The AAPS Journal 2007; 9 (2) Article 14 (http://www.aapsj.org).

Themed Issue: Bioanalytical Method Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays
Guest Editors - Mario L. Rocci Jr., Vinod P. Shah, Mark J. Rose, Jeffrey M. Sailstad

Procedural Elements Involved in Maintaining Bioanalytical Data Integrity for Good Laboratory Practices Studies and Regulated Clinical Studies

Submitted: February 11, 2007; Accepted: April 2, 2007; Published: April 27, 2007

Christopher A. James[1] and Howard M. Hill[2]

[1]Pharmacokinetics and Drug Metabolism, Amgen Inc, Thousand Oaks, CA 91320
[2]Pharmaceutical Development, Huntingdon Life Sciences, Huntingdon, Cambridge, PE28 4HS, UK

Abstract

This article describes procedural elements involved in ensuring the integrity of bioanalytical data. These elements can be divided into 3 areas. First, there are those ensuring the integrity of the analyte until analysis, through correct sample collection, handling, shipment, and storage procedures. Incorrect procedures can lead to loss of analyte via instability, addition of analyte through contamination or instability of related metabolites, or changes in the matrix composition that may adversely affect the performance of the analytical method. Second, the integrity of the sample identity needs to be maintained to ensure that the final result reported relates to the individual sample that was taken. Possible sources of error include sample mixup or mislabeling, or errors in data handling. Finally, there is the overall integrity of the documentation that supports the analysis, and any prestudy validation of the method. This includes a wide range of information, from paper and electronic raw data, through standard operating procedures and analytical procedures and facility records, to study plans and final reports. These are critical to allow an auditor or regulatory body to reconstruct the study.

Keywords: Sample integrity, audit trails, bioanalytical methods validation, GLP

Introduction

Many aspects of bioanalytical method validation focus on the performance of a method as it is used in the analytical laboratory. However, the laboratory analysis is only 1 component controlling the overall quality of the data; several other procedural elements can also affect data integrity. We can divide these procedural or nonanalytical elements into 3 main categories. First, there are factors that could affect the actual, or apparent, measured amount of the analyte in the sample. These generally relate to the handling and storage of the samples prior to analysis and can result in either a change in the amount of analyte in a sample or a change in the ability of the analytical method to accurately measure the analyte (eg, a matrix effect in both Liquid Chromatography-Mass Spectrometry-Mass Spectrometry [LC-MS-MS] analysis and ligand binding assays). Second, correct labeling and identification of the sample is critical; an ambiguously or incorrectly labeled sample will automatically result in an incorrect result. Finally, there are the processes, procedures, and documentation that support data security, data integrity, and the ability to reconstruct the analysis.

Corresponding Author: Christopher A. James, Pharmacokinetics and Drug Metabolism, Amgen Inc, One Amgen Center Drive, Mail Stop 30e-2-B, Thousand Oaks, CA 91320-1799. Tel: (805) 447-1771/231-1748; Fax: (805) 480-3057; E-mail: cajames@amgen.com

Factors That Can Affect the Analytical Result

Sample Collection

Some type of processing is required for most biological samples immediately following their collection from an animal or human subject. Most commonly this involves collection of a venous blood sample followed by centrifugation to harvest plasma or serum to be frozen for later analysis. For most analytes this should be a relatively simple procedure, and conditions such as temperature, centrifugation time and force, and maximum time from sampling to freezing the sample are specified in study documentation. While straightforward, these tasks will be performed a large number of times, and for a multisite study, often at many different locations. In contrast to the bioanalysis conducted in the laboratory, there are no calibration or quality control samples being run alongside these sample collection processes to indicate if any problems occurred. The effect of variability in these processes will be highly dependent on the nature of the analyte and matrix. In some cases, sample collection conditions may be particularly important; for example, if an analyte is less stable in whole blood than in plasma, any delay in processing the sample or poor temperature control could result in analyte loss. Some analytes are not stable under standard sample collection conditions and may need stabilizers or other special sample handling conditions to be

applied. There is also the potential for contamination of samples during collection, especially in toxicology studies where high doses of the drug are administered. This has resulted in significant discussion over the last few years, and a European guideline[1] that addresses the issue of contamination in toxicology studies was published.

Because of the lack of quantifiable quality control procedures covering initial sample handling, it is essential that procedures be clearly defined in a protocol or study manual and be readily available to the staff processing the samples. Staff involved in this aspect of the work must also be fully trained and experienced in carrying out these activities. While sample collection and initial processing are often very simple technical procedures, if they are poorly performed, the quality of the samples will be compromised, nullifying any further activities performed in the analytical laboratory.

Sample Stability and Storage Conditions

The current US Food and Drug Administration (FDA) guidance[2] and conference report[3] already describes in some detail how stability of analytes in the biomatrix should be defined during validation and that during analysis of study samples, it is necessary to ensure that samples are stored under the same conditions and analyzed within the period of defined stability. Required stability experiments for the analyte in biomatrix typically include short-term stability at room temperature, freeze/thaw stability, and long-term stability in frozen biomatrix (typically at –20°C or –70°C). Adequate documentation is also needed to track the location of the samples throughout their storage, from receipt until disposal, and to document the temperature in the storage freezers while the samples are stored. Adequate contingencies, including some backup capacity, should also be in place to protect the samples in the event of a failure of a freezer or the main power supply. Freezers, therefore, will require backup power, an alarm system to alert staff of temperature changes outside a prescribed range, and procedural arrangements to call-in staff outside of normal working hours to resolve a problem or transfer samples to another correctly functioning freezer comparable in characteristics to the defective freezer.

Sample Transport

Biomatrix samples are usually shipped frozen in insulated containers with dry ice. The main concern is ensuring that the shipment is still frozen upon arrival. Data loggers can be included within shipment packages to monitor temperature; however, these are not generally used, particularly as shipments are usually packed with sufficient dry ice to last for a significantly greater period than the anticipated shipment time. It is important that the sample condition be accurately recorded on receipt to document that samples were received still frozen and in good condition. Additional attention may be warranted for shipments with particular risks, for example, where delays could occur in customs clearance. The increasing sensitivity of modern analytical methods has resulted in sample volumes tending to be reduced. Often, samples are split into 2 aliquots at the collection site, for additional security; a set of reserve aliquots can then be safely stored until the first set is received for analysis.

Sample Identification and Labeling

The *FDA Compliance Program Guidance Manual* (Chapter 48, Bioresearch Monitoring: Human Drugs)[4] refers to the possibility of sample mixup on at least 3 separate occasions. Sample labeling and traceability is critical, because once a sample has been mislabeled, it will always provide an incorrect analytical result. Given the hundreds, often thousands, of samples collected in preclinical and clinical studies, it is almost inevitable that some labeling errors may occur, most likely when samples are transferred from 1 tube to another. These errors may be minimized by clear, simply described study designs, clear label design, and adequate workspace and procedures to minimize the risk of confusing samples during their processing.

Other types of errors can occur when labels are ambiguous, do not match the protocol or case report forms, or have been altered or incorrectly completed. A key step when samples are received at the analytical laboratory is to reconcile their identity against the study protocol, sample accession list, and/or other study documentation. It is more likely that an error can be resolved if a problem is noted shortly after collection of the sample rather than many months later. Some laboratories relabel tubes on receipt to provide their own unique number, especially when barcodes are to be used to monitor the audit trail; the additional labels should not obscure the original labels.

It is important to remember that sample labeling issues cannot be resolved by bioanalysis; repeat analysis can be used only to confirm whether an anomalous value may have been caused by an analytical error. There is some tendency to want to proceed with the analysis of ambiguously labeled samples to "see what you get." We believe that analysis of poorly labeled samples should not be performed until or unless the labeling issue can be resolved.

Documentation

Any analysis supporting a good laboratory practices (GLP) or regulated clinical study needs to be fully documented, so that the study could be reconstructed. A variety of documents, from laboratory raw data, laboratory notebooks and worksheets, and facility and calibration records, to standard operating procedures (SOPs) and method documents, are typically required to fully document a study. At the end of a

validation project, or on completion of sample analysis, a validation or analytical report is normally produced. Such reports not only provide details of study results and methods but also direct the reader to the location of all other records that support the study, including archived paper and electronic records, and references to methods and validations supporting the analysis. A separate validation report is almost always generated on completion of validation experiments; however, for analytical support studies, a separate report may not always be created, and reporting of the bioanalytical results may be integrated with the main study report, or with a sub-report for a related part of the study (for example, as a bioanalysis and toxicokinetic report).

Increasingly, a significant amount of raw data may exist only as electronic records. Special criteria apply to data that are generated and stored as electronic records by computerized systems. In these situations, laboratories must have policies and procedures in place to ensure that they meet the prevailing criteria for the acceptance of the electronic records/signatures as the equivalent of paper records/signatures by the regulatory authorities. FDA regulation 21 CFR Part 11[5] allows for the use of electronic records and for electronic signatures when appropriate.

Data System Validation and Laboratory Information Management Systems

Generation of data in a modern analytical laboratory is likely to involve the use of 1 or more computerized systems. Typically, the analytical instrument used to run the analysis (eg, an LC-MS-MS system, or a plate reader for an enzyme linked immunosorbent assay [ELISA] assay) will be controlled by a computer that will also capture data as it is generated. These data will often subsequently be transferred to a laboratory information management system (LIMS) for further processing and evaluation, storage, and report generation. Further transfer of the data may then take place to allow statistical or pharmacokinetic analysis. While a LIMS is not an absolute requirement, it is unlikely that a modern bioanalytical laboratory could function efficiently without one. Computer validation is a major topic that is beyond the scope of this article. Computerized systems used to generate, manipulate, modify, or store electronic data should be validated, and key instrumentation should be appropriately qualified before use. When data are transferred between electronic systems, the link or transfer process should, ideally, be validated. If not, procedural and quality control processes will be needed to demonstrate that data integrity has been maintained.

Reporting

Reporting arrangements will depend on whether the analytical work undertaken formed part of a study or was a separate study. Similar principles apply to bioanalytical support for both preclinical GLP studies and regulated clinical studies, but for full GLP studies, the requirements for multisite studies[1] should be followed. Both the current FDA guidance,[2] and the conference report,[3] contain specific recommendations on the contents of bioanalytical reports. Bioanalytical reports may be an integral part of preclinical or clinical reports or may be appendixes to such reports.

If the analytical work constitutes a complete study, there should be a final report containing the essential information required by the principles of GLP. Any data included in the report that were not generated following GLP principles, or were generated by a facility not claiming GLP compliance, should be fully identified (on the GLP compliance statement) by the study director. If the analytical work was the responsibility of a principal investigator, that person is responsible for producing a report detailing the work performed under his or her supervision and for sending the report to the study director. There should be a statement signed by the principal investigator certifying that the report accurately reflects all of the work performed and results obtained, and that the work was conducted in compliance with the principles of GLP. The principal investigator may present the original raw data as his or her report, accompanied by a statement of GLP compliance. If the work was conducted by a subcontractor laboratory, there should also be a quality assurance statement signed by that laboratory's quality assurance unit.

SOPs

Readily available in every laboratory area should be copies of the SOPs relevant to the activities performed in that area. The following are examples of SOPs relating to laboratory activities:

1. Test and reference items: receipt and handling, labeling and traceability, identification, characterization, storage, measures to prevent cross-contamination
2. Equipment, materials, and reagents
    1. Apparatus: use, maintenance, cleaning, validation, calibration and/or standardization, environmental monitoring of storage facilities
    2. Computerized systems: validation, operation, maintenance, security, change control, backup
    3. Materials and reagents: preparation and labeling
3. Laboratory operations
    1. Sample handling: receipt, preparation, collection of homogeneous samples, storage
    2. Housekeeping and waste disposal, control of methods of analysis

3. Validation of the analytical procedure, method acceptance criteria
4. Adherence to appropriate health and safety laws and guidelines pertaining to that facility

4. Documentation: control and handling of documentation, definition of raw data, data collection, preparation of the analytical report or final report, data storage and retrieval

The conference report[2] makes specific reference to the requirement to have SOPs for run acceptance criteria, assay procedure, reintegration, and reassay. Of these, generation of procedures for objective and consistent reintegration of chromatographic peaks is, in particular, a contentious area. While automatic algorithms often work well, there are inevitably situations where baselines appear to have been set incorrectly by integration software and could readily be "corrected" by, for example, manual redrawing of a baseline. However, allowing operators to perform such actions may introduce unintentional or deliberate bias. An increasingly popular option is to never allow manual reintegration of spurious peaks; this can be approached by careful setting of appropriate integration parameters but may be problematic for methods with less than ideal chromatography. If manual integration is to be implemented, the process to be followed will need to be fully documented in the SOPs. It is essential that operators be fully trained and that any changes be peer reviewed in order to ensure consistency in an area with the potential for bias.

Laboratories should also anticipate the need to have SOPs covering the reanalysis of incurred samples to demonstrate assay reproducibility. An SOP for the investigation of anomalous results (sometimes also termed "out of specification" results) is also an emerging requirement. This SOP needs to address problems that are obvious according to predefined specifications (eg, multiple batch failures) and to identify and investigate situations in which all specifications have been met but there are indications of problems that may affect data quality (eg, contamination or strange pharmacokinetic profiles). Analysts need to be aware that such problems may exist even when defined specifications are met. The SOP should address the need to pinpoint the source of the problem if possible, assess the problem's impact on the study, and discuss procedures for eliminating or minimizing this impact.

Study Director and Principal Investigator

As discussed below, bioanalysis is typically conducted according to the principles of GLP but can only be claimed to be in full compliance with GLP regulations when it supports GLP toxicology studies. This has led to some confusion in the terminology used for the roles and responsibilities associated with bioanalytical projects. For (preclinical) GLP studies (ie, bioanalytical support for toxicokinetic assessment), the role of the (bioanalytical) principal investigator is clearly defined in Organization for Economic Cooperation and Development (OECD) guidelines[1] ("acts on behalf of the Study Director for the delegated phase and is responsible for ensuring compliance with the Principles of GLP for that phase"). Outside of this clear definition, the principles of GLP are applied and a senior bioanalytical scientist is appointed, for example, to be the equivalent of a "study director" for a validation study, or to be responsible for the bioanalytical component of a clinical study. However, most laboratories avoid using "principal investigator" in the context of clinical bioanalytical support, as this term is also used to describe the individual with overall responsibility for the clinical study. For bioanalytical validation studies, some labs use "study director" or "principal investigator" to denote the scientist with overall responsibility for the study, while others avoid these terms, as they could indicate that the study is a full GLP study (which it generally is not).

Protocols and Amendments

For validation studies there is typically a separate plan or protocol issued prior to commencement of the validation experiments to describe in detail how the validation will be conducted. As for all such documents generated according to GLP principles, plans or protocols should be altered only by issuing an amendment. Validations of methods are typically regarded as a separate study, and consequently the validation plan would be expected to follow the principles of GLP in terms of not only describing experimental details but addressing issues such as data analysis, reporting, and archiving. For bioanalytical support of preclinical and clinical studies, there is wide variability in whether a separate analysis plan to describe the analysis of samples from an individual study is generated. Anecdotal evidence suggests that this practice has been more common for outsourced studies in Europe than in the United States. At this time, there does not appear to be any absolute regulatory requirement to generate such a plan, provided that key details about responsibility for any bioanalysis are provided in the main study plan or protocol.

Compliance with GLP?

FDA bioanalytical guidelines[2] are applicable to bioanalytical method validation and sample analysis from bioequivalence, pharmacokinetic, and comparability studies in both human and nonhuman subjects, and they indicate that validation and analysis will be performed according to the principles of GLP.

GLP was developed as a consequence of inadequacies in preclinical studies, and as it is now defined, it applies to only nonclinical studies. As a consequence, it is self-evident that the application of a bioanalytical method to a toxicokinetic study should be performed in compliance with GLP. However, for validation of a bioanalytical method, the requirement to carry out these aspects in compliance with GLP is debatable. Indeed, in both UK and Japanese regulations, validation is considered a non-GLP activity, although there is a preference throughout the industry for validation to be performed in a GLP environment, following the principles of GLP.

The issue becomes more complex when one considers which studies must or can be performed in full compliance with GLP regulations. The FDA bioanalytical method validation guidance can be implemented in a GLP or non-GLP environment, but implementation of this guidance is not synonymous with GLP. Indeed, in the context of the FDA, bioanalysis is an integral part of non-GLP-based guidances, such as 21CFR320, Bioavailability and Bioequivalence Requirements (Drugs for Human Use).[6]

The FDA bioanalytical method validation guidance[2] provides some further insight on this point: "The analytical laboratory conducting BA [bioavailability] and BE [bioequivalence] studies should 'closely adhere' to FDA's GLPs and to sound principles of quality assurance throughout the testing process." The legal claim "compliance with GLP" is replaced with "closely adhere to FDA's GLPs." Many laboratories have developed similar statements that do not claim compliance with GLP but state that the claim processes are closely related to it, for instance, "This study was carried out in laboratories that are GLP certified" or "This study was carried out in accordance with the principles of GLP." These terminologies are reflected in the Committee for Proprietary Medicinal Products (CPMP) *Note for Guidance on Investigation of Bioavailability and Bioequivalence*,[7] which states that the bioanalytical part of bioequivalence trials should be conducted according to principles of GLP. While this is not the same as requiring such studies to be done in full compliance with GLP regulations, there is an expectation by the inspecting agencies that there will be close adherence to GLP, although a specific claim to compliance with GLP in the case of clinical studies cannot be made.

Conclusion

Regardless of guidances and GLP, the quality of any analytical data is a function of the need to ensure sample integrity and stability from the time it leaves the "subject" to the time it is analyzed using a validated and fully documented analytical procedure that is suited to the study. Documentation must be available to reconstruct, if necessary, all processes and procedures used to generate the final analytical result, from sample collection through laboratory analysis and generation of the final authorized study report.

Acknowledgments

The authors gratefully acknowledge the input of colleagues at Amgen and Huntingdon Life Sciences.

References

1. Organisation for Economic Co-operation and Development. *OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring No 13*. Paris, France: ENV,JM,MONO. 2002.

2. Food and Drug Administration. *Guidance for Industry: Bioanalytical Method Validation.* Rockville, MD: US Department of Health and Human Services, FDA, CDER; 2001.

3. Viswanathan CT, Bansal S, Booth B, et al. Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. *AAPS J*. 2007;9:E30-E42. serial online.

4. Food and Drug Administration. *FDA Compliance Program Guidance Manual. Chapter 48: Program 7348.001.* Rockville, MD: FDA; 2000.

5. Food and Drug Administration. *Guidance for Industry Part 11, Electronic Records; Electronic Signatures—Scope and Application.* Rockville, MD: US Department of Health and Human Services, FDA, CDER; 2003.

6. Food and Drug Administration. *21CFR320—Bioavailability and Bioequivalence Requirements (Drugs for Human Use).* Rockville, MD: US Department of Health and Human Services, FDA, CDER; 2002.

7. Committee for Proprietary Medicinal Products. *Note for Guidance on Investigation of Bioavailability and Bioequivalence*. Canary Wharf, London: CPMP,EWP,QWP-1401/98. 2001.