

	Title of Document	Purpose	Located in Files of	
			Investigator/ Institution	Sponsor
8.3.1	**INVESTIGATOR'S BROCHURE UPDATES**	To document that investigator is informed in a timely manner of relevant information as it becomes available	X	X
8.3.2	**ANY REVISION TO:** - protocol/amendment(s) and CRF - informed consent form - any other written information provided to subjects - advertisement for subject recruitment (if used)	To document revisions of these trial related documents that take effect during trial	X	X

	Title of Document	Purpose	Located in Files of	
			Investigator/ Institution	Sponsor

8.3.3	**DATED, DOCUMENTED APPROVAL/FAVOURABLE OPINION OF INSTITUTIONAL REVIEW BOARD (IRB) /INDEPENDENT ETHICS COMMITTEE (IEC) OF THE FOLLOWING:**
	- protocol amendment(s)
	- revision(s) of:
	  - informed consent form
	  - any other written information to be provided to the subject
	  - advertisement for subject recruitment (if used)
	- any other documents given approval/favourable opinion
	- continuing review of trial (where required)
		To document that the amendment(s) and/or revision(s) have been subject to IRB/IEC review and were given approval/favourable opinion. To identify the version number and date of the document(s).	X	X

8.3.4	**REGULATORY AUTHORITY(IES) AUTHORISATIONS/APPROVALS/NOTIFICATIONS WHERE REQUIRED FOR:** - protocol amendment(s) and other documents	To document compliance with applicable regulatory requirements	X (where required)	X
8.3.5	**CURRICULUM VITAE FOR NEW INVESTIGATOR(S) AND/OR SUB-INVESTIGATOR(S)**	(see 8.2.10)	X	X

	Title of Document	Purpose	Located in Files of	
			Investigator/ Institution	Sponsor
8.3.6	**UPDATES TO NORMAL VALUE(S)/RANGE(S) FOR MEDICAL/ LABORATORY/ TECHNICAL PROCEDURE(S)/TEST(S) INCLUDED IN THE PROTOCOL**	To document normal values and ranges that are revised during the trial (see 8.2.11)	X	X
8.3.7	**UPDATES OF MEDICAL/LABORATORY/ TECHNICAL PROCEDURES/TESTS** - certification or - accreditation or - established quality control and/or external quality assessment or - other validation (where required)	To document that tests remain adequate throughout the trial period (see 8.2.12)	X (where required)	X
8.3.8	**DOCUMENTATION OF INVESTIGATIONAL PRODUCT(S) AND TRIAL-RELATED MATERIALS SHIPMENT**	(see 8.2.15.)	X	X
8.3.9	**CERTIFICATE(S) OF ANALYSIS FOR NEW BATCHES OF INVESTIGATIONAL PRODUCTS**	(see 8.2.16)		X
8.3.10	**MONITORING VISIT REPORTS**	To document site visits by, and findings of, the monitor		X

	Title of Document	Purpose	Located in Files of	
			Investigator/ Institution	**Sponsor**
8.3.11	**RELEVANT COMMUNICATIONS OTHER THAN SITE VISITS** - letters - meeting notes - notes of telephone calls	To document any agreements or significant discussions regarding trial administration, protocol violations, trial conduct, adverse event (AE) reporting	X	X
8.3.12	**SIGNED INFORMED CONSENT FORMS**	To document that consent is obtained in accordance with GCP and protocol and dated prior to participation of each subject in trial. Also to document direct access permission (see 8.2.3)	X	
8.3.13	**SOURCE DOCUMENTS**	To document the existence of the subject and substantiate integrity of trial data collected. To include original documents related to the trial, to medical treatment, and history of subject	X	
8.3.14	**SIGNED, DATED AND COMPLETED CASE REPORT FORMS (CRF)**	To document that the investigator or authorised member of the investigator's staff confirms the observations recorded	X (copy)	X (original)
8.3.15	**DOCUMENTATION OF CRF CORRECTIONS**	To document all changes/additions or corrections made to CRF after initial data were recorded	X (copy)	X (original)
8.3.16	**NOTIFICATION BY ORIGINATING INVESTIGATOR TO SPONSOR OF SERIOUS ADVERSE EVENTS AND RELATED REPORTS**	Notification by originating investigator to sponsor of serious adverse events and related reports in accordance with 4.11	X	X

	Title of Document	Purpose	Located in Files of	
			Investigator/ Institution	Sponsor
8.3.17	**NOTIFICATION BY SPONSOR AND/OR INVESTIGATOR, WHERE APPLICABLE, TO REGULATORY AUTHORITY(IES) AND IRB(S)/IEC(S) OF UNEXPECTED SERIOUS ADVERSE DRUG REACTIONS AND OF OTHER SAFETY INFORMATION**	Notification by sponsor and/or investigator, where applicable, to regulatory authorities and IRB(s)/IEC(s) of unexpected serious adverse drug reactions in accordance with 5.17 and 4.11.1 and of other safety information in accordance with 5.16.2 and 4.11.2	X (where required)	X
8.3.18	**NOTIFICATION BY SPONSOR TO INVESTIGATORS OF SAFETY INFORMATION**	Notification by sponsor to investigators of safety information in accordance with 5.16.2	X	X
8.3.19	**INTERIM OR ANNUAL REPORTS TO IRB/IEC AND AUTHORITY(IES)**	Interim or annual reports provided to IRB/IEC in accordance with 4.10 and to authority(ies) in accordance with 5.17.3	X	X (where required)
8.3.20	**SUBJECT SCREENING LOG**	To document identification of subjects who entered pre-trial screening	X	X (where required)
8.3.21	**SUBJECT IDENTIFICATION CODE LIST**	To document that investigator/institution keeps a confidential list of names of all subjects allocated to trial numbers on enrolling in the trial. Allows investigator/institution to reveal identity of any subject	X	

	Title of Document	Purpose	Located in Files of	
			Investigator/ Institution	Sponsor
8.3.22	**SUBJECT ENROLMENT LOG**	To document chronological enrolment of subjects by trial number	X	
8.3.23	**INVESTIGATIONAL PRODUCTS ACCOUNTABILITY AT THE SITE**	To document that investigational product(s) have been used according to the protocol	X	X
8.3.24	**SIGNATURE SHEET**	To document signatures and initials of all persons authorised to make entries and/or corrections on CRFs	X	X
8.3.25	**RECORD OF RETAINED BODY FLUIDS/ TISSUE SAMPLES (IF ANY)**	To document location and identification of retained samples if assays need to be repeated	X	X

8.4. After Completion or Termination of the Trial

After completion or termination of the trial, all of the documents identified in Sections 8.2 and 8.3 should be in the file together with the following

	Title of Document	Purpose	Located in Files of	
			Investigator/ Institution	**Sponsor**
8.4.1	**INVESTIGATIONAL PRODUCT(S) ACCOUNTABILITY AT SITE**	To document that the investigational product(s) have been used according to the protocol. To documents the final accounting of investigational product(s) received at the site, dispensed to subjects, returned by the subjects, and returned to sponsor	X	X
8.4.2	**DOCUMENTATION OF INVESTIGATIONAL PRODUCT DESTRUCTION**	To document destruction of unused investigational products by sponsor or at site	X (if destroyed at site)	X
8.4.3	**COMPLETED SUBJECT IDENTIFICATION CODE LIST**	To permit identification of all subjects enrolled in the trial in case follow-up is required. List should be kept in a confidential manner and for agreed upon time	X	
8.4.4	**AUDIT CERTIFICATE** (if available)	To document that audit was performed		X
8.4.5	**FINAL TRIAL CLOSE-OUT MONITORING REPORT**	To document that all activities required for trial close-out are completed, and copies of essential documents are held in the appropriate files		X

	Title of Document	Purpose	Located in Files of	
			Investigator/ Institution	**Sponsor**
8.4.6	**TREATMENT ALLOCATION AND DECODING DOCUMENTATION**	Returned to sponsor to document any decoding that may have occurred		X
8.4.7	**FINAL REPORT BY INVESTIGATOR TO IRB/IEC WHERE REQUIRED, AND WHERE APPLICABLE, TO THE REGULATORY AUTHORITY(IES)**	To document completion of the trial	X	
8.4.8	**CLINICAL STUDY REPORT**	To document results and interpretation of trial	X (if applicable)	X

BIOANALYTICAL METHOD VALIDATION AND STUDY SAMPLE ANALYSIS

Guidance for Industry

Adopted from the International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use
(Updated version)

ICH-M10-November 2022

PREAMBLE

The Ministry of Public Health (MOPH) recognizes the importance of the International Council for Harmonisation (ICH) guidelines and adopts them as part of its regulatory framework. By aligning with these guidelines, the MOPH aims to ensure that the regulatory processes and standards for pharmaceuticals in their jurisdiction are consistent with international best practices.

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) is an organization that aims to achieve global regulatory harmonization in the development, registration, and maintenance of pharmaceutical products. Its mission is to ensure that safe, effective, and high-quality medicines are produced and made available to patients in a resource-efficient manner. The ICH operates through a consensus-driven process that involves the participation of technical experts from regulatory authorities and industry stakeholders. These experts collaborate on detailed technical and science-based harmonization work to develop international guidelines that provide recommendations for pharmaceutical development, registration, and post-approval practices.

The commitment of regulators, including the MOPH, to adopting the consensus-based ICH guidelines helps foster regulatory harmonization and contributes to improvements in the quality of global drug development, manufacturing, and the availability of pharmaceutical products to patients.

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BIOANALYTICAL METHOD VALIDATION AND STUDY SAMPLE ANALYSIS

I. INTRODUCTION (1)

A. Objective (1.1)

This guidance is intended to provide recommendations for the validation of bioanalytical methods for chemical and biological drug quantification and their application in the analysis of study samples. Adherence to the principles presented in this guidance will ensure the quality and consistency of the bioanalytical data in support of the development and market approval of both chemical and biological drugs.

B. Background (1.2)

Concentration measurements of chemical and biological drug(s) and their metabolite(s) in biological matrices are an important aspect of drug development. The results of studies employing such methods contribute to regulatory decisions regarding the safety and efficacy of drug products. It is therefore critical that the bioanalytical methods used are well characterized, appropriately validated, and documented in order to ensure reliable data to support regulatory decisions.

C. Scope (1.3)

This guidance describes the validation of bioanalytical methods and study sample analysis that are expected to support regulatory decisions. The guidance is applicable to the bioanalytical methods used to measure concentrations of chemical and biological drug(s) and their metabolite(s) in biological samples (e.g., blood, plasma, serum, other body fluids or tissues) obtained in nonclinical toxicokinetic (TK) studies conducted according to the principles of Good Laboratory Practice (GLP), nonclinical pharmacokinetic (PK) studies conducted as surrogates for clinical studies, and all phases of clinical trials, including comparative bioavailability/bioequivalence (BA/BE) studies, in regulatory submissions. Full method validation is recommended for the primary matrix intended to support regulatory submissions. Additional matrices should be validated as necessary.

For studies that are not submitted for regulatory approval or not considered for regulatory decisions regarding safety, efficacy, or labeling (e.g., exploratory investigations), applicants may decide on the level of qualification that supports their own internal decision-making.

The information in this guidance applies to the quantitative analysis by ligand binding assays (LBAs) and chromatographic methods such as liquid chromatography (LC) or gas chromatography (GC), which are typically used in combination with mass spectrometry (MS) detection.

For studies that are subject to GLP or Good Clinical Practice (GCP) requirements, the bioanalysis of study samples must conform to those requirements. The bioanalysis of biomarkers and bioanalytical methods used for the assessment of immunogenicity are not within the scope of this guidance.

II. GENERAL PRINCIPLES (2)

A. Method Development (2.1)

The purpose of bioanalytical method development is to define the design, operating conditions, limitations, and suitability of the method for its intended purpose and to ensure that the method is ready for validation.

Before or during the development of a bioanalytical method, the applicant is encouraged to, if feasible, understand the analyte of interest (e.g., the physicochemical properties of the drug, in vitro and in vivo metabolism, preferential distribution between red blood cells and plasma, and protein binding) and consider aspects of any prior analytical methods that may be applicable.

Method development involves identifying the procedures and conditions involved with quantifying the analyte. Method development can include the characterization of the following bioanalytical elements: reference standards, critical reagents, calibration curve, quality control samples (QCs), selectivity and specificity, sensitivity, accuracy, precision, recovery, stability of the analyte, and minimum required dilution (MRD).

Bioanalytical method development does not require extensive record keeping or notation. Once the method has been developed, bioanalytical method validation proves that the method is suited to the analysis of the study samples.

If a problem is encountered with the method during the analysis of nonclinical or clinical study samples that requires that the analysis be stopped, any changes to the method and the rationale should be documented.

B. Method Validation (2.2)

1. Full Validation (2.2.1)

Bioanalytical method validation is important to ensure the acceptability of assay performance and the reliability of analytical results. A bioanalytical method is defined as a set of procedures used for measuring analyte concentrations in biological samples. A full validation of a bioanalytical method should be performed when establishing a bioanalytical method for the quantification of an analyte in clinical and in applicable nonclinical studies. Full validation should also be performed when implementing an analytical method that is reported in the literature and when a commercial kit is repurposed for bioanalytical use in drug development. Usually, one analyte has to be determined, but on occasion it may be appropriate to measure more than one analyte. This may involve two different drugs, a parent drug with its metabolites or the enantiomers or isomers of a drug. In these cases, the principles of validation and analysis apply to all analytes of interest. For chromatographic methods, a full validation should include

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the following elements, unless otherwise justified: selectivity, specificity, matrix effect, calibration curve (response function), range (lower limit of quantification (LLOQ) to upper limit of quantification (ULOQ)), accuracy, precision, carryover, dilution integrity, stability, and reinjection reproducibility.

For LBAs, the following elements should be evaluated, unless otherwise justified: specificity, selectivity, calibration curve (response function), range (LLOQ to ULOQ), accuracy, precision, carryover, dilution linearity, and stability. If necessary, parallelism can be conducted when appropriate study samples are available.

The assessments that are performed during validation should be relevant to the sample analysis workflow. The matrix used for bioanalytical method validation should be the same as the matrix of the study samples, including anticoagulants and additives. In cases in which it may be difficult to obtain an identical matrix to that of the study samples (e.g., rare matrices such as tissue, cerebrospinal fluid, bile or in cases where free drug is measured), surrogate matrices may be acceptable for analytical method validation.

The choice of surrogate matrix should be scientifically justified. Matrix differences within species (e.g., age, ethnicity, gender) are generally not considered different when validating a method.

A specific, detailed, written description of the bioanalytical method and validation procedure should be established *a priori*. This description may be in the form of a protocol, study plan, report, notebook, or Standard Operating Procedure (SOP).

2. Partial Validation (2.2.2)

Modifications to a fully validated analytical method may be evaluated by partial validation. Partial validation can range from as little as one accuracy and precision determination to a nearly full validation (refer to section VI.A (6.1)). The items in a partial validation should be determined according to the extent and nature of the changes made to the method.

3. Cross Validation (2.2.3)

Cross validation is required to demonstrate how the reported data are related when multiple bioanalytical methods and/or multiple bioanalytical laboratories are involved (refer to section VI.B (6.2)).

III. CHROMATOGRAPHY (3)

A. Reference Standards (3.1)

During method validation and the analysis of study samples, a blank biological matrix is spiked with the analyte(s) of interest using solutions of reference standard(s) to prepare calibration standards and QCs. Calibration standards and QCs should be prepared from separate stock solutions. However, calibration standards and QCs may be prepared from the same stock solution provided the accurate preparation and stability of the stock solution should have been verified.

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A suitable internal standard (IS) should be added to all calibration standards, QCs, and study samples during sample processing. The absence of an IS should be justified.

It is important that the reference standard is well characterized and the quality (e.g., purity, identity) of the reference standard and the suitability of the IS is ensured, as the quality will affect the outcome of the analysis and, therefore, the study data. The reference standard used during validation and study sample analysis should be obtained from an authentic and traceable source. The reference standard should be identical to the analyte. If this is not possible, an established form (e.g., salt or hydrate) of known quality should be used.

Suitable reference standards include compendial standards, commercially available standards or sufficiently characterized standards prepared in-house or by an external organization. A certificate of analysis (CoA) or an equivalent alternative is recommended to ensure quality and to provide information on the purity, storage conditions, retest/expiration date and batch number of the reference standard.

A CoA is not required for the IS as long as the suitability for use is demonstrated, e.g., a lack of analytical interference is shown for the substance itself or any impurities thereof.

When MS detection is used, the use of the stable isotope-labeled analyte as the IS is recommended whenever possible. However, it is important that the labeled standard is of high isotope purity and that no isotope exchange reaction occurs. The presence of unlabeled analyte should be checked and if unlabeled analyte is detected, the potential influence should be evaluated during method validation.

Stock and working solutions should only be prepared from reference standards that are within the stability period as documented in the CoA (either expiration date or the retest date).

B. Validation (3.2)

1. Selectivity (3.2.1)

Selectivity is the ability of an analytical method to differentiate and measure the analyte in the presence of potential interfering substances in the blank biological matrix.

Selectivity should be evaluated using blank samples (matrix samples processed without addition of an analyte or IS) obtained from at least six individual sources/lots (non-hemolyzed and non-lipemic). Use of fewer sources may be acceptable in the case of rare matrices. Selectivity for the IS should also be evaluated.

The evaluation of selectivity should demonstrate that no significant response attributable to analyte response at the LLOQ and not more than 5% of the IS response in the LLOQ sample for each matrix.

For the investigation of selectivity in lipemic matrices at least one source of matrix should be used. To be scientifically meaningful, the matrix used for these tests should be representative as much as possible of the expected study samples. A naturally lipemic matrix with abnormally high levels of triglycerides should be obtained from donors. Although it is recommended to use lipemic matrix from donors, if this is difficult to obtain, matrix can be spiked with triglycerides

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even though it may not be representative of study samples. However, if the drug impacts lipid metabolism or if the intended patient population is hyperlipidemic, the use of spiked samples is discouraged. This evaluation is not necessary for nonclinical studies unless the drug impacts lipid metabolism or is administered in a particular animal strain that is hyperlipidemic.

For the investigation of selectivity in hemolyzed matrices at least one source of matrix should be used. Hemolyzed matrices should be obtained by spiking matrix with hemolyzed whole blood (at least 2% V/V) to generate a visibly detectable hemolyzed sample.

2. *Specificity (3.2.2)*

Specificity is the ability of a bioanalytical method to detect and differentiate the analyte from other substances, including its related substances (e.g., substances that are structurally similar to the analyte, metabolites, isomers, impurities, degradation products formed during sample preparation, or concomitant medications that are expected to be used in the treatment of patients with the intended indication).

If the presence of related substances is anticipated in the biological matrix of interest, the impact of such substances should be evaluated during method validation, or alternatively, in the pre-dose study samples. In the case of LC-MS based methods, to assess the impact of such substances, the evaluation may include comparing the molecular weight of a potential interfering related substance with the analyte and chromatographic separation of the related substance from the analyte.

Responses detected and attributable to interfering components should not be more than 20% of the analyte response at the LLOQ and not more than 5% of the IS response in the LLOQ sample.

The possibility of back-conversion of a metabolite into the parent analyte during the successive steps of the analysis (including extraction procedures or in the MS source) should also be evaluated when relevant (e.g., potentially unstable metabolites such as ester analytes to ester/acidic metabolites, unstable N-oxides or glucuronide metabolites, lactone-ring structures). It is acknowledged that this evaluation will not be possible in the early stages of drug development of a new chemical entity when the metabolism is not yet evaluated. However, this issue should be investigated, and partial validation performed, if needed. The extent of back-conversion, if any, should be established and the impact on the study results should be discussed in the Bioanalytical Report.

3. *Matrix Effect (3.2.3)*

A matrix effect is defined as an alteration of the analyte response due to interfering and often unidentified component(s) in the sample matrix. During method validation the matrix effect between different independent sources/lots should be evaluated.

The matrix effect should be evaluated by analyzing at least three replicates of low and high QCs, each prepared using matrix from at least six different sources/lots. For each individual matrix sources/lots evaluated, the accuracy should be within ±15% of the nominal concentration and the precision (percent coefficient of variation (%CV)) should not be greater than 15%. Use of fewer sources/lots may be acceptable in the case of rare matrices.

The matrix effect should also be evaluated in relevant patient populations or special populations (e.g., hepatically impaired or renally impaired) when available. An additional evaluation of the matrix effect is recommended using hemolyzed or lipemic matrix samples during method validation on a case-by-case basis, especially when these conditions are expected to occur within the study.

4. Calibration Curve and Range (3.2.4)

The calibration curve demonstrates the relationship between the nominal analyte concentration and the response of the analytical platform to the analyte. Calibration standards, prepared by spiking matrix with a known quantity of analyte(s), span the calibration range and comprise the calibration curve. Calibration standards should be prepared in the same biological matrix as the study samples. The calibration range is defined by the LLOQ, which is the lowest calibration standard, and the ULOQ, which is the highest calibration standard. There should be one calibration curve for each analyte studied during method validation and for each analytical run.

A calibration curve should be generated with a blank sample, a zero sample (blank sample spiked with IS), and at least six concentration levels of calibration standards, including the LLOQ and the ULOQ.

A simple regression model that adequately describes the concentration-response relationship should be used. The selection of the regression model should be directed by written procedures. The regression model, weighting scheme, and transformation should be determined during the method validation. Blank and zero samples should not be included in the determination of the regression equation for the calibration curve. Each calibration standard may be analyzed in replicate, in which case data from all acceptable replicates should be used in the regression analysis.

The calibration curve parameters should be reported (e.g., slope and intercept in the case of a linear model). The back-calculated concentrations of the calibration standards should be presented together with the calculated mean accuracy and precision values. All acceptable curves obtained during validation, based on a minimum of three independent runs over several days, should be reported. The accuracy of the back-calculated concentrations of each calibration standard should be within ±20% of the nominal concentration at the LLOQ and within ±15% at all the other levels. At least 75% of the calibration standards with a minimum of six calibration standard levels should meet the above criteria.

In the case that replicates are used, the criteria (within ±15% or ±20% for LLOQ) should also be fulfilled for at least 50% of the calibration standards tested per concentration level. In the case that a calibration standard does not comply with these criteria, this calibration standard sample should be rejected, and the calibration curve without this calibration standard should be reevaluated, including regression analysis. For accuracy and precision runs, if all replicates of the LLOQ or the ULOQ calibration standard in a run are rejected, then the run should be rejected, the possible source of the failure should be determined and the method revised, if necessary. If the next validation run also fails, then the method should be revised before restarting validation.

The calibration curve should be prepared using freshly spiked calibration standards in at least one assessment. Subsequently, frozen calibration standards can be used within their defined period of stability.

5. Accuracy and Precision (3.2.5)

a. Preparation of quality control samples (3.2.5.1)

The QCs are intended to mimic study samples and should be prepared by spiking matrix with a known quantity of analyte, storing them under the conditions anticipated for study samples and analyzing them to assess the validity of the analytical method.

Calibration standards and the QCs should be prepared from separate stock solutions in order to avoid biased estimations which are not related to the analytical performance of the method. If calibration standards and the QCs may be prepared from the same stock solution, the accuracy and stability of the stock solution should be verified. A single source of blank matrix may be used, which should be free of interference or matrix effects, as described in section III.B.3 (3.2.3).

During method validation the QCs for accuracy and precision runs should be prepared at a minimum of 4 concentration levels within the calibration curve range: the LLOQ, within 3 times of the LLOQ (low QC), around 30% to 50% of the calibration curve range (medium QC) and at least 75% of the ULOQ (high QC).

For non-accuracy and precision validation runs, low, medium, and high QCs may be analyzed in duplicate. These QCs, along with the calibration standards, will provide the basis for the acceptance or rejection of the run.

b. Evaluation of accuracy and precision (3.2.5.2)

Accuracy and precision should be determined by analyzing the QCs within each run (within-run) and in different runs (between-run). Accuracy and precision should be evaluated using the same runs and data.

Within-run accuracy and precision should be evaluated by analyzing at least five replicates at each QC concentration level in each analytical run. Between-run accuracy and precision should be evaluated by analyzing each QC concentration level in at least three analytical runs over at least two days. To enable the evaluation of any trends over time within one run, it is recommended to demonstrate accuracy and precision of the QCs over at least one of the runs in a size equivalent to a prospective analytical run of study samples. Reported method validation data and the determination of accuracy and precision should include all results obtained, including individual QCs outside of the acceptance criteria, except those cases where errors are obvious and documented. Within-run accuracy and precision data should be reported for each run. If the within-run accuracy or precision criteria are not met in all runs, an overall estimate of within-run accuracy and precision for each QC level should be calculated. Between-run (intermediate) precision and accuracy should be calculated by combining the data from all runs.

The calibration curves for these assessments should be prepared using freshly spiked calibration standards in at least one run. If freshly spiked calibration standards are not used in the other runs, stability of the frozen calibration standards should be demonstrated.

The accuracy at each concentration level should be within ±15% of the nominal concentration, except at the LLOQ, where it should be within ±20%. The precision (%CV) of the concentrations

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determined at each level should not exceed 15%, except at the LLOQ, where it should not exceed 20%. For non-accuracy and precision validation runs, at least 2/3 of the total QCs and at least 50% at each concentration level should be within ±15% of the nominal values.

6. Carryover (3.2.6)

Carryover is an alteration of a measured concentration due to residual analyte from a preceding sample that remains in the analytical instrument.

Carryover should be assessed and minimized during method development. During validation carryover should be assessed by analyzing blank samples after the calibration standard at the ULOQ. Carryover in the blank samples following the highest calibration standard should not be greater than 20% of the analyte response at the LLOQ and 5% of the response for the IS. If it appears that carryover is unavoidable, study samples should not be randomized. Specific measures should be considered, validated, and applied during the analysis of the study samples, so that carryover does not affect accuracy and precision. This could include the injection of blank sample(s) after samples with an expected high concentration, before the next study sample.

7. Dilution Integrity (3.2.7)

Dilution integrity is the assessment of the sample dilution procedure, when required, to confirm that it does not impact the accuracy and precision of the measured concentration of the analyte. The same matrix from the same species used for preparation of the QCs should be used for dilution.

Dilution QCs should be prepared with analyte concentrations in matrix that are greater than the ULOQ and then diluted with blank matrix. At least five replicates per dilution factor should be tested in one run to determine if concentrations are accurately and precisely measured within the calibration range. The dilution factor(s) and concentrations applied during study sample analysis should be within the range of the dilution factors and concentrations evaluated during validation. The mean accuracy of the dilution QCs should be within ±15% of the nominal concentration and the precision (%CV) should not exceed 15%.

In the cases of rare matrices, use of a surrogate matrix for dilution may be acceptable. It should be demonstrated that this does not affect precision and accuracy.

8. Stability (3.2.8)

Stability evaluations should be carried out to ensure that every step taken during sample preparation, processing, and analysis as well as the storage conditions used do not affect the concentration of the analyte.

The storage and analytical conditions applied to the stability tests, such as the sample storage times and temperatures, sample matrix, anticoagulant and container materials, should reflect those used for the study samples. Reference to data published in the literature is not considered sufficient. Validation of storage periods should be performed on QCs that have been stored for a time that is equal to or longer than the study sample storage periods.

Stability of the analyte in the matrix is evaluated using low and high concentration QCs. Aliquots

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of the low and high QCs are analyzed at time zero and after the applied storage conditions that are to be evaluated. One bulk QC should be prepared at each concentration level. For each concentration tested, the bulk sample should be divided into a minimum of three aliquots that will be stored, stressed, and analyzed.

The QCs should be analyzed against a calibration curve, obtained from freshly spiked calibration standards in a run with its corresponding freshly spiked QCs or QCs for which stability has been proven. The mean concentration at each QC level should be within ±15% of the nominal concentration. If the concentrations of the study samples are consistently higher than the ULOQ of the calibration range, the concentration of the high QC should be adjusted to reflect these higher concentrations. It is recognized that this may not be possible in nonclinical studies due to solubility limitations.

For fixed dose combination products and specifically labeled drug regimens, the freeze-thaw, bench top, and long-term stability tests of an analyte in matrix should be conducted with the matrix spiked with all of the dosed compounds.

The following stability tests should be evaluated:

1. **Stability of the Analyte in Matrix**

Freeze-Thaw Stability in Matrix

To assess the impact of repeatedly removing samples from frozen storage, the stability of the analyte should be assessed after multiple cycles of freezing and thawing. Low and high QCs should be thawed and analyzed according to the same procedures as the study samples. QCs should be kept frozen for at least 12 hours between the thawing cycles. QCs for freeze-thaw stability should be assessed using freshly prepared calibration standards and QCs, or QCs for which stability has been proven. The number of freeze-thaw cycles validated should equal or exceed that of the freeze-thaw cycles undergone by the study samples, but a minimum of three cycles should be conducted.

Bench Top (Short-Term) Stability in Matrix

Bench top matrix stability experiments should be designed and conducted to cover the laboratory handling conditions for the study samples.

Low and high QCs should be thawed in the same manner as the study samples and kept on the bench top at the same temperature and for at least the same duration as the study samples.

The total time on the bench top should be concurrent; additive exposure to bench top conditions should not be used (i.e., time from each freeze-thaw evaluation should not be added up).

Long-Term Stability in Matrix

The long-term stability of the analyte in matrix stored in the freezer should be established. Low and high QCs should be stored in the freezer under the same storage conditions and at least for the same duration as the study samples.

For chemical drugs, the stability at one temperature (e.g., -20°C) to can be extrapolated to lower temperatures (e.g., -70°/-80°C).

For biological drugs, a bracketing approach can be applied, e.g., in the case that the stability has been demonstrated at -70°/-80°C and at -20°C, then it is not necessary to investigate the stability at temperatures in between those two points at which study samples will be stored.

2. Stability of the Analyte in Processed Samples

The stability of processed samples, including the time until completion of analysis (in the autosampler/instrument), should be determined. For example:

- Stability of the processed sample under the storage conditions to be used during the analysis of study samples (dry extract or in the injection phase)

- On-instrument/autosampler stability of the processed sample at injector or autosampler temperature

The total time that a processed sample is stored should be concurrent (i.e., autosampler and other storage times should not be added together).

3. Stability of the Analyte and IS in Stock and Working Solutions

The stability of the stock and working solutions of the analyte and IS should be determined under the storage conditions used during the analysis of study samples by using the lowest and the highest concentrations of these solutions. They should be assessed using the response of the detector. Stability of the stock and working solutions should be tested with an appropriate dilution, taking into consideration the linearity and measuring range of the detector. If the stability varies with concentration, then the stability of all concentrations of the stock and working solutions should be assessed. If no isotopic exchange occurs for the stable isotopically labeled IS under the same storage conditions as the analyte for which the stability is demonstrated, then no additional stability determinations for the IS are necessary.
If the reference standard expires, or it is past the retest date, the stability of the stock solutions made previously with this lot of reference standard are defined by the expiration or retest date established for the stock solution. Stock and working solutions should not be made from reference standards solely for extending the expiration date for the use of the reference standard.

In addition, the following test should be performed, if applicable:

4. Stability of the Analyte in Whole Blood

Sufficient attention should be paid to the stability of the analyte in the sampled matrix (blood) directly after collection from subjects and prior to preparation for storage to ensure that the concentrations obtained by the analytical method reflect the concentrations of the analyte in the subject's blood at the time of sample collection.

If the matrix used is plasma, the stability of the analyte in blood should be evaluated during

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method development (e.g., using an exploratory method in blood) or during method validation. The results should be provided in the Validation Report.

9. Reinjection Reproducibility (3.2.9)

Reproducibility of the method is assessed by replicate measurements of the QCs and is usually included in the assessment of precision and accuracy. However, if samples could be reinjected (e.g., in the case of instrument interruptions or other reasons such as equipment failure), reinjection reproducibility should be evaluated to establish the viability of the processed samples and to support their storage prior to reinjection.

Reinjection reproducibility is assessed by reinjecting a run that is comprised of calibration standards and a minimum of five replicates of the low, middle, and high QCs after storage. The precision and accuracy of the reinjected QCs establish the viability of the processed samples.

The results should be included in the Validation Report or provided in the Bioanalytical Report of the study where it was conducted.

C. Study Sample Analysis (3.3)

The analysis of study samples can be carried out after validation has been completed, however, it is understood that some parameters may be completed at a later stage (e.g., long-term stability). By the time the data are submitted to a regulatory authority, the bioanalytical method validation should have been completed. The study samples, QCs, and calibration standards should be processed in accordance with the validated analytical method. If system suitability is assessed, a predefined specific study plan, protocol, or SOP should be used. System suitability, including apparatus conditioning and instrument performance, should be determined using samples that are independent of the calibration standards and QCs for the run. Subject samples should not be used for system suitability. The IS responses of the study samples should be monitored to determine whether there is systemic IS variability. Refer to Table 1 for recommendations regarding documentation.

1. Analytical Run (3.3.1)

An analytical run should consist of a blank sample (processed matrix sample without analyte and without IS), a zero sample (processed matrix with IS), calibration standards at a minimum of six concentration levels, at least three levels of QCs (low, medium, and high) in duplicate (or at least 5% of the number of study samples, whichever is higher) and the study samples to be analyzed. The QCs should be interspersed in the run in such a way that the accuracy and precision of the whole run is ensured. Study samples should always be bracketed by QCs.

The calibration standards and QCs should be spiked independently using separately prepared stock solutions unless the accuracy and stability of the stock solutions have been verified. All samples (calibration standards, QCs, and study samples) should be processed and extracted as one single batch of samples in the order in which they are intended to be analyzed. Analyzing samples that were processed as several separate batches in a single analytical run is discouraged.
If such an approach cannot be avoided, for instance due to bench top stability limitations, each

batch of samples should include low, medium, and high QCs.

For comparative BA/BE studies, it is advisable to analyze all samples of one subject together in one analytical run to reduce variability.

The impact of any carryover that occurs during study sample analysis should be assessed and reported (refer to section III.B.6 (3.2.6)). If carryover is detected, its impact on the measured concentrations should be mitigated (e.g., non-randomization of study samples, injection of blank samples after samples with an expected high concentration) or the validity of the reported concentrations should be justified in the Bioanalytical Report.

2. *Acceptance Criteria for an Analytical Run (3.3.2)*

Criteria for the acceptance or rejection of an analytical run should be defined in the protocol, in the study plan, or in an SOP. In the case that a run contains multiple batches, acceptance criteria should be applied to the whole run and to the individual batches. It is possible for the run to meet acceptance criteria, even if a batch within that run is rejected for failing to meet the batch acceptance criteria. Calibration standards in a failed batch should not be used to support the acceptance of other batches within the analytical run.

The back-calculated concentrations of the calibration standards should be within ±15% of the nominal value, except for the LLOQ for which it should be within ±20%. At least 75% of the calibration standard concentrations, which should include a minimum of six concentration levels, should fulfill these criteria. If more than six calibration standard levels are used and one of the calibration standards does not meet the criteria, this calibration standard should be rejected and the calibration curve without this calibration standard should be reevaluated, and a new regression analysis performed.

If the rejected calibration standard is the LLOQ, the new lower limit for this analytical run should be the next lowest acceptable calibration standard of the calibration curve. This new lower limit calibration standard should retain its original acceptance criteria (i.e., ±15%). If the highest calibration standard is rejected, the ULOQ for this analytical run should be the next acceptable highest calibration standard of the calibration curve. The revised calibration range should cover at least three QC concentration levels (low, medium, and high). Study samples outside of the revised range should be reanalyzed. If replicate calibration standards are used and only one of the LLOQ or ULOQ standards fails, the calibration range is unchanged.

At least 2/3 of the total QCs and at least 50% at each concentration level should be within ±15% of the nominal values. If these criteria are not fulfilled the analytical run should be rejected. A new analytical batch should be prepared for all study samples within the failed analytical run for subsequent analysis. In the cases where the failure is due to an assignable technical cause, samples may be reinjected.

Analytical runs containing samples that are diluted and reanalyzed should include dilution QCs to verify the accuracy and precision of the dilution method during study sample analysis. The concentration of the dilution QCs should exceed that of the study samples being diluted (or of the ULOQ) and they should be diluted using the same dilution factor. If multiple dilution factors are used in one analytical run, then dilution QCs need only be diluted by the highest and lowest dilution factors. The within-run acceptance criteria of the dilution QC(s) will only affect

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the acceptance of the diluted study samples and not the outcome of the analytical run.

When several analytes are assayed simultaneously, there should be one calibration curve for each analyte studied. If an analytical run is acceptable for one analyte but has to be rejected for another analyte, the data for the accepted analyte should be used. The determination of the rejected analyte requires reextraction and analysis only for the analyte that is reanalyzed. Only data for this reanalyzed analyte needs to be reported.

The back-calculated concentrations of the calibration standards and QCs of passed and accepted runs should be reported. The overall (between-run) accuracy and precision of the QCs of all accepted runs should be calculated at each concentration level and reported in the analytical report (refer to section VIII. (8) Documentation and Table 1). If the overall mean accuracy and/or precision fails the 15% criterion, an investigation to determine the cause of the deviation should be conducted. In the case of comparative BA/BE studies, it may result in the rejection of the data.

3. Calibration Range (3.3.3)

If a narrow range of analyte concentrations of the study samples is known or anticipated before the start of study sample analysis, it is recommended to either narrow the calibration curve range, adapt the concentrations of the QCs, or add new QCs at different concentration levels as appropriate, to adequately reflect the concentrations of the study samples.

At the intended therapeutic dose(s), if an unanticipated clustering of study samples at one end of the calibration curve is encountered after the start of sample analysis, the analysis should be stopped and either the standard calibration range narrowed (i.e., partial validation), existing QC concentrations revised, or QCs at additional concentrations added to the original curve within the observed range before continuing with study sample analysis. It is not necessary to reanalyze samples analyzed before optimizing the calibration curve range or QC concentrations.

The same applies if a large number of the analyte concentrations of the study samples are above the ULOQ. The calibration curve range should be changed, if possible, and QC(s) added, or their concentrations modified. If it is not possible to change the calibration curve range or the number of samples with a concentration above the ULOQ is not large, samples should be diluted according to the validated dilution method.

At least two QC levels should fall within the range of concentrations measured in study samples. If the calibration curve range is changed, the bioanalytical method should be revalidated (partial validation) to verify the response function and to ensure accuracy and precision.

4. Reanalysis ofStudy Samples (3.3.4)

Possible reasons for reanalysis of study samples, the number of replicates, and the decision criteria to select the value to be reported should be predefined in the protocol, study plan, or SOP before the actual start of the analysis of the study samples. For study samples in which multiple analytes are being analyzed, a valid result for one analyte should not be rejected if the otheranalyte fails the acceptance criteria.

The number of samples (and percentage of total number of samples) that have been reanalyzed

should be reported and discussed in the Bioanalytical Report. For comparative BA/BE studies, a separate table should report values from rejected runs.

Some examples of reasons for study sample reanalysis are:

a. Rejection of an analytical run because the run failed the acceptance criteria with regard to accuracy of the calibration standards and/or the precision and accuracy of the QCs

b. IS response significantly different from the response for the calibration standards and QCs (as predefined in an SOP)

c. The concentration obtained is above the ULOQ

d. The concentration observed is below the revised LLOQ in runs where the lowest calibration standard has been rejected from a calibration curve, resulting in a higher LLOQ compared with other runs

e. Improper sample injection or malfunction of equipment

f. The diluted study sample is below the LLOQ

g. Identification of quantifiable analyte levels in pre-dose samples, control, or placebo samples

h. Poor chromatography (as predefined in an SOP)

For comparative BA/BE studies, reanalysis of study samples for a PK reason (e.g., a sample concentration does not fit with the expected profile) should not be done, as it may bias the study result.

Any reanalyzed samples should be identified in the Bioanalytical Report and the initial value, the reason for reanalysis, the values obtained in the reanalyzes, the final accepted value and a justification for the acceptance should be provided. Further, a summary table of the total number of samples that have been reanalyzed for each reason should be provided. In cases where the first analysis yields a nonreportable result, a single reanalysis is considered sufficient (e.g., concentration above the ULOQ or equipment malfunction). In cases where the value needs to be confirmed (e.g., pre-dose sample with measurable concentrations) replicate determinations are required if sample volume allows.

The safety of trial subjects should take precedence over any other aspect of the trial. Consequently, there may be other circumstances when it is necessary to reanalyze specific study samples for the purpose of a safety investigation.

5. Reinjection of Study Samples (3.3.5)

Reinjection of processed samples can be made in the case of equipment failure if reinjection reproducibility has been demonstrated during validation or provided in the Bioanalytical Report where it was conducted. Reinjection of a full analytical run or of individual calibration standards or QCs simply because the calibration standards or QCs failed, without any identified analytical

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cause, should not be done.

6. Integration of Chromatograms (3.3.6)

Chromatogram integration and reintegration should be described in a study plan, protocol, or SOP. Any deviation from the procedures described *a priori* should be discussed in the Bioanalytical Report. The list of chromatograms that required reintegration, including any manual integrations, and the reasons for reintegration should be included in the Bioanalytical Report. Original and reintegrated chromatograms and initial and repeat integration results should be kept for future reference and submitted in the Bioanalytical Report for comparative BA/BE studies.

IV. LIGAND BINDING ASSAYS (4)

A. Key Reagents (4.1)

1. Reference Standard (4.1.1)

The reference standard should be well characterized and documented (e.g., CoA and origin). A biological drug has a highly complex structure and its reactivity with binding reagents for bioanalysis may be influenced by a change in the manufacturing process of the drug substance. It is recommended that the manufacturing batch of the reference standard used for the preparation of calibration standards and QCs is derived from the same batch of drug substance as that used for dosing in the nonclinical and clinical studies whenever possible. If the reference standard batch used for bioanalysis is changed, bioanalytical evaluation should be carried out with QCs from the original material and the new material prior to use to ensure that the performance characteristics of the method are within the acceptance criteria.

2. Critical Reagents (4.1.2)

Critical reagents, including binding reagents (e.g., binding proteins, aptamers, antibodies, or conjugated antibodies) and those containing enzymatic moieties, have direct impact on the results of the assay and, therefore, their quality should be assured. Critical reagents bind the analyte and, upon interaction, lead to an instrument signal corresponding to the analyte concentration. The critical reagents should be identified and defined in the assay method.

Reliable procurement of critical reagents, whether manufactured in-house or purchased commercially, should be considered early in method development. The data sheet for the critical reagent should include at a minimum identity, source, batch/lot number, purity (if applicable), concentration (if applicable), and stability/retest date/storage conditions (refer to Table 1). Additional characteristics may be warranted.

A critical reagent lifecycle management procedure is necessary to ensure consistency between the original and new batches of critical reagents. Reagent performance should be evaluated using the bioanalytical method. Minor changes to critical reagents would not be expected to influence the method performance, whereas major changes may significantly impact the performance. If the change is minor (e.g., the source of one reagent is changed), a single comparative accuracy and precision assessment is sufficient f or characterization. If the change is major, then additional

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validation experiments are recommended. Ideally, assessment of changes should compare the method with the new reagents to the method with the old reagents directly. Major changes include, but are not limited to, change in production method of antibodies, additional blood collection from animals for polyclonal antibodies, and new clones or new supplier for monoclonal antibody production.

Retest dates and validation parameters should be documented in order to support the extension or replacement of the critical reagent. Stability testing of the reagents should be based upon the performance in the bioanalytical method and upon general guidance for reagent storage conditions. It can be extended beyond the expiration date from the supplier. The performance parameters should be documented in order to support the extension or replacement of the critical reagent.

B. Validation (4.2)

Most often, microtiter plates are used for LBAs and study samples can be analyzed using an assay format of one or more well(s) per sample. The assay format should be specified in the protocol, study plan or SOP. If method development and method validation are performed using one or more well(s) per sample, then study sample analysis should also be performed using one or more well(s) per sample, respectively. If multiple wells per sample are used, the reportable sample concentration should be determined either by calculating the mean of the responses from the replicate wells or by averaging the concentrations calculated from each response. Data evaluation should be performed on reportable concentrations.

1. Specificity (4.2.1)

Specificity is related to the concept of cross-reactivity in LBA. It is important that the binding reagent specifically binds to the target analyte but does not cross-react with coexisting structurally related molecules (e.g., endogenous compounds, isoforms, or structurally related concomitant medication). Specificity is evaluated by spiking blank matrix samples with related molecules at the maximal concentration(s) of the structurally related molecule anticipated in study samples.

The accuracy of the target analyte at the LLOQ and at the ULOQ should be investigated in the presence of related molecules at the maximal concentration(s) anticipated in study samples. The response of blank samples spiked with related molecules should be below the LLOQ. The accuracy of the target analyte in presence of related molecules should be within ±25% of the nominal values.

In the event of nonspecificity, the impact on the method should be evaluated by spiking increasing concentrations of interfering molecules in blank matrix and measuring the accuracy of the target analyte at the LLOQ and ULOQ. It is important to determine the minimum concentration of the related molecule where interference occurs. Appropriate mitigation during sample analysis should be employed, e.g., it may be necessary to adjust the LLOQ/ULOQ accordingly or consider a new method.

During method development and early method validation, these “related molecules” are frequently not available. Additional evaluation of specificity may be conducted after the original validation is

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completed.

2. *Selectivity (4.2.2)*

Selectivity is the ability of the method to detect and differentiate the analyte of interest in the presence of nonspecific matrix components. The matrix can contain nonspecific matrix component such as degrading enzymes, heterophilic antibodies or rheumatoid factor which may interfere with the analyte of interest.

Selectivity should be evaluated at the low end of an assay where problems occur in most cases, but it is recommended that selectivity is also evaluated at higher analyte concentrations. Therefore, selectivity should be evaluated using blank samples obtained from at least 10 individual sources and by spiking the individual blank matrices at the LLOQ and at the high QC level. Use of fewer sources may be acceptable in the case of rare matrices. The response of the blank samples should be below the LLOQ in at least 80% of the individual sources.

The accuracy should be within ±25% at the LLOQ and within ±20% at the high QC level of the nominal concentration in at least 80% of the individual sources evaluated.

Selectivity should be evaluated in lipemic samples and hemolyzed samples (refer to section (3.2.1)). For lipemic and hemolyzed samples, tests can be evaluated once using a single source of matrix. Selectivity should be assessed in samples from relevant patient populations (e.g., renally or hepatically impaired patients, inflammatory or immune-oncology patients if applicable). In the case of relevant patient populations, there should be at least five individual patients.

3. *Calibration Curve and Range (4.2.3)*

The calibration curve demonstrates the relationship between the nominal analyte concentration and the response of the analytical platform to the analyte. Calibration standards, prepared by spiking matrix with a known quantity of analyte, span the calibration range and comprise the calibration curve. Calibration standards should be prepared in the same biological matrix as the study samples. The calibration range is defined by the LLOQ, which is the lowest calibration standard, and the ULOQ, which is the highest calibration standard. There should be one calibration curve for each analyte studied during method validation and for each analytical run. If needed, the use of surrogate matrix should be scientifically justified.

A calibration curve should be generated with at least six concentration levels of calibration standards, including LLOQ and ULOQ standards, plus a blank sample. The blank sample should not be included in the calculation of calibration curve parameters. Anchor point samples at concentrations below the LLOQ and above the ULOQ of the calibration curve may also be used to improve curve fitting. The relationship between response and concentration for a calibration curve is most often fitted by a 4- or 5-parameter logistic model if there are data points near the lower and upper asymptotes. Oher models should be suitably justified.

A minimum of six independent runs should be evaluated over several days considering the factors that may contribute to between-run variability.

The accuracy and precision of back-calculated concentrations of each calibration standard should be within ±25% of the nominal concentration at the LLOQ and ULOQ, and within ±20% at all

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other levels. At least 75% of the calibration standards excluding anchor points, and a minimum of 6 concentration levels of calibration standards, including the LLOQ and ULOQ, should meet the above criteria. The anchor points do not require acceptance criteria since they are beyond the quantifiable range of the curve.

The calibration curve should preferably be prepared using freshly spiked calibration standards. If freshly spiked calibration standards are not used, the frozen calibration standards can be used within their defined period of stability.

4. Accuracy and Precision (4.2.4)

a. Preparation of quality control samples (4.2.4.1)

The QCs are intended to mimic study samples and should be prepared by spiking matrix with a known quantity of analyte, stored under the conditions anticipated for study samples, and analyzed to assess the validity of the analytical method.

The dilution series for the preparation of the QCs should be completely independent from the dilution series for the preparation of calibration standard samples. If they are prepared from the same stock solution (or working stock) the accurate preparation and stability should be verified. The QCs should be prepared at a minimum of five concentration levels within the calibration curve range: The analyte should be spiked at the LLOQ, within three times of the LLOQ (low QC), around the geometric mean of the calibration curve range (medium QC), and at least at 75% of the ULOQ (high QC) and at the ULOQ.

For non-accuracy and precision validation runs, low, medium, and high QCs may be analyzed in duplicate. These QCs, along with the calibration standards, should provide the basis for the acceptance or rejection of the run.

b. Evaluation of accuracy and precision (4.2.4.2)

Accuracy and precision should be determined by analyzing the QCs within each run (within-run) and in different runs (between-run). Accuracy and precision should be evaluated using the same runs and data.

Accuracy and precision should be determined by analyzing at least three replicates per run at each QC concentration level (LLOQ, low, medium, high, ULOQ) in at least six runs over 2 or more days. Reported method validation data and the determination of accuracy and precision should include all results obtained, except those cases where errors are obvious and documented.
Within-run accuracy and precision data should be reported for each run. If the within-run accuracy or precision criteria are not met in all runs, an overall estimate of within-run accuracy and precision for each QC level should be calculated. Between-run (intermediate) precision and accuracy should be calculated by combining the data from all runs.

The overall within-run and between-run accuracy at each concentration level should be within ±20% of the nominal values, except for the LLOQ and ULOQ, which should be within ±25% of the nominal value. Within-run and between-run precision of the QC concentrations determined at each level should not exceed 20%, except at the LLOQ and ULOQ, where it should not exceed 25%.

For non-accuracy and precision validation runs, at least 2/3 of the total QCs and at least 50% at each concentration level should be within ±20% of the nominal values.

Furthermore, the total error (i.e., sum of absolute values of the errors in accuracy (%) and precision (%)) should be evaluated. The total error should not exceed 30% (40% at LLOQ and ULOQ).

5. *Carryover (4.2.5)*

Carryover is generally not an issue for LBA analyses. However, if the analytical platform is prone to carryover, the potential of carryover should be investigated by placing blank samples after the calibration standard at the ULOQ. The response of blank samples should be below the LLOQ.

6. *Dilution Linearity and Hook Effect(4.2.6)*

Due to the narrow assay range in many LBAs, study samples may require dilution in order to achieve analyte concentrations within the range of the assay. Dilution linearity should be assessed to confirm: (i) that measured concentrations are not affected by dilution within the calibration range and (ii) that sample concentrations above the ULOQ of a calibration curve are not impacted by hook effect (i.e., a signal suppression caused by high concentrations of the analyte), whereby yielding an erroneous result.

The same matrix as that of the study sample should be used for preparation of the QCs for dilution.

Dilution linearity should be demonstrated by generating a dilution QC, i.e., spiking the matrix with an analyte concentration above the ULOQ, analyzed undiluted (for hook effect) and diluting this sample (to at least three different dilution factors) with blank matrix to a concentration within the calibration range. For each dilution factor tested, at least three independently prepared dilution series should be performed using the number of replicates that will be used in sample analysis. The absence or presence of response reduction (hook effect) should be checked in the dilution QCs and, if observed and unable to be eliminated with reasonable measures, steps should be taken to mitigate this effect during the analysis of study samples.

The calculated mean concentration for each dilution should be within ±20% of the nominal concentration after correction for dilution and the precision should not exceed 20%.

The dilution factor(s) applied during study sample analysis should be within the range of dilution factors evaluated during validation.

7. *Stability (4.2.7)*

Stability evaluations should be carried out to ensure that every step taken during sample preparation, processing, and analysis as well as the storage conditions used do not affect the concentration of the analyte.

The storage and analytical conditions applied to the stability tests, such as the sample storage times and temperatures, sample matrix, anticoagulant, and container materials should reflect those used for the study samples. Reference to data published in the literature is not considered

sufficient. Validation of storage periods should be performed on QCs that have been stored for a time that is equal to or longer than the study sample storage periods.

Stability of the analyte in the studied matrix should be evaluated using low and high concentration QCs. Aliquots of the low and high QCs should be analyzed at time zero and after the applied storage conditions that are to be evaluated. One bulk QC should be prepared at each concentration level. For each concentration tested, the bulk sample should be divided into a minimum of three aliquots that will be stored, stressed, and analyzed.

The QCs should be analyzed against a calibration curve, obtained from freshly spiked calibration standards in a run with its corresponding freshly spiked QCs or QCs for which stability has been proven. While the use of freshly prepared calibration standards and QCs is the preferred approach, it is recognized that in some cases, for macromolecules, it may be necessary to freeze them overnight. In such cases, valid justification should be provided, and freeze-thaw stability demonstrated. QCs should be kept frozen for at least 12 hours between the thawing cycles. The mean concentration at each QC level should be within ±20% of the nominal concentration.

Since sample dilution may be required for many LBA methods due to a narrow calibration range, the concentrations of the study samples may be consistently higher than the ULOQ of the calibration curve. If this is the case, the concentration of the QCs should be adjusted, considering the applied sample dilution, to represent the actual sample concentration range.

For fixed dose combination products and specifically labeled drug regimens, the freeze-thaw, bench top and long-term stability tests of an analyte in matrix should be conducted with the matrix spiked with all of the dosed compounds, on a case-by-case basis.

As mentioned in section III.B.8 (3.2.8), the investigation of stability should cover bench top (short-term) stability at room temperature or sample preparation temperature and freeze-thaw stability. In addition, long-term stability should be studied.

For chemical drugs, the stability at one temperature (e.g., -20°C) can be extrapolated to lower temperatures (e.g., -70°/-80°C).

For biological drugs, a bracketing approach can be applied, e.g., in the case that the stability has been demonstrated at -70°/-80°C and at -20°C, then it is not necessary to investigate the stability at temperatures in between those two points at which study samples will be stored.

C. Study Sample Analysis (4.3)

The analysis of study samples can be carried out after validation has been completed, however, it is understood that some parameters may be completed at a later stage (e.g., long-term stability). By the time the data are submitted to a regulatory authority, the bioanalytical method validation should have been completed. The study samples, QCs, and calibration standards should be processed in accordance with the validated analytical method. Refer to Table 1 for recommendations regarding documentation.

1. Analytical Run (4.3.1)

An analytical run should consist of a blank sample, calibration standards at a minimum of six

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concentration levels, at least three levels of QCs (low, medium, and high) applied as two sets (or at least 5% of the number of study samples, whichever is higher) and the study samples to be analyzed. The blank sample should not be included in the calculation of calibration curve parameters. The QCs should be placed in the run in such a way that the accuracy and precision of the whole run is ensured taking into account that study samples should always be bracketed by QCs.

Most often, microtiter plates are used for LBAs. An analytical run may comprise of one or more plate(s). Typically, each plate contains an individual set of calibration standards and QCs. If each plate contains its own calibration standards and QCs, then each plate should be assessed on its own. However, for some platforms the sample capacity may be limited. In this case, sets of calibration standards may be placed on the first and the last plate, but QCs should be placed on every single plate. QCs should be placed at least at the beginning (before) and at the end (after) of the study samples of each plate. The QCs on each plate and each calibration curve should fulfill the acceptance criteria for an analytical run (refer to section IV.C.2 (4.3.2)). For the calculation of concentrations, the calibration standards should be combined to conduct one regression analysis. If the combined calibration curve does not pass the acceptance criteria the whole run fails.

2. *Acceptance Criteria for an Analytical Run (4.3.2)*

Criteria for the acceptance or rejection of an analytical run should be defined in the protocol, in the study plan, or in an SOP. In the case that a run contains multiple batches, acceptance criteria should be applied to the whole run and to the individual batches. It is possible for the run to meet acceptance criteria, even if a batch within that run is rejected for failing to meet the batch acceptance criteria. Calibration standards in a failed batch should not be used to support the acceptance of other batches within the analytical run.

The back-calculated concentrations of the calibration standards should be within ±20% of the nominal value at each concentration level, except for the LLOQ and the ULOQ, for which it should be within ±25%. At least 75% of the calibration standards, with a minimum of 6 concentration levels, should fulfill this criterion. This requirement does not apply to anchor calibration standards. If more than six calibration standards are used and one of the calibration standards does not meet these criteria, this calibration standard should be rejected and the calibration curve without this calibration standard should be reevaluated, and a new regression analysis performed.

If the rejected calibration standard is the LLOQ, the new lower limit for this analytical run should be the next lowest acceptable calibration standard of the calibration curve. If the highest calibration standard is rejected, the new upper limit for this analytical run should be the next acceptable highest calibration standard of the calibration curve. The new lower and upper limit calibration standard will retain their original acceptance criteria (i.e., ±20%). The revised calibration range should cover all QCs (low, medium, and high). The study samples outside of the revised assay range should be reanalyzed.

Each run should contain at least three levels of QCs (low, medium, and high). During study sample analysis, the calibration standards and QCs should mimic the analysis of the study sample with regard to the number of wells used per study sample. At least 2/3 of the QCs and 50% at each concentration level should be within ±20% of the nominal value at each

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concentration level. Exceptions to these criteria should be justified and predefined in the SOP or protocol.

The overall mean accuracy and precision of the QCs of all accepted runs should be calculated at each concentration level and reported in the analytical report. In the case that the overall mean accuracy and/or precision exceeds 20%, additional investigations should be conducted to determine the cause(s) of this deviation. In the case of comparative BA/BE studies, it may result in the rejection of the data.

3. Calibration Range (4.3.3)

At least two QC sample levels should fall within the range of concentrations measured in study samples. At the intended therapeutic dose(s), if an unanticipated clustering of study samples at one end of the calibration curve is encountered after the start of sample analysis, the analysis should be stopped and either the standard calibration range narrowed (i.e., partial validation), existing QC concentrations revised, or QCs at additional concentrations added to the original curve within the observed range before continuing with study sample analysis. It is not necessary to reanalyze samples analyzed before optimizing the calibration curve range or QC concentrations.

4. Reanalysis ofStudy Samples (4.3.4)

Possible reasons for reanalysis of study samples, the number reanalyzed, and the decision criteria to select the value to be reported should be predefined in the protocol, study plan, or SOP, before the actual start of the analysis of the study samples.

The number of samples (and percentage of total number of samples) that have been reanalyzed should be reported and discussed in the Bioanalytical Report. For comparative BA/BE studies, a separate table should report values from rejected runs.

Some examples of reasons for study sample reanalysis are:

- Rejection of an analytical run because the run failed the acceptance criteria with regard to accuracy of the calibration standards and/or the precision and accuracy of the QCs

- The concentration obtained is above the ULOQ

- The concentration obtained is below the LLOQ in runs where the lowest calibration standard has been rejected from a calibration curve, resulting in a higher LLOQ compared with other runs

- Malfunction of equipment

- The diluted sample is below the LLOQ

- Identification of quantifiable analyte levels in pre-dose samples, control, or placebo samples

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- When samples are analyzed in more than one well and nonreportable values are obtained due to one replicate failing the predefined acceptance criteria (e.g., excessive variability between wells, one replicate being above the ULOQ or below the LLOQ)

For comparative BA/BE studies, reanalysis of study samples for a PK reason (e.g., a sample concentration does not fit with the expected profile) should not be done, as it may bias the study result.

The reanalyzed samples should be identified in the Bioanalytical Report and the initial value, the reason for reanalysis, the values obtained in the reanalysis, the final accepted value, and a justification for the acceptance should be provided. Further, a summary table of the total number of samples that have been reanalyzed due to each reason should be provided. In cases where the first analysis yields a nonreportable result, a single reanalysis is considered sufficient (e.g., concentration above the ULOQ or excessive variability between wells). The analysis of the samples should be based on the same number of wells per study sample as in the initial analysis. In cases where the value needs to be confirmed, (e.g., pre-dose sample with measurable concentrations) multiple determinations are recommended where sample volume allows.

The safety of trial subjects should take precedence over any other aspect of the trial. Consequently, there may be other circumstances when it is necessary to reanalyze specific study samples for the purpose of an investigation.

V. INCURRED SAMPLE REANALYSIS (5)

The performance of study samples may differ from that of the calibration standards and QCs used during method validation, which are prepared by spiking blank matrix. Differences in protein binding, back-conversion of known and unknown metabolites, sample inhomogeneity, concomitant medications, or biological components unique to the study samples may affect measured concentrations of the analyte in study samples. Incurred sample reanalysis (ISR) is intended to verify the reliability of the reported sample analyte concentrations.

ISR should be performed at least in the following situations:

- For nonclinical studies within the scope of this guidance, ISR should, in general, be performed at least once per species.

- All pivotal comparative BA/BE studies.

- First clinical trial in subjects.

- Pivotal early patient trial(s), once per patient population.

- First or pivotal trial in patients with impaired hepatic and/or renal function.

ISR is conducted by repeating the analysis of a subset of samples from a given study in separate (i.e., different to the original) runs on different days using the same bioanalytical method.

The extent of ISR depends upon the analyte and the study samples and should be based upon an

in-depth understanding of the analytical method and analyte. However, as a minimum, if the total number of study samples is less than or equal to 1000, then 10% of the samples should be reanalyzed; if the total number of samples is greater than 1000, then 10% of the first 1000 samples (100) plus 5% of the number of samples that exceed 1000 samples should be assessed. Objective criteria for choosing the subset of study samples for ISR should be predefined in the protocol, study plan, or an SOP. While the subjects/animals should be picked as randomly as possible from the dosed study population, adequate coverage of the concentration profile is important. Therefore, it is recommended that the samples for ISR be chosen around the maximum concentration (C_{max}) and some in the elimination phase. Additionally, the samples chosen should be representative of the whole study.

Samples should not be pooled, as pooling may limit anomalous findings. ISR samples and QCs should be processed and analyzed in the same manner as in the original analysis. ISR should be performed within the stability window of the analyte, but not on the same day as the original analysis.

The percent difference between the initial concentration and the concentration measured during the repeat analysis should be calculated in relation to their mean value using the following Equation:

$$\%\ \text{difference} = \frac{\text{repeat value} - \text{initial value}}{\text{mean value}}\ \text{x100}$$

For chromatographic methods, the percent difference should be within ±20% for at least 2/3 of the repeats. For LBAs, the percent difference should be within ± 30% for at least 2/3 of the repeats.

If the overall ISR results fail the acceptance criteria, an investigation should be conducted and the causes remediated. There should be an SOP that directs how investigations are triggered and conducted. If an investigation does not identify the cause of the failure, the potential impact of an ISR failure on study validity should also be provided in the Bioanalytical Report. If ISR meets the acceptance criteria yet shows large or systemic differences between results for multiple samples, this may indicate analytical issues and it is advisable to investigate this further.

Examples of trends that are of concern may include:

- All ISR samples from one subject fail
- All ISR samples from one run fail

All aspects of ISR evaluations should be documented to allow reconstruction of the study and any investigations. Individual samples that are quite different from the original value (e.g., > 50%, "flyers") should not trigger reanalysis of the original sample and do not need to be investigated. ISR sample data should not replace the original study sample data.

VI. PARTIAL AND CROSS VALIDATION (6)

A. Partial Validation (6.1)

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Partial validations evaluate modifications to already fully validated bioanalytical methods. Partial validation can range from as little as one within-run accuracy and precision determination to a nearly full validation. If stability is established at one facility it does not necessarily need to be repeated at another facility.

For chromatographic methods, typical bioanalytical method modifications or changes that fall into this category include, but are not limited to, the following situations:

- Analytical site change using same method (i.e., bioanalytical method transfers between laboratories)
- A change in analytical method (e.g., change in detection systems, platform)
- A change in sample processing procedures
- A change in sample volume (e.g., the smaller volume of pediatric samples)
- Changes to the calibration concentration range
- A change in anticoagulant (but not changes in the counter-ion) in biological fluids (e.g., heparin to ethylenediaminetetraacetic acid (EDTA))
- Change from one matrix within a species to another (e.g., switching from human plasma to serum or cerebrospinal fluid) or changes to the species within the matrix (e.g., switching from rat plasma to mouse plasma)
- A change in storage conditions

For LBAs, typical bioanalytical method modifications or changes that fall into this category include, but are not limited to, the following situations:

- Changes in LBA critical reagents (e.g., lot-to-lot changes)
- Changes in MRD
- A change in storage conditions
- Changes to the calibration concentration range
- A change in analytical method (e.g., change in detection systems, platform)
- Analytical site change using same method (i.e., bioanalytical method transfers between laboratories)
- A change in sample preparation
- A change in anticoagulant (but not changes in the counter-ion) in biological fluids (e.g.,

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heparin to ethylenediaminetetraacetic acid (EDTA))

The parameters of the partial validations should meet the full validation criteria. If these criteria are not satisfied, additional investigation and validation is warranted.

B. Cross Validation (6.2)

Cross validation is required to demonstrate how the reported data are related when multiple bioanalytical methods and/or multiple bioanalytical laboratories are involved.

Cross validation is required under the following situations:

- Data are obtained from different fully validated methods within a study.
- Data are obtained within a study from different laboratories with the same bioanalytical method.
- Data are obtained from different fully validated methods across studies that are going to be combined or compared to support special dosing regimens, or regulatory decisions regarding safety, efficacy, and labeling.

If data are obtained from different fully validated methods, and these data are not to be combined across studies, cross validation is not generally required.

Cross validation should be performed in advance of study samples being analyzed, if possible. Cross validation should be assessed by measuring the same set of QCs (low, medium, and high) at least in triplicate and study samples (if available) that span the study sample concentration range ($n \geq 30$) with both methods, or in both laboratories.

Bias can be assessed by Bland-Altman plots or Deming regression. Other methods appropriate for assessing agreement between two methods (e.g., concordance correlation coefficient) may be used too. Alternatively, the concentration vs. time curves for study samples could be plotted for samples analyzed by each method to assess bias.

The use of multiple bioanalytical methods for the measurement of the same analyte in the conduct of one comparative BA/BE study is strongly discouraged.

VII. ADDITIONAL CONSIDERATIONS (7)

A. Methods for Analytes That Are Also Endogenous Molecules (7.1)

For analytes that are also endogenous molecules (e.g., replacement therapies), the accuracy of the measurement of the analytes poses a challenge when the method cannot distinguish between the therapeutic agent and the endogenous molecule. Furthermore, the endogenous levels of the analyte may vary because of age, gender, race, diurnal variations, illness, or as side effect of drug treatment. This section describes some of the approaches that may be used to assess concentrations of analytes that are also endogenous molecules. As a reminder, biomarkers are outside of the scope of this guidance.

If available, biological matrix to prepare calibration standards and QCs should be the same as the study samples (i.e., authentic biological matrix) and it should be free of matrix effect and interference, as described in sections III. (3) and IV. (4). The endogenous concentration in the biological matrix chosen should be low enough to obtain an adequate signal-to-noise ratio (e.g., <20% of the LLOQ). In those cases where matrices without interference are not available, the following approaches can be used to calculate the concentration of the analyte in the study samples: (1) the surrogate matrix approach, (2) the surrogate analyte approach, (3) the background subtraction approach, and (4) the standard addition approach.

(1) Surrogate Matrix Approach:

The matrix for the calibration standards is substituted by a surrogate matrix. Surrogate matrices can vary widely in complexity from simple buffers or artificial matrices that try to mimic the authentic one, to stripped matrices or matrices from other species.

(2) Surrogate Analyte Approach:

Stable isotope labeled analytes are used as surrogate standards in mass spectrometric methods to construct the calibration curve for the quantification of endogenous analytes. In this approach, it is assumed that the physicochemical properties of the authentic and surrogate analytes are the same with the exception of molecular weight. However, isotope standards may differ in retention time and MS sensitivity, therefore, before application of this approach, the ratio of the MS responses (i.e., the response factor) of the labeled to unlabeled analyte should be close to unity and remain constant over the entire calibration range. If the response factor does not comply with these requirements, it should be incorporated into the regression equation of the calibration curve.

(3) Background Subtraction Approach:

The concentration of the endogenous analyte observed in a pooled/representative matrix is subtracted from the concentration observed in the spiked standards, subsequently the net differences are used to construct the calibration curve.

When the background concentrations are lowered by dilution of the blank matrices before spiking with the standards (e.g., if a lower LLOQ is required) the composition of the matrices in the study samples and the calibration standards is different, which may cause different recoveries and matrix effects. These differences should be considered when validating the method.

(4) Standard Addition Approach:

The standard addition approach should only be applied for analytical platforms with linear responses. Typically, the standard addition method is used to determine the concentration of the endogenous analyte in the authentic matrix to be used for preparation of standards and QCs. However, this approach can be employed for determination of study samples as well. In this approach, every study sample is divided into aliquots of equal volume. All aliquots, but one, are separately spiked with known and varying amounts of the analyte standards to construct a calibration curve for either

the authentic blank matrix or every study sample (e.g., with three to five points). The endogenous blank concentration or the study sample concentration is then determined as the negative x-intercept of the standard calibration curve prepared in that particular study sample.

Validation of an analytical method for an analyte that is also an endogenous compound should include the following considerations, in addition to the validation shown in sections III. (3) and (4).

1. Quality Control Samples for Methods for Analytes That Are Also Endogenous Molecules (7.1.1)

The endogenous concentrations of the analyte in the biological matrix should be evaluated prior to QC preparation. The matrices with the lowest possible level of the interfering endogenous analyte should be used. The concentrations of the QCs should account for the endogenous concentrations in the authentic matrix and be representative of the expected study sample concentrations.

The QCs should resemble study samples and should be prepared in the same matrix. In principle, all QC concentrations used for validation should be aliquots of the authentic biological matrix unspiked (endogenous QC with a concentration between LLOQ and low QC, if possible) and spiked with known amounts of the authentic analyte (low QC, middle QC, and high QC). In spiked samples (e.g., LLOQ, low QC), the added amount should be enough to provide concentrations that are threefold higher or statistically different from the endogenous concentration. If options such as multiple lots, alternative vendors, and matrices of special populations that might contain a lower concentration of the analyte continue to yield matrices in which the endogenous levels are so high that it is not possible to prepare the low QC in the authentic matrix, diluted (surrogate) matrix may be used.

2. Selectivity, Recovery, and Matrix Effects for Methods for Analytes That Are Also Endogenous Molecules (7.1.2)

The assessment of selectivity is complicated by the absence of interference-free matrix. For chromatography, peak purity should be investigated as part of method validation by analyzing matrices obtained from several donors (at least six normal blanks, one hemolyzed blank, and one lipemic blank) using a discriminative detection system (e.g., tandem mass spectrometry (MS/MS)). Other approaches, if justified by scientific principles, may also be considered.

For the Standard Addition and Background Subtraction Approaches, as the same biological matrix and analyte are used for study samples and calibration standards, the same recovery and matrix effect occurs in the study samples and the calibration standards. However, if the endogenous component were not completely identical (e.g., recombinant proteins) the potential difference in recovery should be assessed in a parallelism test. For the Surrogate Matrix and Surrogate Analyte Approaches, the matrix effect and the extraction recovery may differ between calibration standards and study samples. Matrix effect should be evaluated to ensure that it does not impact accuracy and precision, mainly at LLOQ level.

- If the Surrogate Matrix Approach is used, the impact of the different matrix effect

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and recovery in both the surrogate and authentic matrix should be assessed. This should be investigated in an experiment using QCs spiked with analyte in the matrix, the endogenous matrix only and spiked analyte in the surrogate matrix alone against the surrogate calibration curve.

- If the Surrogate Analyte Approach is used in mass spectrometry chromatographic methods, the impact of the different matrix effect and recovery between surrogate and authentic endogenous analytes should be evaluated. This should be investigated in an experiment using QCs spiked with analyte in the matrix, the endogenous matrix only and spiked surrogate analyte in the matrix against the surrogate calibration curve.

- In certain cases, dilution of the QCs with surrogate matrix may be necessary (e.g., for background subtraction methods) where the endogenous level is high and the LLOQ needs to be reduced. In these cases, the recovery and matrix effect experiments should be repeated with authentic biological matrices with endogenous concentrations between LLOQ and low QC, if available.

Refer to sections III. (3) and IV. (4) for the acceptance criteria for chromatography and LBAs, respectively.

Since the composition of the biological matrix might affect method performance, matrices from at least six (chromatographic methods)/10 (LBA) different donors should be investigated, except in the Standard Addition Approach, where each sample is analyzed with its own calibration curve.

3. *Parallelism for Methods for Analytes That Are Also Endogenous Molecules (7.1.3)*

Parallelism assures that observed changes in response per given changes in analyte concentrations are equivalent for the surrogate and the authentic biological matrix across the range of the method. Parallelism should be evaluated in the Surrogate Matrix and Surrogate Analyte Approaches, taking into account that parallelism is assessed differently in LBA and chromatographic methods.

4. *Accuracy and Precision for Methods for Analytes That Are Also Endogenous Molecules (7.1.4)*

Accuracy and precision should meet criteria specified in sections III. (3) and IV. (4) for chromatography and LBAs, respectively.

In case of using a surrogate matrix or analyte approaches, the assessment of accuracy and precision should be performed by analyzing the QCs against the surrogate calibration curve. The concentration of the endogenous molecule in the blank matrix may be determined and subtracted from the total concentrations observed in the spiked samples. Accuracy is recommended to be calculated using the following formula when QCs are spiked with the authentic analyte in the matrix containing endogenous levels of the analyte:

$$(\%) = 100 \times \frac{(\text{Measured concentration of spiked sample} - \text{endogenous concentration})}{\text{Spiked concentration}}$$

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Only the precision can be determined from the analysis of each unspiked/endogenous QC.

5. *Stability for Methods for Analytes That Are Also Endogenous Molecules (7.1.5)*

To mimic study samples as much as possible, stability experiments should be investigated with the authentic analyte in the authentic biological matrix and with unspiked/endogenous QCs (blank matrix with endogenous molecule) as well as spiked low QC and high QCs as defined in section VII.A.1 (7.1.1) However, if a surrogate matrix is used for calibration standards, stability should also be demonstrated for the analyte in the surrogate matrix, as this could differ from stability in the authentic biological matrix.

B. Parallelism (7.2)

Parallelism is defined as a parallel relationship between the calibration curve and serially diluted study samples to detect any influence of dilution on analyte measurement. Although lack of parallelism is a rare occurrence for bioanalytical methods for PK evaluation, parallelism of LBA should be evaluated on a case-by-case basis, e.g., where interference caused by a matrix component (e.g., presence of endogenous binding protein) is suspected during study sample analysis. Parallelism investigations, or the justification for its absence, should be included in the Bioanalytical Report. Some methods may demonstrate parallelism for one patient population but lack it for another population. Generally, these experiments should be conducted during the analysis of the study samples due to the unavailability of study samples during method development or validation. A study sample with a high concentration (preferably close to Cmax) should be diluted to at least three concentrations with blank matrix. The consistency of the back calculated concentrations between samples in a dilution series should not exceed 30% CV.
However, when applying the 30% criterion, data should be carefully monitored as results that pass this criterion may still reveal trends of nonparallelism. In the case that the sample does not dilute linearly (i.e., in a nonparallel manner), a procedure for reporting a result should be defined *a priori*.

C. Recovery (7.3)

For methods that employ sample extraction, the recovery (extraction efficiency) should be evaluated. Recovery should be reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method. Recovery should be determined by comparing the analyte response in a biological sample that is spiked with the analyte and processed, with the response in a biological blank sample that is processed and then spiked with the analyte. Recovery of the analyte does not need to be 100%, but the extent of recovery of an analyte and of the IS (if used) should be consistent. Recovery experiments are recommended to be performed by comparing the analytical results for extracted samples at multiple concentrations, typically three concentrations (low, medium, and high).

D. Minimum Required Dilution (7.4)

MRD is a dilution factor employed in samples that are diluted with buffer solution to reduce the background signal or matrix interference on the analysis using LBA. The MRD should be identical for all samples, including calibration standards and the QCs, and it should be

determined during method development. If MRD is changed after establishment of the method, partial validation should be done. MRD should be defined in the Validation Report of the analytical method.

E. Commercial and Diagnostic Kits (7.5)

Commercial or diagnostic kits (referred to as *kits*) are sometimes codeveloped with new chemical or biological drugs for point-of-care patient diagnosis. The recommendations in this section of the guidance do not apply to the development of kits that are intended for point-of-care patient diagnosis (e.g., companion or complimentary diagnostic kits). Refer to the appropriate guidance documents regarding regulatory recommendations for the development of these kits.

If an applicant repurposes a kit (instead of developing a new method) or utilizes “research use only” kits to measure chemical or biological drug concentrations during the development of a novel drug, the applicant should assess the kit validation to ensure that it conforms to the drug development standards described in this guidance.

Validation considerations for kit assays include, but are not limited to, the following:

- If the reference standard in the kit differs from that of the study samples, testing should evaluate differences in assay performance of the kit reagents. The specificity, accuracy, precision, and stability of the kit assay should be demonstrated under actual conditions of use in the facility conducting the sample analysis. Modifications from kit processing instructions should be completely validated.

- Kits that use sparse calibration standards (e.g., one- or two-point calibration curves) should include in-house validation experiments to establish the calibration curve with a sufficient number of standards across the calibration range.

- Actual QC concentrations should be known. Concentrations of QCs expressed as ranges are not sufficient for quantitative applications. In such cases QCs with known concentrations should be prepared and used, independent of the kit-supplied QCs.

- Calibration standards and QCs should be prepared in the same matrix as the study samples. Kits with calibration standards and QCs prepared in a matrix different from the study samples should be justified and appropriate experiments should be performed.

- If multiple kit assay lots are used within a study, lot-to-lot variability and comparability should be addressed for any critical reagents included in the kits.

- If a kit using multiple assay plates is employed, sufficient replicate QCs should be used on each plate to monitor the accuracy of the assay. Acceptance criteria should be established for the individual plates and for the overall analytical run.

F. New or Alternative Technologies (7.6)

When a new or alternative technology is used as the sole bioanalytical technology from the onset of drug development, cross validation with an existing technology is not required.

The use of two different bioanalytical technologies for the development of a drug may generate data for the same product that could be difficult to interpret. This outcome can occur when one platform generates drug concentrations that differ from those obtained with another platform. Therefore, when a new or alternative analytical platform is replacing a previous platform used in the development of a drug it is important that the potential differences are well understood. The data generated from the previous platform/technology should be cross validated to that of the new or alternative platform/technology. Seeking feedback from the regulatory authorities is encouraged early in drug development. The use of two methods or technologies within a comparative BA/BE study is strongly discouraged.

The use of new technology in regulated bioanalysis should be supported by acceptance criteria established *a priori* based on method development and verified in validation.

1. Dried Matrix Methods (7.6.1)

Dried matrix methods (DMM) is a sampling methodology that offers benefits such as collection of reduced blood sample volumes as a microsampling technique for drug analysis and ease of collection, storage, and transportation. In addition to the typical methodological validation for LC-MS or LBA, use of DMM necessitates further validation of this sampling approach before using DMM in studies that support a regulatory application, such as:

- Hematocrit (especially for spotting of whole blood into cards).
- Sample homogeneity (especially for sub-punch of the sample on the card/device).
- Extraction of the sample from the dried matrix.
- DMM sample collection for ISR:
  - Care should be taken to ensure sufficient sample volumes or numbers of replicates are retained for ISR.
  - Should be assessed by multiple punches of the sample or samples should be taken in duplicate.

When DMM is used for clinical or nonclinical studies in addition to typical liquid approaches described (refer to section VI.B (6.2)). For nonclinical TK studies, refer to section IV.1 (4.1) of the May 2018 ICH guidance for industry *S3A Guidance: Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies: Focus on Micosampling—Questions and Answers* (ICH S3AQ&A). [4] Feedback from the appropriate regulatory authorities is encouraged in early drug development.

VIII. DOCUMENTATION (8)

General and specific SOPs and good record keeping are essential to a properly validated analytical method. The data generated for bioanalytical method validation should be documented and available for data audit and inspection. Table 1 describes the recommended documentation

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for submission to the regulatory authorities and documentation that should be available at the analytical site at times of inspection. This documentation should be stored at the analytical site or at another secure location. In this case, the documentation should be readily available when requested.

All relevant documentation necessary for reconstructing the study as it was conducted and reported should be maintained in a secure environment. Relevant documentation includes, but is not limited to, source data, protocols and reports, records supporting procedural, operational, and environmental concerns, and correspondence records between all involved parties.

Regardless of the documentation format (i.e., paper or electronic), records should be contemporaneous with the event and subsequent alterations should not obscure the original data. The basis for changing or reprocessing data should be documented with sufficient detail, and the original record should be maintained.

A. Summary Information (8.1)

Summary information should include the following items in sections II.F.4 (2.6.4) and II.G.1 (2.7.1) of the Common Technical Document (CTD; or electronic CTD, eCTD)) or reports:

- A summary of methods used for each study should be included. Each summary should provide the method title, method identification code, the assay type, the Bioanalytical Report code, effective date of the method, and the associated Validation Report codes.

- A summary table of all the relevant Validation Reports should be provided for each analyte, including Partial Validation and Cross Validation Reports. The table should include the method identification code, the type of method, the reason for the new method or additional validation (e.g., to lower the limit of quantification). Changes made to the method should be clearly identified.

- A summary table cross-referencing multiple identification codes should be provided when a method has different codes for the method, the Validation Reports, and the Bioanalytical Reports.

- Discussion of method changes (e.g., evolution of methods, reason(s) for revisions, unique aspects).

- For comparative BA/BE studies, a list of regulatory site inspections including dates and outcomes for each analytical site if conducted over the last 3 years, and 1 year post-study completion.

B. Documentation for Validation and Bioanalytical Reports (8.2)

Table 1 describes the recommended documentation for the Validation and Bioanalytical Reports.

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Table 1: Recommended Documentation and Reporting

Items	Documentation at the Analytical Site	Validation Report*	Bioanalytical Report*
Chromatographic System Suitability	• Dates, times, and samples used for suitability testing	• Not applicable	• Not applicable
Synopsis **Overview of Method Evolution**	• History/evolution of methods (e.g., to explain revisions, unique aspects with supportive data, if available)	• Not applicable	• Not applicable
Reference Standards	• CoA or equivalent alternative to ensure quality (including purity), stability/expiration/retest date(s), batch number, and manufacturer or source • Records of receipt, use, and storage conditions • If expired, recertified CoA, or retest of quality and identity with retest dates	• A copy of the CoA or equivalent alternative including batch/lot number, source, quality (including purity), storage conditions, and expiration/retest date, or table with this information • If expired, quality and stability at the time of use and retest dates and retested values	• A copy of the CoA or equivalent alternative including batch/lot number, source, quality (including purity), storage conditions, and expiration/retest date or a table with this information • If expired, quality and stability at the time of use and retest dates and retested values
Internal Standard	• IS quality or demonstration of suitability • Records of receipt, use, and storage conditions	• Name of reagent or standard • Origin	• Name of reagent or standard • Origin

continued

Table 1, continued

Items	Documentation at the Analytical Site	Validation Report*	Bioanalytical Report*
Critical Reagents	• Name of reagent • Batch/Lot number • Source/Origin • Concentration, if applicable • Retest date (expiration date) • Storage conditions	• Name of reagent • Batch/Lot number • Source/Origin • Concentration, if applicable • Retest date (expiration date) • Storage conditions	• Name of reagent • Batch/Lot number • Source/Origin • Concentration, if applicable • Retest date (expiration date) • Storage conditions
Stock/Working Solutions	• Record of preparation and use of stock/working solutions • Storage location and condition	• Notation that solutions were used within stability period • Stock/working solution stability • Storage conditions	• Notation that solutions were used within stability period • Stock/working solution stability • Storage condition
Blank Matrix	• Records of matrix descriptions, lot numbers, receipt dates, storage conditions, and source/supplier	• Description, lot number, receipt dates	• Description, lot number, receipt dates†
Calibration Standards and QCs	• Records and date of preparation • Record of storage temperature (e.g., log of in/out dates, analyst, temperatures, and freezer(s))	• Description of preparation, including matrix • Batch number, preparation dates, and stability period • Storage conditions (temperatures, dates, duration, etc.)	• Description of preparation • Preparation dates and stability period • Storage conditions

continued

Table 1, continued

Items	Documentation at the Analytical Site	Validation Report*	Bioanalytical Report*
Standard Operating Procedures (SOPs; Procedures)	Procedures for all aspects of analysis, such as: • Method/procedure (validation/analytical) • Acceptance criteria (e.g., run, calibration curve, QCs) • Instrumentation • Reanalysis • ISR • Record of changes to SOP (change, date, reason, etc.)	• A detailed description of the method procedures	• A list of procedures/analytical protocols used for the method
Sample Tracking	• Study sample receipt and condition on receipt • Records that indicate how samples were transported and received. Sample inventory and reasons for missing samples • Location of storage (e.g., freezer unit) • Tracking logs of QCs, calibration standards, and study samples • Freezer logs for QCs, calibration standards, and study samples entry and exit	• Not applicable	**For All Studies:** • Dates of receipt of shipments number of samples • Sample condition on receipt • Analytical site storage condition and location • Storage: total duration from sample collection to analysis • List of any deviations from planned storage conditions and potential impact **Additionally, for Comparative BA/BE Studies Also Include:** • The subject ID

continued

Table 1, continued

Items	Documentation at the Analytical Site	Validation Report*	Bioanalytical Report*
Analysis	• Documentation and data for system suitability checks for chromatography • Instrument use log, including dates of analysis for each run • Sample extraction logs, including documentation of processing of calibration standards, QCs, and study samples for each run, including dates of extraction • Identity of QCs and calibration standard lots, and study samples in each run • Documentation of instrument settings and maintenance • Laboratory information management system (LIMS) • Validation information, including documentation and data for: ➢ Selectivity, specificity, sensitivity, precision and accuracy, carryover, dilution, recovery, matrix effect ➢ Bench top, freeze-thaw, long-term, extract, and stock solution stability ➢ Cross/partial validations, if applicable	**For All Studies:** • Table of all runs (including failed runs), and analysis dates • Table of calibration standard concentration and response functions results (calibration curve parameters) of all accepted runs with accuracy and precision • Table of within- and between- run QC results and calibration standards (from accuracy and precision runs). Values outside the acceptance criteria should be clearly marked • Include total error for LBA methods. • Data on selectivity, specificity, dilution linearity, and sensitivity (LLOQ), carryover, recovery. Bench top, freeze-thaw, long-term, extract, and stock solution stability • Partial/cross-validation, if applicable • Append separate report for additional validation, if any **Additionally, for Comparative BA/BE Studies Also Include:** • Instrument ID for each run in comparative BA/BE studies. • 100% of run summary table of accepted and failed runs	**For All Studies:** • Table of all runs, status (accepted and failed), reason for failure, and analysis dates. • Table of calibration standard concentration and response function results (calibration curve parameters) of all accepted runs with accuracy and precision • Table of QCs results of all accepted runs with overall (between-run) accuracy and precision results of the QCs and between-run accuracy and precision results from accepted runs • Table of reinjected runs with results from reinjected runs and reason(s) for reinjection • QCs graphs trend analysis encouraged • Study concentration results table **Additionally, for Comparative BA/BE Studies Also Include:** • Instrument ID for each run in comparative BA/BE studies • IS response plots for each analytical run, including failed runs • 100% of run summary table of accepted and failed runs

continued

Table 1, continued

Items	Documentation at the Analytical Site	Validation Report*	Bioanalytical Report*
Chromatograms and Reintegration	• Electronic audit trail: • 100% e-chromatograms of original and reintegration from accepted and fail runs • Reason for reintegration • Mode of reintegration 100% of run summary tables of accepted and failed runs, including calibration curve, regression, weighting function, analyte and IS response and retention time, response ratio, integration type	**For All Studies:** • Representative chromatograms (original and reintegration) • Reason for reintegration • Chromatograms may be submitted as a supplement **Additionally, for Comparative BA/BE Studies Also Include:** • 100% chromatograms of original and reintegration from accepted and fail runs • 100% of run summary table of accepted and failed runs	**For All Studies:** • Chromatograms may be submitted as a supplement • For studies other than comparative BA/BE, randomly selected chromatograms from 5% of samples submitted in application dossiers • Reason for reintegration • Identification and discussion of chromatograms with manual reintegration • SOP for reintegration, as applicable **Additionally, for Comparative BA/BE Studies Also Include:** • 100% of chromatograms. • Original and reintegrated chromatograms and initial and repeat integration results • 100% of run summary table of accepted and failed runs

continued

Table 1, continued

Items	Documentation at the Analytical Site	Validation Report*	Bioanalytical Report*
Deviations From Procedures	• Contemporaneous documentation of deviations/unexpected events • Investigation of unexpected events • Impact assessment	• Description of Deviations • Impact on study results • Description and supporting data of significant investigations	• Description of deviations • Impact on study results • Description and supporting data of significant investigations
Reanalysis/Repeat Analysis	• Procedures for conducting reanalysis/repeat analysis (define reasons for reanalysis, etc.) • Retain 100% of repeat/reanalyzed data • Contemporaneous records of reason for repeats	• Not applicable	**For All Studies:** • Table of sample IDs, reason for repeat analysis, original and repeat analysis values, reason for reported values, run IDs **Additionally, for Comparative BA/BE Studies Also Include:** • For comparative BA/BE studies, values from rejected runs should be included in a separate table
ISR	• Procedure for ISR • ISR data: Run IDs, run summary sheets, chromatograms, or other electronic instrument data files • Document ISR failure investigations, if any	• Not applicable	• ISR data table (original and reanalysis values and run IDs, percent difference, percent passed) • ISR failure investigations, if any†

continued

Table 1, continued

Items	Documentation at the Analytical Site	Validation Report*	Bioanalytical Report*
Communication	• Between involved parties (Applicant, contract research organizations, and consultants) related to study/method	• Not applicable	• Not applicable
Audits and Inspections	• Evidence of audit and inspections	• Not applicable/Refer to section VIII.A (8.1) for summary information to include in the eCTD	• Not applicable/Refer to section VIII.A (8.1) for summary information to include in the eCTD

*The applicant should maintain data at the analytical site to support summary data submitted in Validation and Bioanalytical Reports. Validation and Bioanalytical Reports should be submitted in the application.

†Submit either in Validation Report or in Bioanalytical Report.

GLOSSARY

Accuracy:
The degree of closeness of the measured value to the nominal or known true value under prescribed conditions (or as measured by a particular method). In this document, accuracy is expressed as percent of the nominal value:

$$\text{Accuracy (\%)} = (\text{Measured Value}/\text{Nominal Value}) \times 100.$$

Analysis:
A series of analytical procedures from sample processing/dilution to measurement on an analytical instrument.

Analyte:
A specific chemical moiety being measured, including an intact drug, a biomolecule or its derivative, or a metabolite in a biological matrix.

Analytical Run (also referred to as *Run*):
A complete set of analytical and study samples with appropriate number of calibration standards and QCs for their validation. Several runs may be completed in one day or one run may take several days to complete.

Anchor Calibration Standards/Anchor Points:
Spiked samples set at concentrations below the LLOQ or above the ULOQ of the calibration curve and analyzed to improve curve fitting in LBAs.

Batch (for Bioanalysis):
A batch is comprised of QCs and study samples, and possibly blanks, zero samples, and calibration standards, which are handled during a fixed period of time and by the same group of analysts with the same reagents under homogenous conditions.

Batch (for Reference Standards and Reagents):
A specific quantity of material produced in a process or series of processes so that it is expected to be homogeneous within specified limits. Also referred to as *Lot.*

Bias:
The tendency of a measurement process to over- or underestimate the value of a population parameter.

Bioanalytical Method:
Analytical method used in the quantitative determination of analytes in biological matrices.

Biological Drugs:
Drugs that are made by living organisms or cells (e.g., therapeutic proteins).

Biological Matrix:
A biological material including, but not limited to, blood, serum, plasma, and urine.

Binding Reagent:
A reagent that binds to the analyte in LBA-based bioanalytical methods.

Blank Sample:
A sample of a biological matrix to which no analyte, no IS, and no additional-alternative matrix or buffer has been added.

Calibration Curve:
The relationship between the instrument response (e.g., peak area, height, or signal) and the concentration (amount) of analyte in the calibration standards within a given range. Also referred to as *Standard Curve.*

Calibration Range:
The interval between the upper and lower concentration (amounts) of analyte in the calibration standards (including these concentrations).

Calibration Standard:
A matrix to which a known amount of analyte has been added or spiked. Calibration standards are used to construct calibration curves.

Carryover:
The appearance of an analyte signal in a sample from a preceding sample.

Chemical Drugs:
Chemically synthesized drugs.

Critical Reagent:
Critical reagents for LBAs include binding reagents (e.g., antibodies, binding proteins, and peptides) and those containing enzymatic moieties that have a direct impact on the results of the assay.

Cross Validation:
Assessment of potential bias between two bioanalytical methods or the same bioanalytical method used in different laboratories in order to determine whether reported data are comparable.

Dilution Integrity:
Assessment of the sample dilution procedure to confirm that the procedure does not impact the measured concentration of the analyte.

Dilution Linearity:
A parameter demonstrating that the method can appropriately analyze samples at a concentration exceeding the ULOQ of the calibration curve without influence of prozone (hook) effect and that the measured concentrations are not affected by dilution within the calibration range in LBAs.

Dilution Factor:
The magnitude by which a sample is diluted.

Full Validation:
Establishment of all validation parameters that ensure the integrity of the method when applied to sample analysis.

Hook Effect:
Suppression of response due to very high concentrations of a particular analyte. A hook effect

may occur in LBAs that use a liquid-phase reaction step for incubating the binding reagents with the analyte. Also referred to as *Prozone Effect.*

Incurred Sample:
A sample obtained from study subjects or animals.

Incurred Sample Reanalysis (ISR):
Reanalysis of a portion of the incurred samples in a separate analytical run on a different day to determine whether the original analytical results are reproducible.

Interfering Substance:
A substance that is present in the matrix that may affect the quantification of an analyte.

Internal Standard (IS):
A structurally similar analogue or stable isotope labeled compound added to calibration standards, QCs, and study samples at a known and constant concentration to facilitate quantification of the target analyte.

Ligand Binding Assay (LBA):
A method to analyze an analyte of interest using reagents that specifically bind to the analyte. The analyte is detected using reagents labeled with, e.g., an enzyme, radioisotope, fluorophore, or chromophore. Reactions are carried out in microtiter plates, test tubes, disks, etc.

Lower Limit of Quantification (LLOQ):
The lowest amount of an analyte in a sample that can be quantitatively determined using a method with predefined precision and accuracy.

Matrix Effect:
The direct or indirect alteration or interference in response due to the presence of unintended analytes or other interfering substances in the sample.

Minimum Required Dilution (MRD):
The initial dilution factor by which biological samples are diluted with buffer solution for the analysis by LBAs. The MRD may not necessarily be the ultimate dilution but should be identical for all samples including calibration standards and QCs. However, samples may require further dilution.

Nominal Concentration:
Theoretical or expected concentration.

Parallelism:
Parallelism demonstrates that the serially diluted incurred sample response curve is parallel to the calibration curve. Parallelism is a performance characteristic that can detect potential matrix effects.

Partial Validation:
Validation based on evaluation of selected validation parameters. Applicable to methods that were changed after full validation.

Precision:
The closeness of agreement (i.e., degree of scatter) among a series of measurements. Precision is

expressed as the coefficient of variation (CV), or the relative standard deviation (RSD) expressed as a percentage:

%CV = (Standard Deviation/Mean) x 100.

Processed Sample:
The final sample that has been subjected to various manipulations (e.g., extraction, dilution, concentration).

Quality Control Sample (QC):
A biological matrix spiked with a known quantity of analyte that is used to monitor the performance of a bioanalytical method and assess the integrity and validity of the results of the unknown samples analyzed in an individual batch or run.

Reanalysis:
An additional evaluation of a previously assayed sample. Also referred to as *Repeat Analysis.*

Recovery:
The extraction efficiency of an analytical process, reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method.

Reference Standard:
A well-characterized substance of known purity and identity used to prepare calibration and quality control samples.

Reintegration:
Change of the original integration of a chromatographic peak

Replicate:
One of several determinations or measurements of a sample, calibration standards or QC.

Reproducibility:
The extent to which consistent results are obtained when an experiment is repeated.

Response Function:
A mathematical expression which adequately describes the relationship between instrument response (e.g., peak area or height ratio or signal) and the concentration (amount) of analyte in the calibration standards. Response function is defined within a given range. See also *Calibration Curve.*

Run Summary Table:
Tabular output of all data from individual samples, QCs, and calibration standards within the analytical run (e.g., for chromatography retention times, analyte and IS responses, concentrations, and dilution factors if any; for ligand binding assays analyte responses concentrations, and dilution factors).

Selectivity:
Ability of an analytical method to differentiate and measure the analyte in the presence of interfering substances in the biological matrix (nonspecific interference).

Sensitivity:
The lowest analyte concentration that can be measured with acceptable accuracy and precision

(i.e., LLOQ).

Specificity:
Ability of an analytical method to detect and differentiate the analyte from other substances, including its related substances (e.g., substances that are structurally similar to the analyte, metabolites, isomers, impurities, or concomitant medications).

Stability:
Measure of the intactness of an analyte (lack of degradation) in a given matrix under specific storage and use conditions relative to the starting material for given time intervals.

Standard Curve:
The relationship between the instrument response (e.g., peak area, height, or signal) and the concentration (amount) of analyte in the calibration standards within a given range. Also referred to as *Calibration Curve.*

Standard Operating Procedure (SOP):
Detailed written instructions to achieve uniformity of the performance of a specific function and/or process(es).

Stock Solution:
An analyte in a solvent or mixture of solvents at a known concentration, which is used to prepare calibration standards or QCs.

Study Samples:
Samples from animals or subjects enrolled in nonclinical or clinical studies.

Surrogate Matrix:
An alternative to a study matrix of limited availability (e.g., tissue, cerebrospinal fluid, bile) or where the study matrix contains an interfering endogenous counterpart.

System Suitability:
Determination of instrument performance (e.g., Signal-to-Noise ratio, peak shape, retention time) by analysis of a prepared, spiked sample conducted prior to the analytical run and is not a part of the sample analysis.

Total Error:
The sum of the absolute value of the errors in accuracy (%) and precision (%). Total error is reported as percent (%) error.

Upper Limit of Quantification (ULOQ):
The highest amount of an analyte in a sample that can be quantitatively determined with predefined precision and accuracy.

Validation:
Demonstration that a bioanalytical method is suitable for its intended purpose.

Working Solution:
A non-matrix solution prepared by diluting the stock solution in an appropriate solvent. It is mainly added to matrix to prepare calibration standards and QCs.

Zero Sample:
A blank sample spiked with an IS.